ENCYCLOPEDIA OF TOXICOLOGY, FOUR-VOLUME SET, 1-4

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Second Edition

Philip Wexler, Bethesda, MD
Bruce Anderson
Ann de Peyster
Shayne Gad, Gad Consulting Services, Raleigh, North Carolina, U.S.A.
P.J. Hakkinen, Procter & Gamble Company, Cincinnati, Ohio, U.S.A.
Michael Kamrin
Betty Locey
Harihara Mehendale
Carey Pope
Lee Shugart

Description

The second edition of the **Encyclopedia of Toxicology** continues its comprehensive survey of toxicology. This new edition continues to present entries devoted to key concepts and specific chemicals. There has been an increase in entries devoted to international organizations and well-known toxic-related incidents such as Love Canal and Chernobyl. Along with the traditional

scientifically based entries, new articles focus on the societal implications of toxicological knowledge including environmental crimes, chemical and biological warfare in ancient times, and a history of the U.S. environmental movement. With more than 1150 entries, this second edition has been expanded in length, breadth and depth, and provides an extensive overview of the many facets of toxicology. Also available online via ScienceDirect – featuring extensive browsing, searching, and internal cross-referencing between articles in the work, plus dynamic linking to journal articles and abstract databases, making navigation flexible and easy. For more information, pricing options and availability visit www.info.sciencedirect.com.

Overview of all books

Audience
Toxicologists, pharmacologists, drug companies, toxicology testing labs, libraries, poison control centers, physicians, legal and regulatory professionals (EPA, government), and chemists.

Contents
Esterases Absorption Academy of Toxicological Sciences Acceptable Daily Intake (ADI) Accutane ACE Inhibitors Acenaphthene Acephate Acetaldehyde Acetamide Acetaminophen Acetamiprid Acetic Acid Acetone Acetonitrile Acetylaminofluorene Acetylcholine Acetylene Acetylsalicylic Acid Acids Aconitum Species Acrolein Acrylamide Acrylic Acid Acrylonitrile Adamsite Adiponitrile Aerosols Aflatoxin Agency for Toxic Substances and Disease Registry Agent 15 Agent Orange Aggregate Exposures Alachlor Alar Albuterol Alcoholic Beverages and Alcoholism Aldicarb Aldrin Algae Alkalies Alkyl Halides Allyl Alcohol Allyl Formate a-Methylfentanyl a-Naphthyl Thiourea Aluminum (Al) Aluminum Phosphide Amdro American Academy of

Clinical Toxicology American Association of Poison Control Centers American Board of Toxicology American College of Medical Toxicology American College of Toxicology American Conference of Governmental Industrial Hygienists American Industrial Hygiene Association Ames Test 4-Aminobiphenyl Aminoglycosides 4-Aminopyridine Amiodarone Amitraz Ammonia Ammonium Nitrate Ammonium Perchlorate Amphetamine Amphibians Amyl Nitrate Anabolic Steroids Analytical Toxicology Androgens Anesthetic Agents Aniline Animal Models "Animals, Poisonous and Venomous" Antagonism Anthracene Anthrax Anticholinergics Antimony (Sb) Antimony Trioxide Anxiolytics Apoptosis Aquatic Ecotoxicology Aramite Arsenic (As) Arsine Arum Asbestos Ascorbic Acid Aspartame Astemizole Atrazine Atropine Avermectin Avian Ecotoxicology Azamethiphos Azothioprine Azinphos-Methyl Bacillus cereus Bacillus thuringiensis BAL (British Antilewisite) Baneberry "Barbiturates, Long-Acting" "Barbiturates, Short-Acting" Barium (Ba) Baycol Baygon BCNU (Bischloroethyl Nitrosourea) Behavioral Toxicology Belladonna Alkaloids Benadryl Benchmark Dose Benomyl Benzene Benzene Hexachloride Benzidine Benzo(ghi)perylene Benzodiazepines Benzo[a]pyrene Benzyl Alcohol Benzyl Benzoate Benz[a]anthracene Beryllium (Be) Beta Blockers Biguanides Bioaccumulation Biocompatibility Bioconcentration Bioinformatics Biological Exposure Index Biological Warfare and Terrorism: Toxins and Other Agents Biomagnification "Biomarkers, Environmental" "Biomarkers, Human Health" Biomonitoring Biotransformation Bismuth (Bi) Bisphenol A Bleach Blood Boric Acid Boron (B) Botulinum Toxin Brodifacoum Bromethalin Bromine Bromobenzene Bromobenzylcyanide Bromadialone Bromoform Bromotrichloromethane

x

Phosphoric Acid Phosphorus Photoallergens Photochemical Oxidants Phthalate Ester Plasticizers Physical Hazards Picloram Picric Acid Piperazine Piperonyl Butoxide "Plants, Poisonous" Platinum (Pt) Plutonium (Pu) Poinsettia Poisoning Emergencies in Humans Pokeweed Pollutant Release and Transfer Registries (PRTRs) Pollution Prevention Act "Pollution, Air" "Pollution, Air Indoor" "Pollution, Soil" "Pollution, Water" Polybrominated Biphenyls (PBBs) Polybrominated Diphenyl Ethers (PBDEs) Polychlorinated Biphenyls (PCBs) Polycyclic Aromatic Amines Polycyclic Aromatic Hydrocarbons (PAHs) Polyethylene Glycol Polymers Potassium (K) Potassium Iodide Primidone Procainamide Prometryn Propachlor Propane Propanil Propargite Propazine Propene Propionic Acid Proposition 65 Propoxur Propoxyphene Propylene Glycol Propylene Oxide Prostaglandins Proteomics Prunus Species Pseudoephedrine Psychological Indices of Toxicity Public Health Service Puromycin PUVA Pyrene Pyrethrins/Pyrethroids Pyridine Pyridostigmine Pyridoxine Pyriminil Pyrrolizidine Alkaloids QT Interval Quinidine Quinine Quinoline Quinone "Radiation Toxicology, Ionizing and Non-Ionizing" Radium Radon Ranitidine Red Dye No. 2 Red Phosphorous Red Squill Red Tide Reference Concentration (RfC) Reference Dose (RfD) "Reproductive System, Female" "Reproductive System, Male" Research Institute for Fragrance Materials (RIFM) Reserpine Resistance to Toxicants Resource Conservation and Recovery Act Respiratory Tract Rhodium Rhododendron Genus Rhubarb Riboflavin Rifampin "Risk Assessment, Ecological" "Risk Assessment, Human Health" Risk Characterization Risk Communication Risk Management Risk Perception Rotenone Saccharin Safe Drinking Water Act Safety Pharmacology Saint John's Wort Salicylates Salmonella Sarin Saxitoxin Scombroid

"Toxicity Testing, Mutagenicity" "Toxicity Testing, Reproductive" "Toxicity Testing, Sensitization" "Toxicity, Acute" "Toxicity, Chronic" "Toxicity, Subchronic" Toxicology "Toxicology, Education and Careers" "Toxicology, History of" Trade Associations Transgenic Animals Triadimefon Trichlorfon Trichloroethane Trichloroethylene Tricyclic Antidepressants Trifluralin Trihalomethanes Trinitrotoluenes Tungsten Turpentine Uncertainty Analysis Uncertainty Factors UNEP Chemicals Uranium (U) Urea Urethane United States Pharmacopoeia (USP) V-Gas Valproic Acid Vanadium Vanillin VE Veterinary Toxicology VG Vinyl Acetate Vinyl Bromide Vinyl Chloride Vinylidene Chloride Virtually Safe Dose (VSD) Vitamin A Vitamin D Vitamin E VM Volatile Organic Compounds (VOC) VX Warfarin Wisteria Workplace Environmental Exposure Levels (WEELs) Xenobiotics Xylene Xyrem Yew Yohimbine Zinc (Zn) Zinc Oxide "Safety Testing, Clinical Studies" "Toxicity Testing, Validation" Genetically Engineered Products Global Environmental Change Pharmaceuticals in the Environment Aneuploidy Tacrine Selamectin Minamata Great Smog of London Itai-Itai N-methylpyrrolidone Peptide Coupling Agents DNA Phosphoramidites Occupational Exposure Limits Arts and Crafts Materials and Processes National Center for Environmental Health (NCEH) Curare Department of Defense Diazoaminobenzene Department of Energy (DOE) Drinking Water Criteria Environmental Crimes Grain Incidents Iatrogenic Disease Immediately Dangerous to Life and Health (IDLH) values "Hazardous Chemicals, Import/Export of" Islip Garbage Barge Mad Cow Disease Oxalates Perfluorooctanoic Acid (PFOA) Persisent Organic Pollutants (POPs) Risk Based Corrective Action (RBCA) Recommended Exposure Limits (REL) Sulfates Texas City Disaster United States Department of Agriculture (USDA) Silent

Spring Love Canal Exxon Valdez Donora Chernobyl Wildlife Toxicology Three Mile Island Cuyahoga River Material Safety Data Sheets and Chemical Hazard Communication Society for Chemical Hazard Communication Killer Lakes Times Beach Valley of the Drums Perfluoroisobutene Riot Control Agents Redbook European Centre for Ecotoxicology and Toxicology of Chemicals Toxicology Excellence for Risk Assessment (TERA) Estrogen Mimics "S-(1,2-dichlorovinyl)-L-cysteine" "Diabetes, Effect of Toxicity" Fetal Alcohol Syndrome Heat Shock Proteins Cell Cycle Trans Fatty Acids Biocides Alkanolamines Lanthanide Series of Metals International Organization of the Flavor Industry (IOFI) International Union of Pure and Applied Chemistry Cesium Nanotechnology Nails (of the Fingers and Toes) Famous Poisoners and Poisoning Cases "Regulation, Toxicology and" "Toxicology, Intuitive" Hair Methyl Isocyanate Bhopal Seveso Ancient Warfare and Toxicology Inert Ingriedients Bioremediation Bioremediation Cancer Chemotherapeutic Agents Homobatrachotoxin Chemical Warfare During WW1 Chemical Warfare Delivery Systems Toxicology Forum Nerve Agents Blister Agents/Vesicants G-Series Nerve Agents V-Series Nerve Agents: Other than VX

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Editor-in-Chief

Philip Wexler, *National Library of Medicine, Bethesda, MD, USA*

Associate Editors

Dedication

For my son Jake and my parents Yetty and Will, with love, appreciation, and respect.

FOREWORD

It gives me great pleasure to once again have the opportunity to introduce the *Encyclopedia of Toxicology* to its users. The second edition is a worthy successor to the first, expanded and refined, which will serve the toxicology community well. Particularly in these days when specialization tends to narrow the individual focus, it brings a real understanding of the entire scope and function of the science of toxicology.

The changes evident at the publication of the first edition have continued at an accelerated pace. At that time it was clear that toxicology, over a period of four or five decades, had changed from a largely descriptive science based on *in vivo* toxicity to one that included all aspects of modern biology and chemistry, from molecular biology to sophisticated instrumental analysis. The philosophical basis had shifted from routine risk analysis based primarily on pathological or *in vivo* toxicological endpoints to one that emphasized mechanisms of toxic action at the organ, cellular, and molecular levels. All of this brought about an explosion in the toxicological literature.

Since then, the techniques of molecular biology have played an increasing role in the elucidation of toxic mechanisms, in the study of xenobiotic metabolism, in the development of safer and more useful drugs and other chemicals, and in the development of biomarkers of exposure and effect, to mention only a few of the more important aspects impacted by these techniques. Analytical chemistry has continued to develop to the point that vanishing small quantities of xenobiotics can be detected, quantities so small that their toxicological impact is likely to remain unknown for the immediate future. While the application of all of this new science to risk assessment remains problematical, since the latter is still largely based on mathematical models rather than toxicological science, progress in both human health risk assessment and environmental risk assessment is also evident.

What has not changed, however, is the need for the toxicological literature to serve many masters. Given the eclectic nature both of the methodological roots and the practical needs served by toxicology, general works are needed more than ever. Works such as the *Encyclopedia of Toxicology* play a critical role at an important intermediate level, more detailed than dictionaries while remaining accessible to the generalist in risk assessment, regulation, teaching, and consultation as well as specialists seeking information beyond the narrow confines of their specialty. It will also serve as an important role for nontoxicologists who need to know more of the philosophy, methods, and uses of this science.

In summary, this is an important and outstanding contribution that no serious toxicologist or library serving toxicologists can afford to be without.

Ernest Hodgson
William Neal Reynolds Professor
Environmental and Molecular Toxicology
North Carolina State University

PREFACE

Time passes, but the need for toxicological understanding persists. As much as we might wish for the end of poverty, ignorance, hunger, and exposure to hazardous chemicals, and as much as we work toward these goals, the challenges are formidable, and the end is not in sight. Chemicals and finished products made from chemicals continue to play an ever-present part in our lives. Although it is not evident that the benefits of chemicals always outweigh their risks, there is little doubt that a wide spectrum of chemicals and drugs has enhanced both the duration and quality of our lives. That said, certain of them, in certain situations, are clearly harmful to certain people. Among the fruits of toxicologists' labors is information on how best to eliminate, reduce, or prevent such harm.

The discipline of toxicology has made considerable strides in the 7 years since the first edition of this encyclopedia was published. The understanding of molecular toxicology continues to advance rapidly. Indeed, it is often much easier to generate the data than to find the time to adequately evaluate it. Genomic, proteomic, and other 'omic' technologies are helping us unravel the complex connection between exposure to environmental chemicals and susceptibility to disease. The US National Center for Toxicogenomics, dedicated to research on informatics and computational toxicology, was established in 2000. As a result of this and other research, much more sophisticated approaches are now available for ascertaining chemical safety, and investigating structure–activity relationships. In addition, analytical instrumentation has become more highly refined and sensitive, making it easier to detect and quantitate even smaller amounts of contaminants in biological systems and the environment.

With greater consumer (especially Western) acceptance of complementary and alternative medicine, more people than ever before are being exposed to a vast array of herbal and other plant-based medicinal products. Although toxicologists have always recognized that 'natural' does not necessarily equate with 'safe', not much has been done to assess the hazards of herbal supplements and their interactions with other chemicals. This is beginning to change.

Chemical, biological, and nuclear warfare have always been subjects of interest, sometimes as practical matters, and more often as academic ones. In the light of the events of September 11, 2001, there has been an increased urgency in learning more about nonconventional warfare and its agents, how they operate, and how to protect ourselves from their effects. Toxicology has found itself broadening its scope to deal with this resurgent type of weaponry.

The scope of what constitutes hazards waste, an ever-present downside of the benefits we derive from the manufacture, processing, and use of chemicals and their products, continues to expand as technology moves forward. In the US two million tons of electronic products, including 50 million computers and 130 million cellphones, are disposed of every year. According to the International Association of Electronic Recylers, this number will more than triple by 2010. With such quantities in landfills and rivers, there are bound to be consequences for our air and water. Potential toxicants include lead, cadmium, and beryllium.

Alternatives to animal studies no longer represent a toxicological sideline. While whole animal testing is unlikely to disappear soon, if ever, other methods of determining hazard and safety are increasingly being embraced by the toxicology community and becoming part of mainstream chemical evaluations. *In vitro* approaches (e.g., using cell culture or skin irritation potential) and *in silico* approaches (i.e., using computer programs to estimate toxic properties based on existing data for similar chemicals with or without supplemental chemical and physical property data) are both generating increasing amounts of toxicity information.

The marketplace is seeing an increase in products utilizing nanotechnologies, and nanotechnology research and development is on the upswing. The United States has had an official National Nanotechnology Initiative since 2001. A start has also been made by federal agencies and universities in assessing the environmental and health effects of nanomaterials.

Greater insight into chemical exposures, both actual and anticipated, is helping to develop a more focused picture of the risks these exposures present to humans and the environment. Growing cooperation between toxicologists and exposure assessors is proving vital to strengthening the scientific basis of risk assessment, thus giving risk assessors and managers more credible tools to address the control of chemical hazards.

At the global level, there have been important strides in the control and management of chemicals. The 10-year followup to the Rio Earth Summit, the World Summit on Sustainable Development, was held in 2002 in Johannesburg, South Africa. Among the targets it set was to use and produce chemicals by 2020 in ways that do not lead to significant adverse effects on human health and the environment.

The Stockholm Convention to protect human health and the environment from persistent organic pollutants (POPs) became binding on May 17, 2004. POPs tend to be toxic, persistent, accumulative, and capable of traveling long distances in the environment. This Convention seeks to eliminate or restrict the production and use of such chemicals. The Kyoto Protocol, designed to decrease greenhouse gas emissions, has now become an international law, despite the resistance of several countries.

The United States hosts a vibrant and growing community of toxicology professionals who perform innovative toxicological research, and scientists in other countries are making their presence felt equally. Global information sharing and collaborations among these investigators are growing, facilitated by the increased accessibility of the Internet and its enhanced technologies. Significant work is proceeding under the auspices of multinational bodies such as Organisation for Economic Co-operation and Development, the European Commission, and the International Program on Chemical Safety.

Efforts to harmonize and link data and information on toxic chemicals throughout the world have been multiplying. The Globally Harmonized System (GHS) of classification and labeling of chemicals has been adopted and is ready for implementation. This will provide a consistent and coherent approach to identifying hazardous chemicals, as well as provide information on such hazards and protective measures to exposed populations. Meanwhile in the European Union, a regulatory framework known as REACH (Registration, Evaluation and Authorization of Chemicals) has been proposed for the registration of chemical substances manufactured or imported in quantities greater than one ton per year.

Last, but not least, the role that poisons played in personal and political intrigues and vendettas, although it may have peaked with Borgias, by no means ended there. A case in point was the 2004 presidential elections in Ukraine. After a bitterly contested battle for the presidency of Ukraine, Viktor Yushchenko emerged victorious and was inaugurated in January 2005, a happy day for democracy, but with a toxic twist. Yushchenko, according to physicians, suffered severe facial disfigurement (chloracne) and other ailments by being poisoned with large dose of dioxins, allegedly mixed in some soup he consumed. Fortunately he is recovering gradually. Although the full story has not yet emerged, political motivations are suspected.

This second edition has grown from 749 entries submitted by 200 authors to 1057 entries contributed by 392 authors. Virtually all the entries from the first edition have been updated and in some cases entirely new versions of these entries have been written. Among the 308 topics appearing for the first time in this edition are avian ecotoxicology, benchmark dose, biocides, computational toxicology, cancer potency factors, metabonomics, chemical accidents, Monte Carlo analysis, nonlethal chemical weapons, invertebrate ecotoxicology, drugs of abuse, cancer chemotherapeutic agents, and consumer products. Many entries devoted to specific chemicals are also brand new to this edition and the international scope of organizations included has been broadened. Entries describing a number of well-known toxin-related incidents, e.g., Love Canal, Times Beach, Chernobyl, and Three-Mile Island, have been added. In addition to the scientific-based entries, others focus on the societal implications of toxicological knowledge. Among them are Toxicology in Culture, Environmental Crimes, Notorious Poisoners and Poisoning Cases Chemical and Biological Warfare in Ancient Times, and a History of the US Environmental Movement. Thus, this new edition has been expanded in length, breadth, and depth and provides an extensive overview of the many facets of toxicology.

Philip Wexler

PREFACE TO THE FIRST EDITION

There are many fine general and specialized monographs on toxicology, most of which are addressed to toxicologists and students in the field and a few to laypeople. This encyclopedia of toxicology does not presume to replace any of them but rather is intended to fulfill the toxicology information needs of new audiences by taking a different organizational approach and assuming a middle ground in the level of presentation by borrowing elements of both primer and treatise.

The encyclopedia is broad-ranging in scope, although it does not aspire to be exhaustive. The idea was to look at basic, critical, and controversial elements in toxicology, which are those elements that are essential to an understanding of the subject's scientific underpinnings and societal ramifications. As such, the encyclopedia had to cover not only key concepts, such as dose response, mechanism of action, testing procedures, endpoint responses, and target sites, but also individual chemicals and classes of chemicals. Despite the strong chemical emphasis of the book, we had to look at concepts such as radiation and noise, and beyond the emphasis on the science of toxicology, we had to look at history, laws, regulation, education, organizations, and databases. The encyclopedia also needed to consider environmental and ecological toxicology to somewhat counterbalance the acknowledged emphasis on laboratory animals and humans because, in the end, all our connections run deep.

In terms of the chemicals, we the editors of this book made a personal selection based on our own knowledge of those with relatively high toxicity, exposure, production, controversy, newsworthiness, or other interest. The chemicals do not represent a merger of regulatory lists or databases of chemicals; they are what we consider to be, for one reason or another, chemicals of concern to toxicology. The book was not intended as a large-scale compendium of toxic chemicals, several of which already exist.

In the tradition of many standard encyclopedias, scientific and otherwise, the encyclopedia is organized entirely alphabetically. Other than in a few useful but smaller scale dictionaries, this style of arrangement has not been done before for toxicology. This organization, along with a detailed index and extensive cross-references, should help the reader quickly arrive at the needed information.

Next, although this book should be of use to the practicing toxicologist, it is geared more to others who, in the course of their work, study, or for general interest, need to know about toxicology. This would include the scientific community in general, physicians, legal and regulatory professionals, and laypeople with some scientific background. Toxicologists needing to brush up on or get a quick review of a subject other than their own specialty would also benefit from it, but toxicologists seeking an in-depth treatment should instead consult a specialized monograph or journal literature.

The encyclopedia is meant to give relatively succinct overviews of sometimes very complex subjects. Formal references and footnotes were dispensed with because these seemed less relevant to the encyclopedia's goals than a simple list of recommended readings designed to lead the reader to more detailed information on a particular subject entry. The entry on Information Resources leads readers to print and electronic sources of information in toxicology.

First and foremost, thanks go to the Associate Editors and contributors, whose efforts are here in print. Yale Altman and Linda Marshall, earlier Acquisitions Editors for the books, were of great assistance in getting the project off the ground. Tari Paschall, the current Acquisitions Editor, and Monique Larson, Senior Production Editor, both of Academic Press, have with great expertise and efficiency brought it to fruition. Organization and formatting of the original entry manuscripts were handled with skill, patience, and poise by Mary Hall with the help of Christen Bosh and Jennifer Brewster.

My work on the *Encyclopedia of Toxicology* was undertaken as a private citizen, not as a government employee. The views expressed are strictly my own. No official support or endorsement by the US National Library of Medicine or any other agency of the US Federal Government was provided or should be inferred.

Philip Wexler

ACKNOWLEDGMENTS

This book, as is all too easy to discern, is not a one-man operation, and doubtlessly could not be one and still encompass the same breadth and depth. Above all, I bow, tip my hat, and throw roses in appreciation, to the nine associate editors Bruce D Anderson, Ann de Peyster, Shayne C Gad, Pertti J Hakkinen, Michael A Kamrin, Betty J Locey, Harihara M Mehendale, Carey N Pope, Lee R Shugart and the authors of this work. There is no exaggerating their importance in this collaboration. We were the prototypical occasionally disputative but affectionate family engaged in a common single-minded goal – self-preservation. Secondarily, we had an encyclopedia to produce cooperatively, and managed to engage in the process with good humor and without punching each other silly. Such are the advantages of online interaction. We survived, relatively intact, in good spirits, and on speaking terms, even after our few in-person meetings. And rest assured, no transfer of funds was involved in Dr Ernie Hodgson's flattering and much appreciated foreword.

On the publisher (Elsevier) end, Tari Paschall, experienced in the production of the first edition, ushered this second edition through its formative stages to the point where we had a stable process and a clear direction. She handed the baton to Judy Meyer, the new Publishing Editor for the encyclopedia, who deftly kept us on course, and hydrated, up to the finish line. Another baton pass shortly before the production process was from Nick Panissidi of Elsevier's San Diego Office to Michael Bevan in Oxford. Nick set up the Encyclopedia Website and initial editorial ground rules. Michael brought the editorial details to fruition and got us into and through production with hardly a scar. I would like to thank the many other unknown to me Elsevier staff who have worked diligently on other aspects of the book, including marketing. I have had great support from many colleagues. Dr Jack Snyder, Associate Director of the Division of Specialized Information Services at the National Library of Medicine, and Jeanne Goshorn, Chief of the Biomedical Information Services Branch of the same division, in particular, have been unflagging boosters of my efforts.

And finally, on the home front, I am certain that my dog, Chi-Chi, barked less than she would have, and my bird, Hercules, moderated his screeching, in consideration of my work on the encyclopedia. As for my teenage son, Jake, he probably bugged me more on account of it, but we are old hands at knowing how to annoy each other with relish.

Notes on the Glossary

Reprinted from the IUPAC 'Glossary for Chemists of Terms used in Toxicology' and the IUPAC 'Glossary of Terms used in Toxicokinetics', with permission from the International Union of Pure and Applied Chemistry.

In order that the *Encyclopedia of Toxicology* may be useful to as wide a readership as possible, a Glossary of key terms has been provided by the publisher. For the purpose of the article text itself, it is important to use the established technical vocabulary of the science of toxicology, in the interest of accuracy, brevity, and consistency.

However, it is possible that some of these technical terms will not be entirely familiar to the nonprofessional readers of this encyclopedia. Therefore, in the interest of greater understanding for those readers – and also for the possible benefit of professional readers consulting material outside their own area of expertise – the Glossary defines a selected group of several hundred terms. These terms occur frequently within a variety of articles in the encyclopedia and thus can be said to represent a core vocabulary of the field of toxicology. The definitions are presented in a concise, accessible format, based on the use of the term in the context of the encyclopedia.

Notes on the Subject Index

To save in the index, the following abbreviations have been used:

ADI	acceptable daily intake
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CSAF	chemical-specific adjustment factors
DDT	dichloro-diphenyl-trichloro-ethane
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
GCP	good clinical practice
GLP	good laboratory practice
ICH	International Conference on Harmonization
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
OPIDN	organophosphate-induced delayed neurotoxicity
QSARs	quantitative structure-activity relationships
SSRIs	selective serotonin reuptake inhibitors
WHO	World Health Organization

A

Aberrations of Chromosomes *See* Chromosome Aberrations.

Absorption

Jules Brodeur and Robert Tardif

Introduction

Absorption is the process by which a chemical crosses the various membrane barriers of the body before it enters the bloodstream. The main sites of entry are the gastrointestinal tract, the lungs, and the skin. In drug therapy, other convenient, but more rarely used, portals of entry are the intravenous, subcutaneous, and intramuscular routes.

The absorption of a chemical from the site of exposure is regulated by the biologic membrane surrounding the various cells that line the tissue compartments of the body. The membrane is composed principally of phospholipids forming an oriented bilayer, 7–9 nm thick. The more polar hydrophilic (attracted to water) ends of the phospholipids project into the aqueous media on each side of the membrane, and the hydrophobic (repelled by water) fatty acid tails form a barrier to water in the inner space of the membrane. Proteins are embedded throughout the lipid bilayer and have various functions. One of these is to act as active carriers for certain molecules across the membrane. Proteins can also form pathways or small pores through the membrane, serving as aqueous channels and allowing passage of water across them.

Before discussing absorption in more detail, it is important to consider mechanisms by which chemicals cross membranes. These mechanisms are of interest not only for absorption but also for all other processes (distribution, biotransformation, and excretion) involved in the disposition of chemicals because they also require passage through membranes.

Chemicals can cross membranes by one or more of the following mechanisms: passive diffusion, facilitated diffusion, active transport, filtration, and endocytosis.

Passive Diffusion

This is the mechanism by which lipophilic (hydrophobic) uncharged molecules find a passage across the membrane by solubilizing within the lipids of the membrane. The driving force for this process is the concentration gradient of the chemical between each side of the membrane, allowing molecules to be transported from the side with higher concentration to the side with lower concentration. Passive diffusion, therefore, requires no energy expenditure by the cell; it is not saturable or subject to competition between molecules.

Factors that govern passive diffusion are:

1. *The lipid solubility of a chemical*: This is a characteristic that is usually expressed in terms of the ability of the chemical to distribute between separate oil and water phases. The more a chemical dissolves in oil, or its substitute octanol, the more lipid-soluble it is and the more easily it will cross membranes.
2. *The electrical charge (degree of ionization) of a chemical*: As a rule, chemicals that are electrically neutral permeate more easily through the lipid phase of a membrane by virtue of their higher degree of lipid solubility. For several therapeutic agents that are weakly charged molecules, the pH of the aqueous environment will have considerable influence on the degree of ionization of the chemicals and hence on their lipid solubility and membrane permeation.
3. *The molecular size of a chemical*: Passive diffusion is normally limited to molecules whose molecular weight does not exceed 500 Da. However, a small molecule will cross membranes more rapidly than a larger one of equal lipophilicity.

Facilitated Diffusion

Facilitated diffusion is very similar to passive diffusion with the difference that transfer across membranes is assisted by the participation of carrier proteins embedded in the membrane bilayer. Again, the direction of passage will be from the side of the membrane with high concentration of a chemical to the side with low concentration; this also occurs without energy expenditure by the cell. Such a process is somewhat specific in the sense that it applies to molecules that are able to bind to a carrier protein. Absorption of nutrients such as glucose and amino acids across the epithelial membrane of the gastrointestinal tract occurs by facilitated diffusion. Since a finite number of carriers are available for transport, the process is saturable at high concentrations of the transported molecules and competition for transport may occur between molecules of similar structure.

Active Transport

Active transport requires a specialized carrier molecule, a protein, and the expenditure of cellular energy; transfer across membranes can therefore occur against a concentration gradient. The carrier system is selective for certain structural features of chemicals, namely their ionized state, whether anionic, cationic, or neutral. Recent advances in the understanding of active transport have led to the characterization of several families of carriers. Such carrier systems are saturable. In addition, molecules with similar structural features may compete for transport by a given carrier.

Active transport is of limited importance for absorption of chemicals; it plays an important role, however, in the elimination of chemicals by the liver and the kidneys.

Filtration

Small water-soluble and small charged molecules, such as methanol and salts, respectively, may cross the gastrointestinal epithelial membrane through minute pores or water channels (<4 nm) in the membrane. Filtration is also an important function for urinary excretion. Renal glomeruli possess rather large pores (~ 70 nm) that allow passage into the urine of various solutes contained in blood, including small proteins.

Endocytosis

Endocytosis is a specialized form of transport by which very large molecules and insoluble materials are engulfed by invagination of the absorptive cell membrane, forming intracellular vesicles. This process is responsible for the absorption of certain dyes by mucosal cells of the duodenum (pinocytosis). In the lung, alveolar macrophages scavenge insoluble particles, such as asbestos fibers, and may transport them into the lymphatic circulation (phagocytosis).

Absorption by the Gastrointestinal Tract

The major role of the gastrointestinal tract is to provide for efficient absorption of essential nutrients contained in ingested foods and liquids. It is also an important route for absorption of drugs and toxicants. The entire surface of the gastrointestinal tract is very large, being 200 times that of the body surface; the barrier between the contents of the tract and the blood vessels is easily crossed, consisting essentially of an epithelium only one cell thick. The anatomy of the gastrointestinal tract is illustrated in **Figure 1**. Absorption occurs mostly by passive diffusion of lipid-soluble, electrically neutral (nonionized) molecules.

The degree of ionization of many therapeutic drugs, which are usually weak electrolytes, is directly dependent upon the pH of the gastrointestinal content. The pH will therefore have considerable influence on the absorption of such chemicals; absorption will occur at sites where the drugs are present as neutral molecules. At the low acidic pH of the stomach (1–3), most weak organic acids such as

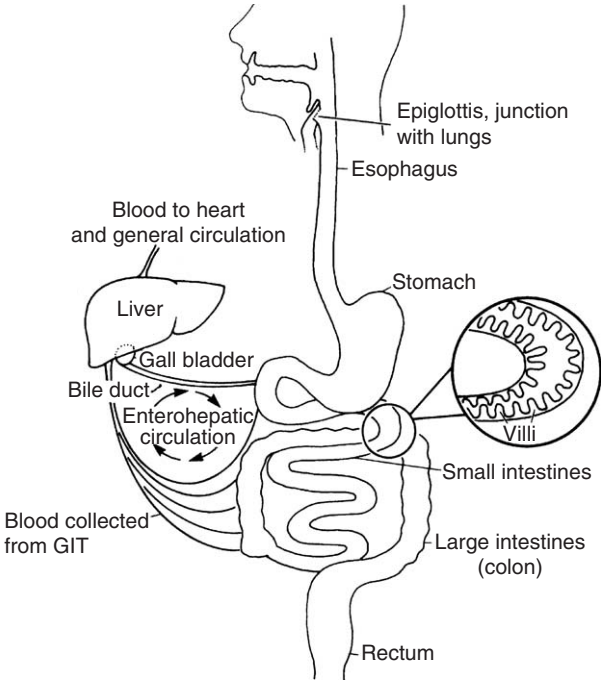

Figure 1 The anatomy of the gastrointestinal tract. (Reproduced from Smith RP (1992) The anatomy of the gastrointestinal tract. *A Primer of Environmental Toxicology*, p. 70. Philadelphia: Lea & Febiger, with permission from Lea & Febiger.)

acetylsalicylic acid will be nonionized and will diffuse passively across the gastric mucosa at a rate that will be proportional to the concentration gradient of the nonionized form. On the other hand, weak organic bases will diffuse more easily through the mucosa of the small intestine in which pH is higher (5–8). However, the bulk of absorption does not necessarily occur at the site where pH is optimal for electrical neutrality of the molecules. The very large surface area of the small intestine, due to the presence of finger-like projections, namely the villi and the microvilli, favors the diffusion of substances even at pH values for which the degree of ionization is not maximal; as a consequence, the small intestine is the region of the gastrointestinal tract that is most effective in the absorption of chemicals.

A small number of chemicals may be absorbed using facilitated diffusion (antimetabolic nucleotides), active transport (lead and 5-fluouracil), or pinocytosis (dyes and bacterial endotoxins).

Chemicals that reach the bloodstream by absorption through the gastrointestinal tract will move, via the portal circulation, directly to the liver, where they will normally undergo metabolic biotransformation to more or less active chemical forms, even before they gain access to the various tissues of the body; this phenomenon is known as the first-pass effect.

Among factors that may modify gastrointestinal absorption of ingested chemicals, the presence of food in the tract is one of the most important. The presence of food in the stomach will delay the absorption of weak organic acids at that site. The presence of lipid-rich food will delay the emptying of the gastric content into the intestine and thus also delay the absorption of chemicals. Conversely, an empty stomach facilitates absorption, a situation that is almost always beneficial in drug therapy.

Chemical interactions in the gastrointestinal tract between nutrients and drugs may considerably reduce the absorption of some drugs: calcium ions from dairy products form insoluble and therefore nonabsorbable complexes with the antibiotic tetracycline. On the other hand, certain drugs are irritants to the gastrointestinal tract (nonsteroidal anti-inflammatory drugs and potassium chloride tablets) and must be ingested with food.

Enterohepatic circulation provides an example of a special case of intestinal absorption. Certain chemicals, like methyl mercury, after undergoing biotransformation in the liver, are excreted into the intestine via the bile. They then can be reabsorbed in the intestine, sometimes after enzymatic modification by intestinal bacteria. This process can markedly prolong the stay of chemicals in the body. It can be interrupted by antibiotics that destroy the intestinal bacterial flora.

Absorption through the Skin

Normal skin represents an effective, but not perfect, barrier against the entry of chemicals present in the environment. There are two major structural components to the skin – the epidermis and the dermis (**Figure 2**).

The epidermis is formed of several layers of cells, with the outermost layers, $\sim 10\,\mu m$ thick, consisting of dried dead cells forming the stratum corneum. The latter, whose cells are rich in a filament-shaped protein called keratin, represents the major structural component of the barrier to passage of chemicals through the skin. Chemicals may move through the various cell layers of the epidermis by passive diffusion, more slowly through the stratum corneum, but more rapidly through the inner layers of live epidermal cells (stratum granulosum, stratum spinosum, and stratum germinativum).

The epidermis rests upon and is anchored onto a much thicker base of connective and fatty tissues, the dermis, whose major structural components are proteins called collagen and elastin; these proteins provide the skin with tensile strength and elasticity. The dermis also contains small blood vessels (capillaries), nerve endings, sebaceous glands, sweat glands, and hair follicles. Small pores in the epidermis that allow passage for sweat and sebum glands, as well as hair shafts, are not an important route of entry for chemicals. Once a chemical has crossed the epidermis by passive diffusion and gained access to the dermis, diffusion into the bloodstream occurs rapidly.

The stratum corneum is much thicker in areas where considerable pressure and repeated friction occur, like palms and soles; absorption is therefore much slower in these areas. Conversely, the stratum corneum is extremely thin on the skin of the scrotum. In general, skin surfaces of the ventral aspect of the body represent barriers that are easier to cross than those of the dorsal aspect.

Mechanical damage to the stratum corneum by cuts or abrasions of the skin or chemical injury by local irritation with acids or alkalis, for example, is likely to facilitate the entry of chemicals through the skin. This may also be the case in subjects suffering from certain skin diseases.

Lipid-soluble chemicals like organophosphate insecticides, tetraethyl lead, certain organic solvents, and certain dyes like aniline are relatively well absorbed through the skin. Percutaneous absorption is facilitated by increasing peripheral dermal blood

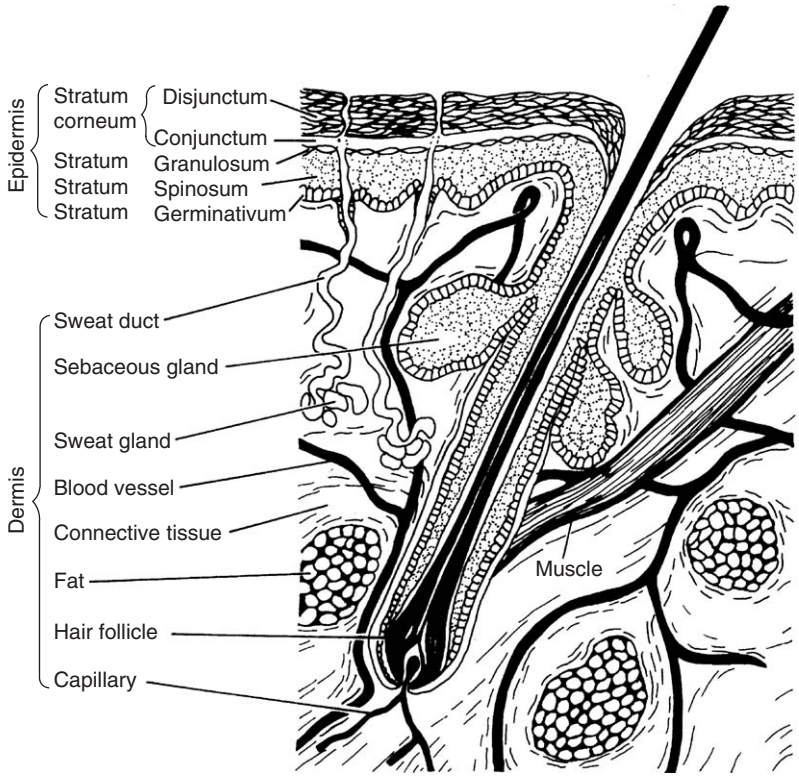

Figure 2 The organization of the skin as a biologic barrier. (Reproduced from Smith RP (1992) The organization of the skin as a biological barrier. *A Primer of Environmental Toxicology*, p. 73. Philadelphia: Lea & Febiger, with permission from Lea & Febiger.)

flow, as might occur when the ambient temperature is elevated. Under the same conditions, and in the presence of elevated sweating, the degree of hydration of the skin will increase considerably, enhancing the permeability of the stratum corneum to foreign chemicals; this observation is of special interest to workers in occupational settings.

Absorption by the Lung

The fundamental physiologic role of the lung is to allow gas exchange, extracting oxygen from the ambient air and eliminating carbon dioxide as a catabolic waste. When performing this function, the human adult lung is exposed each day to $\sim 10\,000$ l of more or less contaminated air. The lung can therefore become an important portal of entry for airborne chemicals present in the environment.

Extraneous substances are presented to the lung as gases or vapors or as liquid or solid particles; following inhalation, they may reach various regions of the respiratory tract, where some fraction of them will undergo absorption into the bloodstream; the remaining part will be either deposited locally or eliminated by exhalation even before being absorbed.

In terms of its anatomical and functional relationship with the contaminated atmospheric environment, the respiratory tract can be divided into three regions: the nasopharyngeal, the tracheobronchiolar, and the alveolar regions (**Figure 3**). The major part of the absorptive process takes place in the alveolar region, due principally to its large surface area ($80\,m^2$ in an adult human) and the extreme thinness of the cellular barrier ($<1\,\mu m$) between the air-side of the alveolar sac (lined with epithelial cells) and the lumen of the lung capillaries (lined with endothelial cells).

When discussing absorption of chemicals through the respiratory tract, it is practical to consider separately gases and vapors, on the one hand, and particles on the other hand.

Gases and Vapors

How much and at what location a contaminant gas or vapor will be absorbed in the respiratory tract is determined primarily by the solubility of the contaminant. The more water-soluble agents (sulfur dioxide and ketonic solvents) may dissolve in the aqueous fluid lining the cells of the more proximal region of the respiratory tree, even before they reach the alveolar region. They may then undergo absorption by passive diffusion or passage through membrane pores. When, in addition, water-soluble contaminants

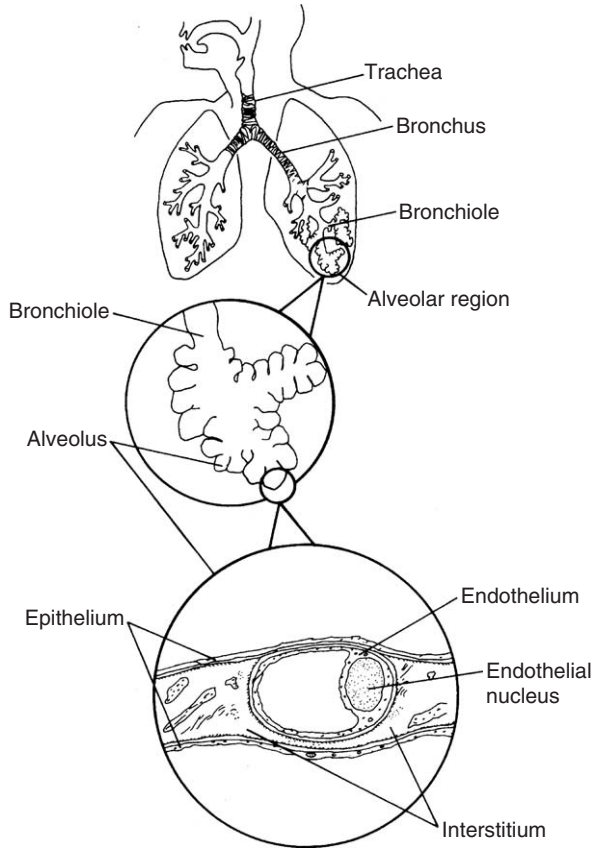

Figure 3 The anatomy of the respiratory tract from trachea to alveolus. (Reproduced from Smith RP (1992) The anatomy of the respiratory tract from trachea to alveolus. *A Primer of Environmental Toxicology*, p. 67. Philadelphia: Lea & Febiger, with permission from Lea & Febiger.)

are very reactive substances, like formaldehyde, they may form stable molecular complexes with cell components as proximally as the nasopharyngeal region. By virtue of these mechanisms, the alveolar region of the lung is partially protected against potential injury by certain gases and vapors.

Lipid-soluble contaminants diffuse passively through the thin alveolar–vascular cell barrier of the alveolar sac and then dissolve into the blood according to the ability of the contaminant to partition between alveolar air and circulating blood. Substances that are very soluble in blood are rapidly transported into the bloodstream. For these substances, like styrene and xylene, the amount absorbed will be greatly enhanced by increasing the rate and the depth of respiration, as is likely to happen when doing strenuous physical work. On the other hand, substances that are poorly soluble in blood have limited capacity for absorption due to rapid saturation of blood. For these substances, like the solvents cyclohexane and methyl chloroform, the amount absorbed may be increased only by increasing the blood perfusion rate in the lung; that is, by enhancing the replacement of saturated blood circulating in the lung capillaries. This can be achieved, for example, when doing work requiring heavy muscular activity.

Particles

Liquid (sulfuric acid and cutting fluids) and solid (silica dusts, asbestos fibers, and microorganisms) particles may become airborne and form respirable aerosols. According to their size and diameter, inhaled particles may be deposited in different anatomical regions of the respiratory system. Once deposited, particles may dissolve locally or may undergo removal to other regions of the respiratory tree.

The surface of the cells lining the tracheobronchial tree and the surface of most of the cells lining the nasopharyngeal region are covered with a layer of relatively thick mucous material; in the alveolar region, cells are lined with a thin film of fluid. The aqueous environment provided by these surface liquids favors at least partial dissolution and eventually absorption of water-soluble particles, especially those present as liquid droplets. Various defense mechanisms may help to remove less soluble particles from their site of deposition.

Particles larger than 5 μm in diameter are usually deposited by inertial impaction on the surface of the nasopharyngeal airways. They may be removed by coughing, sneezing, or nose wiping.

Particles with diameters between 1 and 5 μm are deposited in the tracheobronchial region as a result of either inertial impaction at airway bifurcations or gravitational sedimentation onto other airway surfaces. Undissolved particles may then be removed by the action of the mucociliary defense system working as an escalator; particles trapped in the mucus are propelled toward the pharynx by the action of thin cilia located on the surface membrane of specialized cells. Once in the pharynx, the particles may be swallowed. The efficiency of the escalator defense system may be greatly impaired by various environmental contaminants, like sulfur dioxide, ozone, and cigarette smoke that are known to paralyze the activity of the ciliated cells and consequently the upward movement of the mucus.

Particles ranging between 0.1 and 1.0 μm in diameter reach the alveolar region, where they finally hit cellular walls as a result of their random movement within minute air sacs. Removal of particles in this region of the lung is much less efficient. Some of the particles may eventually reach the tracheobronchiolar escalator system, either as engulfed

material within alveolar macrophages or as naked particles transported by the slow movement of the fluid lining the alveoli. Other possible mechanisms involve transport of the particles into the lymphatic system, either within macrophages or by direct diffusion through the intercellular space of the alveolar wall.

Particles smaller than 0.1 μm are not usually deposited in the lung, entering and exiting the airways together with inhaled and exhaled air.

Often, particulate matter acts as a carrier for gases, vapors, and fumes adsorbed onto their surface (solid particles) or dissolved within them (liquid particles); this increases the residence time of such pollutants in specific areas of the lung and imposes an additional task on the pulmonary defense mechanisms.

The most striking example of this synergistic effect is the one observed between sulfur dioxide, a respiratory tract irritant, and suspended particles, both being typical components of urban air pollution. This explains why current guideline values for exposure to sulfur dioxide in the presence of particulate matter are lower than those for exposure to sulfur dioxide alone. Similar concerns can be expressed for combinations comprising exhaust particles from diesel engines and certain carcinogens like polycyclic aromatic hydrocarbons, as well as cigarette smoke and certain other carcinogens like aromatic amines.

Chemicals absorbed by the lung reach the systemic circulation directly and are therefore immediately available for distribution to the various tissues of the body – brain, kidneys, liver, muscles, skin, bones, and others.

See also: Biotransformation; Distribution; Excretion; Exposure; Gastrointestinal System; Modifying Factors of Toxicity; Pharmacokinetics/Toxicokinetics; Respiratory Tract; Skin; Toxicity Testing, Dermal; Toxicity Testing, Inhalation.

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Acceptable Daily Intake (ADI)

Jaya Chilakapati and Harihara M Mehendale

The acceptable daily intake (ADI) is commonly defined as the amount of a chemical to which a person can be exposed, on a daily basis over an extended period of time, usually a lifetime without suffering a deleterious effect. It represents a daily intake level of a chemical in humans that is associated with minimal or no risk of adverse effects. It is a numerical estimate of daily oral exposure to the human population, including sensitive subgroups such as children, that is not likely to cause harmful effects during a lifetime. The ADI is expressed in milligrams of the chemical, as it appears in the food, per kilogram of body weight per day ($mg\,kg^{-1}\,day^{-1}$). The Environmental Protection Agency (EPA) refers to such an exposure level as the risk reference dose (RfD) in order to avoid any implication that any exposure to a toxic material is 'acceptable'. RfDs are generally used for health effects that are thought to have a threshold or low dose limit for producing effects. The ADI concept has often been used as a tool in reaching risk management decisions such as establishing allowable levels of contaminants in foodstuffs and water.

ADI is derived from an experimentally determined 'no-observed-adverse-effect level (NOAEL)'. An NOAEL is an experimentally determined dose at which there is no statistically or biologically significant indication of the toxic effect of concern. In an experiment with several NOAELs, the regulatory focus is normally on the highest one, leading to the common usage of the term NOAEL as the highest experimentally determined dose without a statistically or biologically significant adverse effect. In cases in which a NOAEL has not been demonstrated experimentally, the term 'lowest-observed-adverse-effect level (LOAEL)' is used.

ADI values are typically calculated from NOAEL values by dividing by uncertainty (UF) and/or modifying factors (MFs):

$$\text{ADI (human dose)} = \text{NOAEL (experimental dose)}/(\text{UF} \times \text{MF})$$

In principle, these safety factors (SFs) allow for intraspecies and interspecies (animal to human) variation with default values of 10. An additional uncertainty factor can be used to account for experimental inadequacies; for example, to extrapolate

from short-exposure-duration studies to a situation more relevant for chronic study or to account for inadequate numbers of animals or other experimental limitations. Traditionally, a safety factor of 100 would be used for RfD calculations to extrapolate from a well-conducted animal bioassay (10-fold factor for animal to human) and to account for human variability in response (10-fold factor human-to-human variability).

Modifying factors can be used to adjust the uncertainty factors if data on mechanisms, pharmacokinetics, and the relevance of the animal response to human risk justify such modifications. For example, if there is kinetic information suggesting that rat and human metabolisms are very similar for a particular compound, producing the same active target metabolite, then, rather than using a 10-fold uncertainty factor to divide the NOAEL from the animal toxicity study to obtain a human relevant RfD, a factor of 3 for that uncertainty factor might be used. Of particular interest is the new extra 10-fold Food Quality and Protection Act (FQPA) factor, added to ensure protection of infants and children.

For other chemicals, with databases that are less complete (for example, those for which only the results of subchronic studies are available), an additional factor of 10 might be judged to be more appropriate leading to an SF of 1000. For certain other chemicals, based on well-characterized responses in sensitive humans, an SF as small as 1 might be selected, as in the case of the effect of fluoride on human teeth.

Some scientists interpret the absence of widespread effects in the exposed human populations as evidence of the adequacy of the SFs traditionally employed.

The RfD approach represents a generally accepted (Food and Drug Administration, National Academy of Sciences (NAS), and EPA) method for setting lifetime exposure limits for humans, and the use of 10-fold uncertainty factors has some experimental support.

Limitations of RfD

However, there are several limitations in the RfD approach, the net result of which is that exposures resulting in the same RfD do not imply the same level of risk for all chemicals. In addition, the RfD approach does not make use of dose–response information. There are also difficulties in the implications of specific UFs. The default value of 10 for the interspecies UF is a reasonable assumption in some cases, but in other cases may not be appropriate. Too narrow a focus on the NOAEL means that information on the shape of the dose–response curve is ignored. Such data could be important in estimating levels of concern for public safety. Guidelines have not been developed to take into account the fact that some studies have used larger (smaller) numbers of animals and, hence, are generally more (less) reliable than other studies.

The ADI is generally viewed by risk assessors as a 'soft' estimate, whose bounds of uncertainty can span an order of magnitude. That is, within reasonable limits, while exposures somewhat higher than the ADI are associated with increased probability of adverse effects, that probability is not a certainty. Similarly, while the ADI is seen as a level at which the probability of adverse effects is low, the absence of all risk to all people cannot be assured at this level.

See also: Benchmark Dose.

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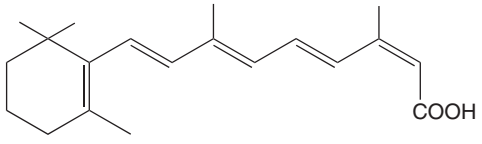
Accutane

Russell Barbare

- Chemical Abstracts Service Registry Number: CAS 4759-48-2
- Synonyms: Isotretinoin; 13-*cis*-Retinoic acid; 2-*cis*-Vitamin A acid; Ro-4-3780; Isotrex
- Chemical Formula: $C_{20}H_{28}O_2$
- Chemical Structure:



Uses

Isotretinoin is approved for use in the treatment of severe recalcitrant nodular acne and psoriasis, and is also used to treat keratinization disorders and some skin cancers.

Background Information

Isotretinoin is a retinoid, the class of natural and synthetic compounds that exhibit vitamin A activity. It is a naturally occurring metabolite of vitamin A that inhibits sebum production. The US Food and Drug Administration classifies it as Pregnancy Risk Category X.

Exposure Routes and Pathways

Ingestion is the most common route of exposure, and capsules are the only form currently produced.

Toxicokinetics

The apparent time lag between oral administration and appearance in systemic circulation is 30 min to 2 h. Absorption is approximately three times greater when taken with a high-fat meal as opposed to fasting, although the half-life is $\sim$21 h either way. Once in the body, isotretinoin binds to plasma proteins, especially albumin, at a rate greater than 99.9%. In humans, it readily undergoes reversible isomerization and irreversible oxidation; the exposure to these metabolites is more than three times greater than to the parent form. *In vitro* studies have indicated that the converted forms may have higher retinoid activity, but the clinical significance of this is unknown. ^{14}C studies have indicated that the half-life of the all drug activity in blood is $\sim$90 h. There was no statistically significant difference in exposure to any of the compounds between adults and patients 12–15 years of age. Excretion occurs in both feces and urine in approximately equal amounts, and overdosage in men can result in trace amounts in their semen. It is unknown whether it is excreted in human breast milk. It is metabolized by the liver, with the parent form having a terminal elimination half-life of 10–20 h.

Mechanism of Toxicity

Retinoids increase cellular mitotic activity, DNA and RNA synthesis, and protein synthesis. The primary toxicity of concern is female-mediated teratogenesis. Isotretinoin alters cell differentiation and placement in developing fetuses that are exposed to it in the first 3 weeks. Any exposed fetus has an increased change of spontaneously aborting or dying and may develop external or internal abnormalities. Cases of IQ less than 85 have been reported without other noted abnormalities. There is no accurate way to determine if a fetus has been exposed, so the safety recommendations are for potentially fertile females to not be pregnant or get pregnant within 30 days before or after exposure or at any time during exposure. External abnormalities have included skull, ear, and eye abnormalities such as cleft palate, absent external auditory canals, or microphthalmia. Noted internal changes have included abnormalities in the central nervous system such as hydrocephalus and microcephaly, abnormalities in the cardiovascular system or thymus gland, and parathyroid hormone deficiency. Even though it is unknown whether isotretinoin is excreted in human breast milk, breastfeeding should be avoided for the same period as pregnancy.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats and mice the oral LD_{50} of isotretinoin is $>4000\,mg\,kg^{-1}$; in rabbits it is $\sim1960\,mg\,kg^{-1}$.

Human

Overdosage can produce headache or abdominal pain, vomiting, dizziness, irregular muscular coordination, facial flushing, or drying and cracking of the lips, but all symptoms pass quickly and with no known long-term effects. An acute toxic dose has not been established – doses up to 1600 mg in an adult and 63 mg kg^{-1} in a child have resulted in only mild toxicity.

Chronic Toxicity (or Exposure)

Animal

Accutane is a potent rat and rabbit developmental toxin (teratogen). Testicular atrophy and evidence of lower spermatogenesis was noted in dogs given isotretinoin for 30 weeks at 20 or 60 $mg\,kg^{-1}\,day^{-1}$. Fischer 344 rats dosed at 8 or 32 $mg\,kg^{-1}\,day^{-1}$ for over 18 months had a dose-related raised incidence of pheochromocytoma, an adrenal gland tumor. The relevance in man is unknown since this animal develops spontaneous pheochromocytoma at a significant rate.

Human

Any level of exposure may be teratogenic, so potentially fertile females must not be pregnant or get

pregnant within 30 days before, during, and after exposure (see Mechanism of Toxicity). Other effects that often require monitoring are psychiatric disorders, including depression and suicidal thoughts, and benign intercranial hypotension, which can lead to headache, visual disturbances, or nausea and vomiting. These disorders may not stop upon discontinuation and should be evaluated by a professional. Dose-dependent adverse effects on the skin and mucous membranes may include inflammation or cracking of the lips, dry eyes, nosebleeding, irritation of the palpebral conjunctiva, and redness or dryness of the skin. Less common effects on the same organ systems include hair loss, photosensitivity, formation of granular tissue, or dark adaptation dysfunction. Colonization and, rarely, infection by *Staphylococcus aureus* can also occur. Hyperlipidemia is reported in 25% of treated patients during therapeutic courses of treatment on a systemic level, with the most common effect being increased triglyceride levels. There may also be increased cholesterol levels, raising of low-density lipoprotein levels, or lowering of high-density lipoprotein levels. Long-term treatments can generate several skeletal side effects including joint or lower back pain, bone hypertrophy, ossification at tendinous insertions, and lowered bone density. Children may experience premature closure of the epiphyseals. Tests of sperm count and motility in man have shown no significant changes.

Clinical Management

Roche Pharmaceuticals has produced the System to Manage Accutane Related Teratogenicity™ (S.M.A.R.T.™) and the Accutane Pregnancy Prevention Protocol (PPP) to be used in conjunction with the prescription of Accutane. Management of toxic effects involves monitoring by the appropriate specialist and discontinuation of the exposure where indicated. Isotretinoin-related depression may require long-term monitoring.

Exposure Standards and Guidelines

The recommended therapeutic dosage is 0.5–1.0 $mg\,kg^{-1}\,day^{-1}$ in two doses per day taken with food for 15–20 weeks.

See also: Developmental Toxicology; Photoallergens; Vitamin A.

Further Reading

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Relevant Website

http://www.rocheusa.com – Roche Pharmaceuticals, Accutane® Website for the United States.

ACE Inhibitors

Henry A Spiller

This article is a revision of the previous print edition article by Daniel J Cobaugh, volume 1, pp. 7–9, © 1998, Elsevier Inc.

- Representative Chemicals: Benazepril, Lotensin®; Capropril, Capoten®; Enalapril, Vasotec®; Enalaprilat, Vasotec IV®; Fosinopril, Monopril®; Lisinopril, Prinivil®; Zestril®; Quinapril, Accupril®; Ramipril, Altace®
- Chemical Abstracts Service Registry Numbers: CAS 86541-75-5; CAS 62571-86-2; CAS 75847-73-3; CAS 84680-54-6; CAS 888 89-14-9; CAS 76547-98-3; CAS 85441-61-8; CAS 87333-19-5
- Chemical/Pharmaceutical/Other Class: Angiotensin-converting enzyme (ACE) inhibitors
- Chemical Formulas: Benazepril, $C_{24}H_{28}N_2O_5$; Captopril, $C_9H_{15}NO_3S$; Enalapril, $C_{20}H_{28}N_20_5$; Enalaprilat, $C_{18}H_{24}N_2O_5 \cdot 2H_2O$; Fosinopril, $C_{30}H_{46}NO_7P$; Lisinopril, $C_{21}H_{31}N_3O_5 \cdot 2H_2O$; Quinapril, $C_{25}H_{30}N_2O_5$; Ramipril, $C_{23}H_{32}N_2O_5$

Uses

Angiotensin-converting enzyme (ACE) inhibitors are used in the management of hypertension and congestive heart failure.

Exposure Routes and Pathways

Ingestion is the most common route for both accidental and intentional exposures. Enalaprilat is

available for parenteral administration and toxicity could occur via this route.

Toxicokinetics

The extent of oral absorption varies from 25% (lisinopril) to 75% (captopril). The rate of absorption also varies from 0.5 h (captopril and enalopril) to 7 h (lisinopril). Reported volumes of distribution range from $0.7\,l\,kg^{-1}$ (captopril) to $1.8\,l\,kg^{-1}$ (lisinopril). All of the ACE inhibitors, except for captopril and lisinopril, are metabolized in the liver to active metabolites. Excretion is via both the urine and the feces. The half-life ranges from 1.3 h (enalapril) to 17 h (ramipril).

Mechanism of Toxicity

The ACE inhibitors affect the rennin–angiotensin system. This system has effects on blood pressure as well as fluids and electrolyte balance. Renin modulates the formation of angiotensin I from angiotensinogen. Angiotensin I is then converted via angiotensin-converting enzyme to angiotensin II. Angiotensin II is a potent vasoconstrictor that also causes increased aldosterone secretion. Aldosterone is responsible for sodium and water retention. The ACE inhibitors interfere with the conversion of angiotensin I to angiotensin II and, therefore, cause vasodilation as well as loss of sodium and water. Literature supporting a relationship between angiotensin and the beta endorphins exists. Angiotensin II is thought to be inhibited by endogenous beta endorphin. *In vitro* studies have demonstrated that captopril can inhibit encephalinase, the enzyme that degrades endorphins. Interference with endorphin metabolism may result in prolonged effects from these opiate-like neurotransmitters. Also, the opiate antagonist naloxone is thought to interfere with beta-endorphin inhibition of angiotensin II. An interaction between angiotensin and bradykinin may also exist. ACE is identical to kinase IT, which is responsible for inactivation of bradykinins. Accumulation of bradykinins may cause a decrease in blood pressure by a direct vasodilatory mechanism or through stimulation of prostaglandin release and/or synthesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

There are limited data, but accidental ingestion of small amount of ACE inhibitors by companion animals would not expected to be a problem.

Human

The clinical effects observed following ACE inhibitor poisoning or overdose are a direct extension of their therapeutic effects and would be expected to manifest in 1–2 h postingestion. Ingestions involving small amounts of ACE inhibitors may result in limited or no toxic effects. Clinical effects that may occur include hypotension with or without a reflex tachycardia and changes in level of consciousness that are directly related to vascular changes. Only a few cases of profound hypotension have been reported. In each of these cases, blood pressure returned to normal within 24 h of ingestion. One death has been attributed to an ACE inhibitor. This was in a 75-year-old male who ingested captopril and the calcium channel blocker diltiazem. Because this was a coingestion, it is not certain that captopril was the primary cause of death.

Chronic Toxicity (or Exposure)

Animal

Carcinogenicity studies carried out over years have not demonstrated any increased tumor incidence. No teratogenic effects have been documented in mice despite large chronic doses (e.g., 625 times the maximum daily dose of lisinopril on days 6–15 of gestation).

Human

Adverse effects observed at therapeutic doses include cough, dermal reactions, blood dyscrasias, bronchospasm, and hypogeusia. Angioedema has been reported, but does not appear to be an IgG related immune response. Reversible renal failure has been reported with chronic therapy. Clinical effects that may occur include hypotension with or without a reflex tachycardia, changes in level of consciousness that are directly related to vascular changes, and hyperkalemia. Hyperkalemia can occur as a response to sodium loss. Delayed hypotension, at 19 and 25 h, has been observed following ingestion of captopril.

In Vitro Toxicity Data

Lisinopril, captopril, quinapril, and benazepril have been studied for mutagenicity using a variety of methods and none have documented evidence of mutagenicity.

Clinical Management

Supportive care, including airway management as well as cardiac and blood pressure monitoring,

should be provided to unstable patients. Ingestion of small amounts of an ACE inhibitor in children can be managed with observation at home. Following ingestion of a toxic amount of these agents or recent ingestions involving toxic coingestants, activated charcoal can be utilized to decontaminate the stomach. Hypotension following ACE inhibitor ingestion has been managed with fluids alone or in combination with vasopressors such as dopamine. A limited number of case reports exist that describe a need for dopamine to treat hypotension. If profound hypotension resistant to dopamine were to occur, other vasopressors, such as epinephrine and norepinephrine, can be used. Laboratory analysis should be used to monitor electrolytes, especially sodium and potassium. ACE inhibitor serum concentrations are not readily available and have little if any clinical utility. Because ACE inhibitors may potentiate the effects of the opiate-like beta endorphins, some authors have suggested the use of naloxone to reverse their toxicities. Successes and failures with naloxone have been described in case reports. Because naloxone has limited adverse effects, its use could be considered in the management of serious ACE inhibitor toxicity. One case report describes the use of the experimental exogenous angiotensin II to counter severe ACE inhibitor toxicity. The pharmacokinetic characteristics of the ACE inhibitors, limited protein binding, and small volume of distribution make them amenable to hemodialysis. Because major morbidity is rare with these agents, the need for dialysis is questionable.

Angioedema with potential for airway obstruction may not respond to epinephrine and antihistamines. Rapid intubation to protect the airway may be necessary.

Environmental Fate

No information is currently available on breakdown in soil, groundwater, or surface water. ACE inhibitors are excreted into breast milk in trace amounts. Captopril is distributed into milk in concentrations of ~1% of those in maternal blood.

See also: Charcoal; Prostaglandins.

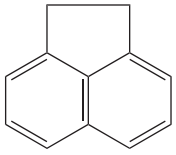
Further Reading

Augenstein WL, Kulig KW, and Rumack BH (1988) Captopril overdose resulting in hypotension. *Journal of the American Medical Association* 259: 3302–3305.

Acenaphthene

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 83-32-9
- Synonyms: 1,2-Dihydroacenaphthylene; 1,8-Dihydroacenaphthalene; 1,8-Ethylenenaphthalene; Acenaphthylene; Naphthyleneethylene; Periethylenenaphthalene; Ethylenenaphthylene
- Chemical/Pharmaceutical/Other Class: Arene belonging to the class of polycyclic aromatic hydrocarbons
- Chemical Structure:



Uses

Acenaphthene is a chemical intermediate used to produce naphthalimide dyes, which are used as fluorescent whitening agents, and used in manufacturing plastics, insecticides, and fungicides.

Background Information

Acenaphthene is a component of crude oil and a product of combustion, which may be produced and released to the environment during natural fires. Emissions from petroleum refining, coal tar distillation, coal combustion, and diesel fueled engines are the major contributors of acenaphthene to the environment. Acenaphthene is used as a chemical intermediate and may be released to the environment via manufacturing effluents and the disposal of manufacturing waste by-products. Because of the widespread use of acenaphthene in a variety of products, acenaphthene may also be released to the environment through landfills, municipal waste

water treatment facilities, and waste incinerators. Acenaphthene should biodegrade rapidly in the environment. The reported biodegradation half-lives for acenaphthene in aerobic soil and surface waters range from 10 to 60 and from 1 to 25 days, respectively. However, acenaphthene may persist under anaerobic conditions or at high concentration due to toxicity to microorganisms. Acenaphthene is not expected to hydrolyze or bioconcentrate in the environment; yet, it should undergo direct photolysis in sunlight environmental media. Acenaphthene is expected to exist entirely in the vapor phase in ambient air.

Exposure Routes and Pathways

Skin contact is the most common accidental exposure pathway. Acenaphthene may irritate or burn skin. Exposure can also be through ingestion or inhalation. Its vapor can be poisonous if inhaled.

Toxicokinetics

The half-life of acenaphthene in the bluegill fish is less than 1 day. A *Beijerinckia* species and a mutant strain, *Beijerinckia* species strain B8/36, were shown to oxidize acenaphthene. Both organisms oxidize acenaphthene to the same spectrum of metabolites, which included 1-acenaphthenol, 1-acenaphthenone, 1,2-acenaphthenediol, acenaphthenequinone, and a compound that was tentatively identified as 1,2-dihydroxyacenaphthylene.

Mechanism of Toxicity

5-Nitroacenaphthene causes toxicity by the reduction of the nitro function to the corresponding hydroxylamine. These arylhydroxylamines may be either direct-acting mutagens or may become so following nonenzymic conversion to aryl nitronium ions or they may be esterified to the corresponding electrophilic hydroxamic acid esters. Acenaphthene can bind to hemoglobin to cause methemoglobinemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acenaphthene can cause hepatotoxicity in rats and mice. Little information is available regarding acute exposure to acenaphthene. It is biotransformed in the liver. On the basis of a mouse oral subchronic study in which hepatotoxicity was seen as the major effect, the no-observed-adverse-effect level and the lowest-observed-adverse-effect level were 175 and $350\,mg\,kg^{-1}\,day^{-1}$, respectively.

Human

Acenaphthene can be irritating to eyes, skin, and mucous membrane. Acenaphthene may be poisonous if inhaled or absorbed through skin. The vapor may cause dizziness or suffocation. Acenaphthene may cause vomiting if swallowed in large quantity. It can cause methemoglobinemia.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to acenaphthene at a level of $12 \pm 1.5\,mg\,m^{-3}$ for 4 h a day, 6 days per week for 5 months showed toxic effects on the blood, lung, and glandular constituents. The bronchial epithelium showed hyperplasia and metaplasia, which may have been symptoms of the pneumonia that killed a large number of rats during the study. No signs of malignancy appeared during the 8 month postexposure observation period.

Human

Chronic human exposure data are not available. Currently, acenaphthene is under review by US Environmental Protection Agency for evidence of human carcinogenic potential. This does not imply that this agent is necessarily a carcinogen. The nitroderivative of acenaphthene (5-nitroacenaphthene) is a possible carcinogen to humans.

In Vitro Toxicity Data

Acenaphthene is devoid of any mutagenic activity in *Salmonella typhimurium* (TA 98) assay. The nitroderivatives of acenaphthene have tumorigenic potential.

Clinical Management

The victim should be moved to fresh air and emergency medical care should be provided. If the victim is not breathing, artificial respiration should be provided; if breathing is difficult, oxygen should be administered. In case of contact with the eyes, the eyes should be flushed immediately with running water for at least 15 min. Affected skin should be washed with soap and water. Contaminated clothing and shoes should be removed and isolated at the site. If methemoglobinemia occurs and is severe, treatment with methylene blue and oxygen is recommended.

Environmental Fate

The reported biodegradation half-lives for acenaphthene in aerobic soil and surface waters range from 10 to 60 and from 1 to 25 days, respectively. However, acenaphthene may persist under anaerobic conditions or at high concentrations due to toxicity to microorganisms. Acenaphthene is not expected to hydrolyze or bioconcentrate in the environment; yet, it should undergo direct photolysis in sunlit environmental media. A calculated K_{oc} range of 2065–3230 indicates acenaphthene will be slightly mobile in soil. In aquatic systems, acenaphthene can partition from the water column to organic matter contained in sediments and suspended solids. A Henry's law constant of 1.55×10^{-4} atm m^3 mol^{-1} at 25°C suggests volatilization of acenaphthene from environmental waters may be important. The volatilization half-lives from a model river and a model pond, the latter considers the effect of adsorption, have been estimated to be 11 h and 39 days, respectively. Acenaphthene is expected to exist entirely in the vapor phase in ambient air. In the atmosphere, the reaction with photochemically produced hydroxyl radicals (half-life of 7.2 h) is likely to be an important fate process. The most probable human exposure would be occupational exposure, which may occur through dermal contact or inhalation at places where acenaphthene is produced or used. Atmospheric workplace exposures have been documented. Nonoccupational exposures would most likely occur via urban atmospheres, contaminated drinking water supplies, and recreational contaminated waterways.

Ecotoxicology

Treatment of cherry-mazzard hybrid seeds with acenaphthene powder for 10 h inhibited the seed germination and seedling growth. Treatment of *Allium cepa* root meristem cells with acenaphthene vapor for 12–96 h caused anomalies leading to random development of cells. Acute toxicity value for bluegill fish was 1700 UG l^{-1} in freshwater and the toxicity to sheepshed minnow was 2230 UG l^{-1} in saltwater.

Exposure Standards and Guidelines

CERCLA reportable quantities: Persons in charge of vessels or facilities are required to notify the National Response Center (NRC) immediately, when there is a release of this designated hazardous substance, in an amount equal to or greater than its reportable quantity of 100 lb or 45.4 kg. State drinking water guidelines: Minnesota 400 µg l^{-1} and Florida 20 µg l^{-1}.

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

Byrne M *et al.* (1985) Government Reports, Announcements & Index, Issue 21. *Exposure and Risk Assessment for Benzo(a)pyrene and Other Polycyclic Aromatic Hydrocarbons*, vol. 3.

Harris J, Perwak J, and Coons S (1985) Government Reports, Announcements & Index Issue 21. *Exposure and Risk Assessment for Benzo(a)pyrene and Other Polycyclic Aromatic Hydrocarbons*, vol. 1.

Health & Welfare Canada (1979) *Polycyclic Aromatic Hydrocarbons*, Report No. 80-EHD-50.

Staples CA and Werner AF (1985) Priority pollutant assessment in the USA: Scientific and regulatory implications. *Toxic Substances* 6(4): 186–200.

US Department of Health & Human Services/Agency for Toxic Substances Disease Registry (1995) *Toxicological Profile for Polycyclic Aromatic Hydrocarbons* (Update), NTIS# PB/95/264370.

USEPA (1979) *Health Assessment Document: Polycyclic Organic Matter*, EPA-600/9-79-008.

USEPA (1980a) *Ambient Water Quality Criteria Doc: Acenaphthene* (Draft).

USEPA (1980b) *Ambient Water Quality Criteria Doc: Polynuclear Aromatic Hydrocarbons* (Draft).

Wilkins ES and Wilkins MG (1985.) Review of toxicity of gases emitted from combustion pyrolysis of municipal and industrial wastes. *Journal of Environmental Science and Health – Part A* 20(2).

Relevant Website

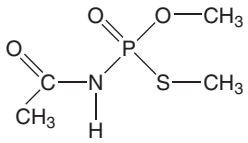
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Acenaphthene.

Acephate

Subramanya Karanth

- Chemical Abstracts Service Registry Number: CAS 30560-19-1
- Synonyms: Asataf; Aimthane; Chrevron RE 12420; Kitron; Orthene; Ortril; Pillarthene
- Chemical/Pharmaceutical/Other Class: Organophosphorus insecticide
- Chemical Formula: $C_4H_{10}NO_3PS$

- Chemical Structure:



Uses

Acephate is registered for use on a variety of field, fruit, and vegetable crops (e.g., cotton, tobacco, cranberries, mint). It is also used commonly in food handling establishments, on ornamental plants (cut flowers), and in and around residential and commercial buildings for the control of roaches and fire ants. It is effective against a wide range of biting and sucking insects, especially aphids.

Exposure Routes and Pathways

Common routes of acephate exposure include ingestion and inhalation.

Toxicokinetics

Acephate is converted to another organophosphorus compound, methamidophos, in the body. Studies with ^{14}C-acephate in mammals have shown 75% of the parent compound eliminated in the urine. Other major metabolites include *O*,*S*-dimethyl phosphorothioate (DMPT, 5%) and *S*-methyl acetyl phosphoramidothioate (5%).

Mechanism of Toxicity

Acephate exerts its toxicity by inhibiting the enzyme acetylcholinesterase in the synapse and neuromuscular junctions, which leads to accumulation of the neurotransmitter acetylcholine and overstimulation of postsynaptic receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acephate is moderately toxic to mammals with an acute oral LD_{50} of 850–950 mg kg^{-1} in rats, whereas its metabolite methamidophos is highly toxic to mammals. The common symptoms of acephate poisoning include salivation, nasal discharge, vomiting, diarrhea, nausea, blurred vision, difficulty in breathing, headache, and muscle weakness. Convulsions, coma, and death may occur in cases of severe acute poisoning.

Human

Acephate exposure can result in cholinesterase inhibition, which causes overstimulation of the nervous system. Acephate is nonirritating to skin and slightly irritating to the eyes but is not a skin sensitizer.

Chronic Toxicity (or Exposure)

Animal

Chronic studies in rats and dogs have shown cholinesterase inhibition. No pathological changes were observed in rats following 3 month exposure to 30 mg kg^{-1} dosage of acephate.

Human

Acephate is classified as a possible human carcinogen. Since acephate is used both on food crops and other common residential areas, risks of human exposures through multiple routes are high. Based on cholinesterase inhibition studies in rats, the no-observed-adverse-effect level for chronic dietary exposure is 0.12 mg kg^{-1} day^{-1}. Agricultural workers who are involved in mixing, formulation, and application may be at higher risk of exposure.

Clinical Management

In case of dermal exposure, the contaminated area should be washed with plenty of water or showered using soap and shampoo. Eyes should be flushed with water repeatedly for several minutes. Contaminated clothing should be removed and the airway cleared. In case of ingestion, vomiting should be induced. Atropine treatment should be initiated immediately to counteract muscarinic effects. Atropine (adults and children >12 years: 2–4 mg; children <12 years: 0.05–0.1 mg) treatment should be repeated every 15 min until oral and bronchial secretions are controlled and atropinization is achieved. The duration and dosage of atropine treatment should be slowly reduced as the condition of the patient improves. Pralidoxime should be administered slowly at the recommended dosage (adults and children >12 years: 1–2 g; children <12 years: 20–50 mg by IV infusion in 100 ml saline at $\sim$0.2 g min^{-1}). This dosage can be repeated at every 1–2 h intervals initially and at 10–12 h intervals later depending on the condition of the patient. Periodic medical examination and care is required depending on the degree of exposure.

Environmental Fate

Acephate is readily degraded in soil by microorganisms and in water it undergoes rapid hydrolysis.

Its half-life is less than 3 and 6 days under aerobic and anaerobic conditions, respectively. CO_2 is the major metabolite following microbial degradation in soil.

Ecotoxicology

Both acephate and its metabolite, methamidophos, pose a high acute and chronic risk to birds. Studies in insects have shown that acephate is highly toxic to honey bees and other beneficial insects. Methamidophos is also very highly toxic to freshwater invertebrates.

Exposure Standards and Guidelines

The chronic reference dose for acephate is $0.0012\,mg\,kg^{-1}\,day^{-1}$ while the accepted daily intake is $0.03\,mg\,kg^{-1}\,day^{-1}$.

See also: Acetylcholine; Methamidophos; Neurotoxicity; Organophosphates; Pesticides.

Relevant Websites

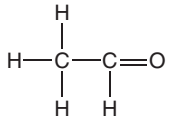
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Acetaldehyde

John Sanseverino

This article is a revision of the previous print edition article by Edward Kerfoot, volume 1, pp. 10–11, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 75-07-0
- Synonyms: Acetic aldehyde; Acetylaldehyde; Ethylaldehyde; Ethanal
- Chemical/Pharmaceutical/Other Class: Aldehydes
- Chemical Formula: C_2H_4O
- Chemical Structure:



Uses

Acetaldehyde is used in the manufacturing of various chemicals such as acetic acid, pyridine, peracetic acid, pentaerythritol, 1,3-butylene glycol, and chloral. It is also used in the silvering of mirrors, leather tanning, fuel compositions, preservatives, paper processing, glues, cosmetics, dyes, plastics, and rubber. Natural sources of acetaldehyde include metabolic intermediate in higher plants, alcohol fermentation, and sugar decomposition in the body. Anthropogenic sources include vehicle exhaust, fuel oil and coal, organic chemical manufacturing.

Exposure Routes and Pathways

Industrial exposures to acetaldehyde are most likely to occur by inhalation with potential for skin and eye contact. Accidental ingestion is also possible. Acetaldehyde is produced from the metabolism of ethanol in the body.

Toxicokinetics

Following inhalation exposure, acetaldehyde is deposited in the nasal cavity and upper respiratory tract, and eventually some traces can be absorbed into the blood and be distributed throughout the body. The uptake of acetaldehyde in the nasal cavity is influenced by its solubility and inspiratory flow rate. Perhaps acetaldehyde uptake in the nasal tissue is dependent on its reaction with tissue substrates that become depleted at high exposure concentrations. Acetaldehyde vapor can be metabolized in the nasal cavity by the mixed-function oxidase and carboxylesterase systems. The first metabolite of ethanol metabolism is acetaldehyde. Metabolism takes place in the liver to a number of metabolites and some unchanged acetaldehyde that can be excreted in the urine. Most of the free acetaldehyde is excreted in the exhaled breath.

Mechanism of Toxicity

Acetaldehyde is soluble in the mucous membranes of the upper respiratory tract causing irritation of the sensory nerve endings. There is also depression of the mucociliary defense system. The direct action of acetaldehyde in the skin and eyes is the result of irritation to these tissues.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for acetaldehyde in rats has been reported to be $1930\,mg\,kg^{-1}$ and the 4 h LC_{50} is

approximately 13 300 ppm. Acetaldehyde is a severe eye irritant to rabbits at 40 mg and mildly irritating to rabbit skin at 500 mg. Rats were exposed to acetaldehyde concentrations ranging from 400 to 5000 ppm in a 4 week subchronic inhalation study at 6 h day^{-1}, 5 day week^{-1}. At 1000 and 2200 ppm, the rats exhibited growth retardation, polyuria, and nasal epithelial degeneration. At 400 ppm, there was slight degeneration of the olfactory epithelium.

Human

Inhalation exposures to acetaldehyde can result in irritation of the upper respiratory tract. Inhalation at concentrations ranging from 100 to 200 ppm can cause irritation to the mucous membranes. Skin and eye contact with liquid acetaldehyde can produce a burning sensation, lacrimation, and blurred vision. Unacclimated subjects experienced eye irritation at 50 ppm after a 15 min exposure. Some more sensitive persons exhibited eye irritation at 25 ppm for a 15 min exposure.

Chronic Toxicity (or Exposure)

Animal

A 52 week chronic inhalation study in hamsters exposed to 1500 ppm acetaldehyde produced growth retardation, slight anemia, increased enzyme and protein content in the urine, and increased kidney weight. There were distinct histopathological changes in the nasal mucosa and trachea, including hyperplasia, squamous cell metaplasia, and inflammation.

Inhalation exposure to acetaldehyde has produced nasal tumors in rats and laryngeal tumors in hamsters. Male and female rats were exposed to acetaldehyde 6 h day^{-1}, 5 day week^{-1} for 28 months at concentrations of 0, 750, 1500, or 3000 ppm. A concentration-related incidence of squamous cell carcinomas of the respiratory epithelium was observed in both male and female rats. A statistically significant number of adenocarcinomas occurred in the olfactory epithelium of both sexes of rats exposed at all three acetaldehyde concentrations. Male and female hamsters were exposed to acetaldehyde 7 h day^{-1}, 5 day week^{-1} at concentrations gradually reduced from 2500 to 1650 ppm for 52 weeks. Both sexes of acetaldehyde-exposed hamsters developed laryngeal tumors consisting of squamous cell carcinomas and adenosquamous cell carcinomas.

Data from studies with rats suggest that acetaldehyde is teratogenic. Fetuses from dams injected intraperitoneally with acetaldehyde concentrations ranging from 50 to 100 mg kg^{-1} on day 10, 11, or 12 of gestation produced a significant increase in fetal resorptions, growth retardation, and an increase in malformations, including digital anomalies, cranial and facial malformations, and delayed skeletogenesis. It was concluded that acetaldehyde interfered with placental function via the maternal–placental nutrient exchange, resulting in retarded growth.

Data from some animal studies suggest that acetaldehyde is teratogenic. According to the American Conference of Governmental Industrial Hygienists (ACGIH), the recent identification of nasal and laryngeal carcinomas indicated that acetaldehyde should be considered an A3 animal carcinogen.

Human

Acetaldehyde, produced from the metabolism of ethanol, may also be responsible for localized cancers, brain damage in prenatal infants, and growth suppression (in chicken embryos). Acetaldehyde, as a direct result of ethanol metabolism in the body, has been implicated in alcoholic cardiomyopathy and cancer of the digestive tract. The levels of acetaldehyde in blood are directly correlated with ethanol consumption.

In Vitro Toxicity Data

Acetaldehyde has been shown to induce mutagenic changes in many assays. In mammalian *in vitro* assays, acetaldehyde produced sister chromatid exchanges and chromosomal breaks and aberrations in mammalian *in vitro* assays.

Clinical Management

Exposures by inhalation should be monitored for respiratory tract irritation, bronchitis, or pneumonitis. Humidified supplemental 100% oxygen should be administered. Gastric lavage may be indicated soon after ingestion of acetaldehyde followed by administration of activated charcoal slurry mixed with a saline cathartic or sorbitol. Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. If eye irritation, pain, swelling, lacrimation, or photophobia persists, the patient should be seen in a health care facility.

Exposure Standards and Guidelines

A short-term exposure limit ceiling of 25 ppm for acetaldehyde was recommended to prevent excessive eye irritation, lacrimation, and potential injury to the respiratory tract.

See also: Respiratory Tract.

Further Reading

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Verschueren K (2000) *Handbook of Environmental Data on Organic Chemicals*, 3rd edn. New York: Wiley.

World Health Organization (1995) *Acetaldehyde*. Geneva: World Health Organization.

Acetamide

Gerald L Kennedy

- Chemical Abstracts Service Registry Number: CAS 60-35-5
- Synonyms: Acetic acid amide; Ethanamide; Methane-carboxamide
- Chemical/Pharmaceutical/Other Class: Amide, aliphatic; Organic solvent; Volatile organic compound
- Chemical Formula: CH_3CONH_2

Uses

As a dipolar solvent, acetamide finds many uses as a solvent for both inorganic and organic compounds. The solvency has led to widespread uses in industry including applications in cryoscopy, soldering, and the textile industry. The neutral and amphoteric characteristics allow its use as an antacid in the lacquer, explosives, and cosmetics industries. Its hygroscopic properties make it useful as a plasticizer in coatings, fixtures, cloth, and leather, and as a humectant for paper. It is also a raw material in organic synthesis of methylamine and thioacetamide and as an intermediate in preparation of medicines, insecticides, and plastics.

Exposure Routes and Pathways

Acetamide may be inhaled, swallowed, or absorbed through the skin. The chemical is considered to be mildly irritating to the skin and eyes. In its usual application, inhalation is the most common route of exposure, although dermal contact is always possible.

Toxicokinetics

Oral administration of acetamide in the rat is followed by absorption and 62% is excreted into the urine unchanged in 24 h. Likewise, a large proportion of an oral dose is excreted in the urine unchanged by the dog and cat. In sheep, absorption of an oral dose is followed by metabolism to CO_2 within 7–12 h. Sequential demethylation of methylacetamide results in acetamide production by rat liver but it is not clear whether this occurs in man. Acetamide is a metabolite of the antiprotozoal drugs metronidazole and ornidazole.

Mechanism of Toxicity

The mechanism of toxicity of acetamide is not known; the response profile is quite different from the better studied dimethyl derivative.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in rodents ranges from 1 to 7 g kg^{-1} and intravenous LD_{50} in mice and rats is 10 g kg^{-1}. No acute lethality information is available following either dermal or inhalation exposures. Acetamide is not a developmental toxicant and is generally inactive in genetic toxicity tests.

Human

No reports could be found in the literature concerning acute toxicity of acetamide in humans.

Chronic Toxicity (or Exposure)

Animal

Liver cancers were produced in rats following oral administration of relatively large amounts of acetamide. The liver appears to be the target of acetamide toxicity although the animal experiments have been limited in the range of endpoints studied.

Human

No reports could be found in the literature concerning the potential human health effects of chronic acetamide exposure.

Clinical Management

Exposed persons should be removed to fresh air and medical attention sought as needed for any breathing difficulty. If swallowed, several glasses of water should be given to dilute the chemical; medical attention is needed if large amounts are ingested. For skin contact, the exposed area should be washed with soap and water; medical attention should be sought if irritation develops. For eye contact, water should be used to flush for at least 15 min while lifting the lower and upper eyelids occasionally; immediate medical attention should be sought.

Environmental Fate

Acetamide will exist as a vapor in the ambient atmosphere. Atmospheric degradation occurs by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 7.6 days. If released to soil, acetamide is expected to have very high mobility and is not expected to adsorb to suspended solids and sediment. Experiments suggest that this chemical may break down in the environment through biodegradation and not through hydrolysis. Volatilization from water surfaces is not expected to be an important fate process based on this compound's estimated Henry's law constant. The potential for bioconcentration in aquatic organisms is low.

Exposure Standards and Guidelines

US Environmental Protection Agency: Listed as a hazardous air pollutant under the Clean Air Act of 1990.

Occupational Safety and Health Administration: No permissible exposure limit (as of October 2003).

International Agency for Research on Cancer: Classified as a 2B carcinogen (probable human carcinogen with sufficient evidence in laboratory animals).

See also: Clean Air Act (CAA), US; Methylamine; Metronidazole; Thioacetamide.

Further Reading

Kennedy GL Jr. (1986) Biological effects of acetamide, formamide, and their monomethyl and dimethyl derivatives. *Critical Reviews in Toxicology* 17: 129–182.

Kennedy GL Jr. (2001) Biological effects of acetamide, formamide, and their monomethyl and dimethyl derivatives: An update. *Critical Reviews in Toxicology* 31: 139–222.

Relevant Website

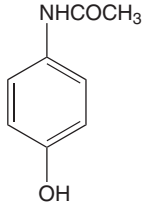
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Acetamide.

Acetaminophen

Kartik Shankar and Harihara M Mehendale

This article is a revision of the previous print edition article by Edward P Krenzelok, volume 1, pp. 11–13, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 103-90-2
- Synonyms: APAP; 4′-Hydroxyacetanilide; *p*-Hydroxyacetanilide; Acetamide *N*-(4-hydroxyphenyl); *N*-Acetyl-*p*-aminophenol; *N*-Acetyl-*p*-aminophenol; *p*-Acetamidophenol; 4-Acetamidophenol; 4-Acetaminophenol; Paracetamol; Tylenol
- Chemical/Pharmaceutical/Other Class: Acetaminophen is a synthetic nonopioid congener of acetanilide in the *p*-aminophenol class
- Chemical Formula: $C_8H_9NO_2$
- Chemical Structure:



Uses

Acetaminophen is a nonnarcotic analgesic and antipyretic drug. It is used to relieve pain of moderate intensity, such as usually occurs in headache and in many muscle, joint, and peripheral nerve disorders. Headaches are one of the most common indications for the use of acetaminophen. Acetaminophen is used to treat acute tension-headaches and mild to moderate

migraine, especially in combination with caffeine and aspirin. Acetaminophen is indicated in chronic pain associated with rheumatoid arthritis, back or hip pain, osteoarthritis, dental pain, or acute pain due to soft-tissue injury. Acetaminophen is a suitable substitute for aspirin for its analgesic or antipyretic uses in cases where aspirin is contraindicated (gastric bleeding) or when the prolongation of bleeding time caused by aspirin would be a disadvantage. Acetaminophen has been used in studies of pain relief following obstetric and gynecological procedures including Caesarean section, hysterectomy, tubal ligation, primary dysmenorrhoea, and termination of pregnancy. Acetaminophen is also used to manage chronic pain of cancer, postpartum and postoperative pain after minor surgery. In a double blind crossover study, the analgesic oral butorphanol and acetaminophen in combination, showed additive analgesic effects against moderate to severe pain due to metastatic carcinoma over that of individual drug. Acetaminophen is also widely used as an antipyretic drug to reduce fever.

Background Information

Acetaminophen can be found as the active ingredient in more than 100 over-the-counter products and a number of prescription drugs, alone or in combination with other drugs. The pharmacology and toxicology of this drug has been extensively studied and reviewed. Acetaminophen has been the subject of more than 30 000 articles in medical literature since 1966. The first clinical use of acetaminophen dates back to 1893 by von Mering (and subsequently by Hinsberg and Treupel, 1894) as an effective antipyretic with comparable pharmacological effects to antipyrine and phenacetin. However, after a hiatus of almost half a century, acetaminophen was rediscovered as the major metabolite of phenacetin and acetanilide in man and was marketed in the United States as a combination with aspirin and caffeine in 1950. In the 1960s and 1970s concerns about gastrointestinal adverse effects of aspirin and methemoglobinemia of acetanilide only led to increased popularity of acetaminophen as a generally safe antipyretic analgesic. Hepatotoxicity of acetaminophen began to be reported in the late 1960s and has been a topic of intense scientific evaluation to this day. The impact of acetaminophen-induced liver toxicity, accidental or otherwise, will be taken up in later sections.

Exposure Routes and Pathways

Acetaminophen is available in several dosage forms including tablets, capsules, syrups, elixirs, and suppositories. Oral ingestion is the most common route for both accidental and intentional exposure to acetaminophen.

Toxicokinetics

Absorption of acetaminophen occurs in the gastrointestinal tract primarily by passive nonionic diffusion and is highly dependent on the several factors including dose, presence of food and other chemicals, mucosal blood flow, age, body weight, time of day, and coexisting disease conditions. At pharmacological doses acetaminophen is absorbed rapidly with $\sim$75–95% of the therapeutic oral dose being recovered in the urine by 12–24 h as unchanged acetaminophen or metabolite. A large number of studies have evaluated the pharmacokinetic parameters of acetaminophen in man after oral or intravenous dosing. Most studies consistently report volume of distribution to be between 0.8 and 1 l kg^{-1}. Total clearance and plasma half-life with therapeutic doses in healthy subjects were usually 3–5 ml min kg^{-1} and 1–3 h, respectively. After suprapharmacological or toxic doses absorption may be delayed after producing peak blood concentrations at $\sim$4 h postingestion. In man, the majority of acetaminophen is metabolized in the liver to glucuronide and sulfate conjugates that are eliminated in the urine. Estimates in man from urinary metabolites report 50–60% as glucuronide conjugate, 25–35% as sulfate conjugate, and between 2% and 5% of cysteine and mercapturate conjugates each. In young children, the sulfate conjugate predominates. The water-soluble glucuronide and sulfate conjugates are eliminated via the kidneys. Approximately 2–5% is eliminated in the urine as unchanged acetaminophen. The half-life of therapeutic dose is 1–3 h. In overdose patients, this may be increased to more than 4 h and may even exceed 12 h in patients with severe acetaminophen-induced liver toxicity.

Mechanism of Toxicity

Although major part of the ingested dose of acetaminophen is detoxified, a very small proportion of acetaminophen is metabolized via the cytochrome P450-mixed function oxidase pathway to a highly reactive *N*-acetyl-*p*-benzoquinoneimine (NAPQI). The toxic intermediate NAPQI is normally detoxified by endogenous glutathione to cysteine and mercapturic acid conjugates and excreted in the urine. Recent studies have shown that hepatic P450 s, CYP2E1 and, to a lesser extent CYP1A2 are responsible for conversion of acetaminophen to NAPQI. In acetaminophen overdosage, the amount of NAPQI

increases and depletes endogenous glutathione stores. Time course studies have shown that covalent binding of reactive NAPQI and subsequent toxicity occurs only after cellular glutathione stores are reduced by 70% or more of normal. Mitochondrial dysfunction and damage can be seen as early as 15 min after a toxic dose in mice, suggesting that this may be a critical to cellular necrosis. The NAPQI is then thought to covalently bind to critical cellular macromolecules in hepatocytes and cause cell death. Recent proteomic studies have identified at least 20 known proteins that are covalently modified by the reactive acetaminophen metabolite. Hepatic necrosis as a consequence of hepatocellular death then results in development of clinical and laboratory findings consistent with liver failure. A similar mechanism is postulated for the renal damage that occurs in some patients who suffer from acetaminophen toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

A large body of evidence is available examining the acute toxicity of acetaminophen in animal models. Mice and rats have been widely used to study the toxic effects of acetaminophen. Since the rat is relatively resistant, the mouse has been the most widely used species to study both the mechanisms of acetaminophen toxicity and to examine chemicals that potentiate or protect from the toxicity. Hepatotoxicity and nephrotoxicity are the two main effects associated with acute overdose of acetaminophen. Of these, death in most species is due to acute hepatic failure. LD_{50} values range from 350 to 4500 mg kg^{-1} depending on the species and the route of acetaminophen administration, mice (LD_{50} 350–600 mg kg^{-1}) being more far more sensitive than rats, guinea pigs, and rabbits ($LD_{50} > 3$ g kg^{-1}). Death occurs within 12 h after acetaminophen exposure. In mice after a toxic dose, general findings in addition to the severe hepatic necrosis, include necrotic changes in the kidney, bronchiolar epithelium, testes, lymphoid follicles of the spleen, and small intestine. Cats are particularly susceptible to acetaminophen intoxication because of their impaired glucuronic acid conjugation mechanism and saturation of their sulfate conjugation pathway. The clinical signs associated with experimental acetaminophen administration to cats included cyanosis followed by anemic hemoglobinuria, icterus, and facial edema. Laboratory findings in acetaminophen poisoned cats include methemoglobinemia and an elevated serum alanine aminotransferase activity.

Human

Hepatotoxicity is the primary toxic insult from acute acetaminophen overdose. Acetaminophen overdose accounts for more than 56 000 emergency room visits and is implicated in ~50% of all acute liver failure in the United States (US Acute Liver Failure (ALF) Study Group). Exposure to toxic doses of acetaminophen may be intentional (suicidal) or unintentional (accidental). Recent data from Parkland Hospital suggests that greater percentages of unintentional overdose victims suffer from fatal consequences compared to persons attempting suicide (with acetaminophen) primarily due to their characteristic late presentation. Data from the US ALF Study Group shows that unintentional overdoses (which are more frequent in liver failure cases) were also larger (median dose of 34 g) compared to suicidal doses, being consumed over several preceding days. There is no clear agreement on a maximum tolerated dose of acetaminophen. Most people tolerate 4–8 g day^{-1} of acetaminophen without any hepatotoxic incidence. However, the risk of severe liver injury may be quite high above the 4 g day^{-1} dose, especially in a group of individuals due to indeterminate idiosyncratic reasons.

The typical clinical manifestations are secondary to hepatic damage. Plasma concentrations should be obtained to determine the probability of acetaminophen-induced hepatotoxicity. The Rumack–Matthews nomogram is used to assess the risk of hepatotoxicity. Levels in excess of 200 μg ml^{-1} of acetaminophen at 4 h postingestion are associated with high probability of development of hepatotoxicity. A second treatment line 25% lower than the original '200' line was added at the request of the Food and Drug Administration (FDA) in 1976. The clinical presentation follows four distinct phases. Gastrointestinal irritation, nausea and vomiting are present in the first 24 h postingestion. The second stage (24–48 h) postingestion is characterized by the resolution of the initial symptoms, accompanied by elevations of hepatic transaminases. Cases that progress to stage three develop hypoglycemia, coagulopathies, jaundice, and symptoms consistent with hepatic failure. Surviving patients go through a fourth stage of recovery. As toxicity develops half-life becomes prolonged and transaminases rise and fall. In instances where reliable history of time of ingestion is not available calculations of body burden may be useful in deciding treatment.

Chronic Toxicity (or Exposure)

Animal

In a 2 year feed study, there was no evidence of carcinogenic activity of acetaminophen in male F344/N

rats that received 600, 3000, or 6000 ppm acetaminophen for 104 weeks. There was equivocal evidence of carcinogenic activity in female F344/N rats based on increased incidences of mononuclear cell leukemia. Overall, there is inadequate evidence in experimental animals for the carcinogenicity of acetaminophen and is not classifiable as to its carcinogenicity. Acetaminophen was nonmutagenic in the *Salmonella*/mammalian microsome assay at concentrations ranging from 0.1 to 50 mg per plate. In a study to examine effect of acetaminophen on reproduction and fertility, no changes in the number of pups/litter, viability, or in adjusted pup weight were found. Acetaminophen in the diet of Swiss mice reduced weight gain during nursing. Fertility endpoints (ability to bear normal numbers of normal-weight young) were generally not affected.

Human

There is inadequate evidence in humans for the carcinogenicity of acetaminophen and is therefore not classifiable. The chronic ingestion of excessive amounts of acetaminophen may produce similar toxicity as a large acute dose but in a more insidious fashion. Age, chronic alcohol abuse, and preexisting disease may be contributing factors. The American Academy of Pediatrics considers use of acetaminophen safe during breast-feeding and is classified as a category B chemical by the FDA (studies in laboratory animals have not demonstrated a fetal risk, but there are no controlled studies in pregnant women). Acetaminophen should be given with care to patients with impaired kidney or liver function. Care should also be taken when giving acetaminophen to patients taking other drugs that affect the liver.

In Vitro Toxicity Data

Acetaminophen causes cytotoxicity in several cell types; however, the most widely studied cytotoxicity of acetaminophen is in primary hepatocytes or hepatocyte cell lines.

Cytotoxicity in Hepatic Cells

Primary hepatocytes from rats, mice, hamsters, rabbits, dogs, pigs, monkeys, and humans have been shown to be susceptible to acetaminophen *in vitro*. The cytotoxicity of acetaminophen varies considerably depending on species, presumably due to differences in bioactivation and glutathione status. The most obvious morphological effect of acetaminophen in isolated primary hepatocytes is blebbing of the cell membrane. However, electron microscopy has shown that toxicity is associated with progressive loss of microvilli, mitochondrial abnormalities and appearance of myeloid bodies. Exposure of primary mouse hepatocytes to concentrations of acetaminophen above 1 mmol l^{-1}, led to significant lactate dehydrogenase leakage as early as 3 h. Cytotoxicity of acetaminophen has also been examined using standard liver cell lines including, PC12 cells, HepG2 cells, H4IIEC3G$^-$ cells, among other cell lines. Immortalized hepatocyte cultures, in many cases, lose their ability to bioactivate acetaminophen and hence are resistant to toxicity. Transient or consistent overexpression of P450 enzymes (CYP2E1 and/or CYP1A2) leads to increased cytotoxicity of acetaminophen. Acetaminophen is also cytotoxic in cultures of rat liver sinusoidal endothelial cells, Kupffer cells and mouse fibroblasts.

Cytotoxicity in Other Cells

The cytotoxicity of acetaminophen has been demonstrated in cultures of HeLa cells, L929 and 3T3 murine fibroblasts, chick embryo neurons, rat embryonic and skeletal muscle, peripheral blood lymphocytes, and lung and dermal cells. In addition cytotoxicity of acetaminophen has been evaluated in BF-2 fish cell line (see section on 'Ecotoxicology').

Clinical Management

Activated charcoal or other gastrointestinal decontamination procedures can be utilized when deemed necessary. Induction of emesis is not recommended as prolonged emesis may interfere with *N*-acetyl cysteine (NAC) therapy. The Rumack–Matthews nomogram is utilized to identify proper course of treatment. Blood acetaminophen concentrations of 200 mg l^{-1} (or higher) at 4 h postingestion indicate severe risk of hepatic failure and are treated with standard NAC treatment regimen. NAC is a glutathione substitute and prevents hepatic damage by quenching the reactive NAPQI. An oral loading dose of 140 mg kg^{-1} (as a 5% solution in soft drink or juice) is followed by 70 mg kg^{-1} given orally as a (5% solution in soft drink or juice) every 4 h for an additional 17 doses. An alternative intravenous dosing protocol (20 h regimen) for NAC (Acetadote) can also be used in patients where oral NAC administration is not possible. A loading dose of 150 mg kg^{-1} NAC (in 200 ml of 5% dextrose in water) is administered over 15 min, followed by 50 mg kg^{-1} NAC (in 500 ml of 5% dextrose) over the next 4 h. A final dose of 100 mg kg^{-1} NAC is administered in 1000 ml of 5% dextrose over a 16 h period. A longer 72 h treatment regimen with intravenous NAC is recommended in the United States.

Basic and advanced life-support measures should be utilized as required by the condition of the patient.

Environmental Fate

Acetaminophen was found to be inherently biodegradable and has no bioaccumulation potential. No other information about the environmental fate of acetaminophen is currently available.

Ecotoxicology

The acute toxicity of acetaminophen has been examined in several aquatic species. LC_{50} value in brine shrimp (*Artemia salina*) examining mortality was reported to be $3820\,\mu mol\,l^{-1}$. EC_{50} for immobility over a 24 h experiment using water flea (*Daphnia magna*) was $367\,\mu mol\,l^{-1}$. Acetaminophen is classified as not toxic or only slightly to moderately toxic to all fish (fathead minnow, *Pimephales promelas*) and zooplankton species tested. The crustacean fairy shrimp (*Streptocephalus proboscideus*) appears to be highly sensitive to acetaminophen (average LC_{50} of $196\,\mu g\,l^{-1}$).

Other Hazards

Acetaminophen is stable under ordinary conditions of use and storage. In the presence of heat and water, acetaminophen will hydrolyze into acetic acid and *p*-aminophenol. Incineration may produce carbon monoxide, carbon dioxide, nitrogen oxides.

Flammability: As with most organic solids, fire is possible at elevated temperatures or by contact with an ignition source.

Explosivity: Fine dust dispersed in air in sufficient concentrations and in the presence of an ignition source, is a potential dust explosion hazard. Minimum concentration for explosion is $0.25\,oz.\,ft^{-3}$. The recommended fire extinguishing media is water spray, dry chemical, alcohol foam, or carbon dioxide. Acetaminophen is capable of generating a static electrical charge. Processes involving dumping of acetaminophen into flammable liquid, inert atmosphere in the vessels, or temperatures of flammable liquid should be maintained below its flashpoint.

Exposure Standards and Guidelines

Therapeutic exposure: Total daily dose of acetaminophen should not exceed 4 g. Dosages of acetaminophen over $4–8\,g\,day^{-1}$ over long periods of time may be associated with higher risk of liver toxicity. Acetaminophen should not be administered for more than 10 days or to young children except upon advice of physician.

Occupational exposure: Mallinckrodt recommends an airborne exposure limit of $5\,mg\,m^{-3}$.

Miscellaneous

A special mention of interaction of acetaminophen with alcohol consumption is warranted. Large numbers of reports in scientific literature and public media suggest that a potentially high risk of liver toxicity due to acetaminophen exists when consumed following alcohol intake. In a recent review, however, Dr. Barry Rumack suggests that only chronic heavy drinkers may be at greater risk following an overdose of acetaminophen and that no potentiation of toxicity occurs at therapeutic doses.

See also: Phenazopyridine.

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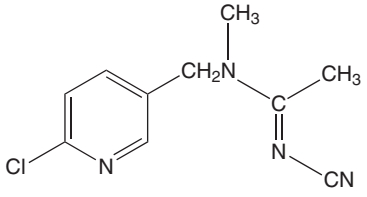
Relevant Websites

http://bulkpharm.mallinckrodt.com – Acetaminophen Material Safety Data Sheet, Mallinckrodt.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Acetaminophen.

Acetamiprid

David Wallace

- Chemical Abstracts Service Registry Number: CAS 135410-20-7
- Synonyms: Mospilan; Assail
- Chemical/Pharmaceutical/Other Class: Neonicotinoid (pyridylmethylamine) insecticide
- Chemical Formula: $C_{10}H_{11}ClN_4$
- Chemical Structure:



Uses

Acetamiprid is used as an insecticide to control sucking-type insects on leafy vegetables and fruits. In many instances, these insects may be resistant to the effects of organophosphorus and other conventional insecticides.

Exposure Routes and Pathways

The primary route of exposure is via diet (food and water). Occupational exposure for individuals who work with this insecticide can occur via dermal contact or inhalation.

Toxicokinetics

Acetamiprid is rapidly and extensively metabolized. Metabolites in urine account for 79–86% of the administered dose. Only 3–7% of acetamiprid is collected unchanged in the urine and feces. Demethylation by phase I biotransformation is the major pathway, with 6-chloronicotinic acid being the major metabolite. Compounds can then undergo phase II transformation with glycine conjugation representing the major pathway.

Mechanism of Toxicity

The primary mechanism of acetamiprid toxicity against insects is due to its action at nicotinic cholinergic receptors. The unique nature of the neonicotinoids as insecticides is that the negatively charged cyano (or nitro) group will specifically interact with a cationic binding region that is unique to insects. This action will convey selectivity of action against insects and leave mammalian nicotinic receptors relatively unaffected.

Acute and Short-Term Toxicity (or Exposure)

There is little evidence for acetamiprid toxicity in vertebrates. There is some evidence for contact exposure, dermal irritation, and stomach poisoning following oral ingestion.

Animal

Acute studies in laboratory animals, mainly rats, have demonstrated relatively low toxicity potential for acetamiprid. Oral ingestion appears to elicit the most severe toxicological responses. At dosages in excess of $140\,mg\,kg^{-1}$, acetamiprid elicited neurotoxic signs, with animals exhibiting disorders of movement and posture. Surviving animals were free of signs on the following day. Acetamiprid was only slightly toxic following inhalation (LC_{50} $>1.15\,mg\,l^{-1}$) and weakly toxic following dermal

administration (LD_{50} $>2000\,mg\,kg^{-1}$). There was minimal or no irritation of eyes or skin. Some metabolites of acetamiprid exhibited greater toxicity than the parent compound.

Human

No evidence is available for assessing human outcomes following acute exposure to acetamiprid. Signs associated with acute exposure and limits of exposure have been established using data from animal studies. Due to the selectivity of acetamiprid for insect nicotinic cholinergic receptors, little human toxicity is expected.

Chronic Toxicity (or Exposure)

Examination of chronic exposure to acetamiprid has utilized animals and little data on chronic human exposure is available. The selectivity of acetamiprid for insect nicotinic receptors would suggest minimal toxicity following chronic exposure.

Animal

Chronic dietary administration of acetamiprid resulted in reduced body and organ weight. Higher doses resulted in neurological dysfunction. There was evidence for teratogenic potential in animal studies.

Human

No evidence is available for assessing human outcomes following chronic exposure to acetamiprid. Symptoms associated with chronic exposure and limits of exposure have been established using data from animal studies. Due to the selectivity of acetamiprid for insect nicotinic cholinergic receptors, little human toxicity is expected however.

In Vitro Toxicity Data

There were no positive results in genotoxicity studies using bacterial or mammalian cell assays.

Clinical Management

There are no guidelines for acetamiprid toxicity outside of symptomatic control.

Environmental Fate

Acetamiprid exhibits a very short half-life in soil. It is rapidly degraded by aerobic metabolism. Acetamiprid is stable to hydrolysis at environmental temperatures and it photodegrades slowly in water. It is transformed moderately rapidly in aerobic aquatic environments, but only slowly in anaerobic aquatic systems. There appears to be minimal effects on drinking water and due to the rapid breakdown has not demonstrated the ability to bioaccumulate in wildlife. Due to the rapid breakdown of acetamiprid it is not expected to be persistent in the environment. Metabolites of acetamiprid will pose a greater risk to the environment, but additional work is needed to determine the fate and toxicity of acetamiprid metabolites.

Ecotoxicology

Due to the rapid breakdown of acetamiprid, there is minimal risk to fish or wildlife. Proper labeling could alleviate any additional risk. Specificity for insects significantly reduces any additional toxicity to nontarget organisms.

Exposure Standards and Guidelines

The Environmental Protection Agency has established guidelines for toxicological dose and endpoints for acetamiprid. Using the no-observed-adverse-effect level (NOAEL) and uncertainty factor (UF), the reference dose (RfD) can be calculated. For acute dietary ingestion for infants and children, $NOAEL = 10\,mg\,kg^{-1}$; $RfD = 0.10\,mg\,kg^{-1}\,day^{-1}$. Chronic dietary exposure for all populations, $NOAEL = 7.1\,mg\,kg^{-1}$; $RfD = 0.07\,mg\,kg^{-1}\,day^{-1}$. Short- and intermediate-term incidental exposure to infants and children, $NOAEL = 15\,mg\,kg^{-1}\,day^{-1}$ and for adults $NOAEL = 17.9\,mg\,kg^{-1}\,day^{-1}$. Long-term dermal exposure $NOAEL = 7.1\,mg\,kg^{-1}\,day^{-1}$ with dermal absorption of 30%. Short- and intermediate-term inhalation exposure $NOAEL = 17.9\,mg\,kg^{-1}\,day^{-1}$ and for long-term inhalation exposure, $NOAEL = 7.1\,mg\,kg^{-1}\,day^{-1}$.

See also: Imidacloprid; Neonicotinoids; Nithiazine; Pesticides.

Further Reading

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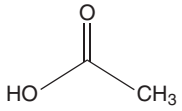
http://www.epa.gov – United States Environmental Protection Agency Federal Register of Environmental Documents; see also United States Environmental Protection Agency: Pesticide Fact Sheet.

Acetic Acid

Sanjay Chanda

- Chemical Abstracts Service Registry Number: CAS 64-19-7
- Synonyms: Glacial acetic acid; Acido acetico; Vinegar acid; Methanecarboxylic acid; Ethanoic acid; Pyroligneous acid
- Chemical/Pharmaceutical/Other Class: Pharmaceutical aid (acidifier)
- Chemical Structure:



Uses

Acetic acid is used in the manufacture of various acetates, acetyl compounds, cellulose acetate, acetate rayons, plastics, and rubber. It is also used in tanning, as laundry sour, in printing calico, and in dyeing silk. It is an acidulant and preservative in food. It is a solvent for gums, resins, volatile oils, and many other substances. Acetic acid is widely used in commercial organic synthesis.

Background Information

Acetic acid is present throughout nature as a normal metabolite of both plants and animals. Acetic acid may also be released to the environment in a variety of waste effluents, in emissions from combustion processes, and in exhaust from gasoline and diesel engines.

Exposure Routes and Pathways

Contact with skin and ingestion are the most common exposure pathways.

Toxicokinetics

Acetic acid is absorbed from the gastrointestinal (GI) tract and through the lung. Acetic acid is readily metabolized by most tissues and may give rise to the production of ketone bodies as intermediates. *In vitro* experiments have demonstrated that acetate is incorporated into phospholipids, neutral lipids, sterols, and saturated and unsaturated fatty acids in a variety of human and animal tissue preparations.

Mechanism of Toxicity

Acetic acid causes toxicity by coagulative necrosis; that is, the acid denatures all tissue protein to form an acid proteinate. As a result, both structural and enzymatic proteins are denatured and cell lysis is blocked. Therefore, cell morphology is not greatly interrupted. In addition, an ester is formed which delays further corrosive damage and helps reduce systemic absorption. Thus, damage, especially with small quantities of acid, is frequently limited to local sites of injury to the skin or the GI tract rather than the systemic response.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acetic acid is corrosive to skin and gastric mucosa. Repetitive exposure to acetic acid may cause erosion of dental enamel, bronchitis, and eye irritation. Bronchopneumonia and pulmonary edema may develop following acute overexposure. LC_{50} in guinea pig and mouse by inhalation is 5000 ppm h^{-1} and LD_{50} in rat by oral route is 3.53 g kg^{-1}.

Human

Acetic acid is corrosive to skin and gastric mucosa. Repetitive exposure to acetic acid may cause erosion of dental enamel, bronchitis, and eye irritation. Bronchopneumonia and pulmonary edema may develop following acute overexposure.

Chronic Toxicity (or Exposure)

Human

Chronic exposure may result in pharyngitis and catarrhal bronchitis. Ingestion, though not likely to

BLANK

Acetone

Lee R Shugart

- Chemical Abstracts Service Registry Number: CAS 67-64-1
- Synonyms: Dimethyl ketone; 2-Propanone; Dimethylketal
- Chemical/Pharmaceutical/Other Class: Ketone
- Chemical Formula: $(CH_3)_2CO$

Uses

Acetone is obtained by fermentation or chemical synthesis and is used to make plastic, fibers, drugs, and other chemicals. It is also used to dissolve fats, oils, waxes, resins, rubber, plastics, lacquers, varnishes, and rubber cements. In the laboratory, it is used to extract various substances from animal and plant tissues and as a dehydrating agent.

Background Information

Acetone occurs naturally in plants, trees, volcanic gases, forest fires, and as a product of the breakdown of body fat. It is present in vehicle exhaust, tobacco smoke, and landfill sites. Industrial processes contribute more acetone to the environment than natural processes.

Exposure Routes and Pathways

Exposure to acetone results mostly from breathing air, drinking water, or coming in contact with products or soil that contains acetone. Significant numbers of workers are potentially exposed to acetone. The general population may be exposed through the use of products such as paints, adhesives, cosmetics, and rubber cement.

Toxicokinetics

Acetone that enters the blood is carried to all organs in the body. Small amounts are enzymatically converted to nonharmful substances in the liver.

Mechanism of Toxicity

Acetone acts mainly as an irritant affecting the eyes, nose, throat, and respiratory tract.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral rat LD_{50}: 5800 mg kg^{-1}; inhalation rat LC_{50}: 50 100 mg m^{-3}; irritation eye rabbit, standard draize, 20 mg severe.

Human

Breathing moderate levels of acetone for short periods of time can cause headaches, light-headedness, and confusion with an increase in pulse rate. Vomiting, unconsciousness, and possibly coma can accompany high levels of exposure. Swallowing very high levels of acetone can result in unconsciousness and damage to the skin in the mouth.

Symptoms following acute acetone ingestion include nausea, vomiting, gastric hemorrhage, sedation, respiratory depression, ataxia, and paresthesia. Depression resembles alcoholic stupor, but its onset is quicker than that with ethanol. Coughing and bronchial irritation may be the only clues to ingestion of quantities that are too small to produce sedation. Hyperglycemia and ketonemia with acidosis that resembles acute diabetic coma may be present.

Chronic Toxicity (or Exposure)

Animal

Kidney, liver, and nerve damage with an increase in birth defects and lowered ability to reproduce (males only) were noted in animals exposed for long periods of time.

Human

It is not know if humans would experience the same effects observed in animals. The relevance to humans of the liver, reproductive, and developmental effects observed in animal studies is not known, and these endpoints have not been sufficiently examined in humans. Not considered genotoxic or mutagenic. Acetone has not been classified as a carcinogen and studies of workers exposed to acetone found no significant risk of death from cancer. Prolonged or repeated skin contact may produce severe dermatitis.

In Vitro Toxicity Data

Acetone (reagent grade) was evaluated by the standard plate incorporation method in the Ames *Salmonella* reverse mutation assay with strains TA98, TA100, TA1535, TA1537, and TA1538.

Experiments were done in triplicate with and without metabolic activation (S9 fractions from Aroclor-treated Sprague–Dawley rats). Results were negative in these strains.

Clinical Management

If inhaled and breathing is difficult, the person is moved to fresh air and administered oxygen. For skin contact, the area is washed with water. For eye contact, water is used for flushing.

Environmental Fate

Acetone is highly volatile and enters the environment mainly via the atmosphere where it may be moderately degraded by photolysis, react with photochemically produced hydroxyl radicals or be removed by wet deposition. Acetone may be biodegraded when released into the soil, but because it is miscible in water, it may leach into existing groundwater but is not expected to significantly bioaccumulate.

Ecotoxicology

Acetone is not expected to be toxic to aquatic life. The LC_{50}/96 h values for fish are over 100 mg l^{-1}.

Other Hazards

Acetone is a volatile and an extremely flammable liquid. Vapor may cause flash fire.

Exposure Standards and Guideline

Occupational Safety and Health Administration Permissible Exposure Limit: 1000 ppm (time-weighted average).

See also: Pollution, Air; Skin.

Further Reading

Arts JH (2002) An analysis of human response to the irritancy of acetone vapors. *Critical Reviews in Toxicology* 32(1): 43–66.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Acetone.
http://www.inchem.org – Acetone: Environmental Health Criteria (from the International Program on Chemical Safety).

Acetonitrile

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 75-05-8
- Synonyms: Cyanomethane; Ethane nitrile; Ethanenitrile; Ethyl nitrile; Methanecarbonitrile; Methyl cyanide
- Chemical/Pharmaceutical/Other Class: Organic solvent; Cyanogen; Nitrile
- Chemical Formula: C_2H_3N

Uses

Acetonitrile is used in the chemical industry as an intermediary in the synthesis of several chemicals and products such as acetophene, thiamine, acetamidine, and α-naphthaleneacetic acid, nitrogen containing compounds, acrylic fibers, nitrile rubber, pesticides, pharmaceuticals, perfumes, and lithium batteries. It is also used as a polar solvent for both organic and inorganic compounds and in non-aqueous titrations.

Exposure Routes and Pathways

Exposure to acetonitrile can occur through the oral, dermal, and inhalation routes. Symptoms of poisoning have been observed in persons exposed through these three routes.

Toxicokinetics

Acetonitrile can be acutely lethal when absorbed in high doses. Acetonitrile is metabolized to a hydroxyl metabolite by cytochrome P450 in the liver. Subsequent metabolism through catalase enzymes produces hydrogen cyanide. Once metabolized, the mechanism of action is the same as expected for

cyanide poisoning. Onset of cyanide poisoning may be delayed 8 or more hours as metabolism is required to produce the cyanide metabolite. Toxicity may be prolonged for up to 3 days in some cases.

Mechanism of Toxicity

Acetonitrile is slowly metabolized by cytochrome P450 in the liver to produce hydrogen cyanide. Toxicity is produced by the combined effect of circulating acetonitrile and cyanide. Cyanide exerts its toxicological effects by disrupting oxygen utilization at the cellular level. The disruption results in decreased oxygen utilization by body tissues and lactic acidosis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal susceptibility to acetonitrile varies by animal species and route of administration. Overall, animal susceptibility is mediated by the animal's ability to absorb and metabolize acetonitrile into its toxic metabolite, hydrogen cyanide.

Atmospheres containing up to 32 000 ppm acetonitrile are lethal to dogs. In rats, the oral LD_{50} has been measured to range from 200 mg kg^{-1} (in young rats) to 3800 mg kg^{-1} (age unspecified), whereas the inhalation LC_{50} has been determined to be 7500 ppm following an 8 h exposure. The acute dermal lethal dose has been investigated in rabbits. The LD_{50} through the dermal route has been determined to be 980 mg kg^{-1}. Subchronic exposures to low acetonitrile concentrations in the air (665 ppm or less) produced pulmonary inflammation and minor changes in body weights, hematocrit, hemoglobin, and liver and kidney functions.

Human

Toxicological effects of acetonitrile are usually delayed as the chemical has to be metabolized to cyanide. However, exposure to high doses may result in rapidly developing loss of consciousness and respiratory failure.

Signs and symptoms of exposure will be determined by the dose of acetonitrile. Onset of symptoms can be expected to be delayed from 2–12 h as acetonitrile is slowly metabolized to its toxic metabolite, cyanide. Exposure to low doses will produce nausea, salivation, vomiting, headache, and lethargy. Exposure to higher doses may produce cyanide intoxication characterized by extreme weakness, lethargy, respiratory depression, metabolic acidosis, tachycardia, shock, coma, seizures, and possibly death.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to acetonitrile in air at concentrations ranging from 166 to 665 ppm for 7 h per day for up to 90 days showed no-observed-effects at doses below 330 ppm. At the maximum dose tested (665 ppm), pulmonary inflammation as well as minor kidney and liver changes were noted in some animals.

Dogs and monkeys exposed to acetonitrile in air for 91 days showed minor variations in body weight, hematocrit, and hemoglobin. The animals were dosed acetonitrile at concentrations averaging 350 ppm for 7 h day^{-1}, 3 days week^{-1}. Autopsy of the animals revealed some cerebral hemorrhaging as well as pigment-bearing macrophages in some animals.

Male and female rats were exposed to acetonitrile by inhalation at doses ranging from 0 to 400 ppm for 6 h day^{-1}, 5 days week^{-1} for 2 years. Results of the study were inconclusive regarding the carcinogenic activity of acetonitrile as there was only a marginal increased incidence of hepatocellular adenomas and carcinomas in male rats. Furthermore, there was no evidence of carcinogenic activity in the female rats even at exposures as high as 400 ppm. In a similar study using male and female mice exposed to acetonitrile at doses ranging from 0 to 200 ppm by inhalation for 6 h day^{-1}, 5 days week^{-1} for 2 years, no carcinogenic activity was noted in the animals and doses tested.

The toxicological effects of acetonitrile have been attributed to the direct effects of the intact molecule combined with the effects of metabolically generated cyanide ions.

Human

No reports were found on the chronic toxicological effects of acetonitrile in humans.

In Vitro Toxicity Data

In vitro studies using rat liver microsomes have demonstrated that the conversion of acetonitrile to cyanide is mediated by cytochrome P450 (P-450IIE1).

Acetonitrile was tested for mutagenicity in the *Salmonella*/microsome preincubation assay. The tests were conducted using up to five *Salmonella* strains and in the presence and absence of rat or hamster liver S-9. All tests were negative for mutagenicity

including those run at the maximum dose tested (10 mg per plate).

Clinical Management

The major goal of treatment is to maintain respiration, blood circulation, and vital signs and to prevent further absorption of acetonitrile into the systemic circulation. If ingested, absorption can be prevented or minimized by instituting gastric lavage or by giving activated charcoal and a cathartic. Gastric lavage is effective only if performed soon after ingestion.

Treatment of acetonitrile poisoning is similar to that of cyanide poisoning. This includes immediate therapy with 100% oxygen and assisted ventilation, if necessary. Seizures can be controlled by giving diazepam, phenobarbital, or phenytoin intravenously at appropriate doses. Therapy should also include correction of the metabolic acidosis and to combat cyanide poisoning. Cyanide poisoning is treated by the intravenous administration of sodium nitrite and sodium thiosulfate. Care should be taken to maintain treatment for as long as acetonitrile is being metabolized to cyanide.

Environmental Fate

If released to ambient air, acetonitrile will remain in the vapor phase where it will be degraded through reaction with photochemically produced hydroxyl radicals. The half-life of acetonitrile in ambient air has been estimated to be $\sim$620 days. If released to soil, acetonitrile is expected to volatilize rapidly. Biodegradation in soil is not expected to be a major degradation pathway. If released to water, acetonitrile is not likely to adsorb to soil and sediment particles. Acetonitrile is expected to be removed from water bodies through volatilization as the chemical hydrolysis and bioaccumulation potential for this chemical are low.

Ecotoxicology

Toxicity thresholds for protozoa, bacteria, and green algae have been measured to range from 520 $mg\,l^{-1}$ for *Microcystis aeruginosa* (algae) to 7300 $mg\,l^{-1}$ for *Scenedesmus quadricauda* (green algae).

The LC_{50} for acetonitrile in fathead minnow (*Pimephales promelas*) has been measured to be $\sim$1640 $mg\,l^{-1}$ per 96 h in a flowthrough bioassay.

Other Hazards

Acetonitrile is highly flammable and will ignite in the presence of flames, sparks, or sufficient heat. Acetonitrile vapors may combine with air to form explosive mixtures.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration permissible exposure limit = 40 ppm (70 $mg\,m^{-3}$).
- American Conference of Governmental Industrial Hygienists (ACGIH) 8 h time-weighted average = 40 ppm.
- ACGIH short-term exposure limit = 60 ppm.
- National Institute for Occupational Safety and Health immediately dangerous to life or health value = 500 ppm.
- The US Environmental Protection Agency's Integrated Risk Information System has published a reference concentration for acetonitrile of 0.06 $mg\,m^{-3}$.
- Florida State Drinking Water Standard = 500 $\mu g\,l^{-1}$.

See also: American Conference of Governmental Industrial Hygienists; Cyanide; National Institute for Occupational Safety and Health; Occupational Safety and Health Act, US.

Further Reading

Ellenhorn MJ and Barceloux DG (eds.) (1988) *Medical Toxicology, Diagnosis and Treatment of Human Poisoning*. New York: Elsevier.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Acetonitrile.

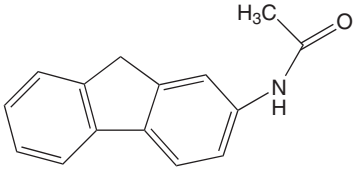
http://www.epa.gov – Chemical Summary for Acetonitrile (from the US Environmental Protection Agency).

http://www.osha-slc.gov – Safety and Health Topics: Acetonitrile (from the US Occupational Safety and Health Administration).

Acetylaminofluorene

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 28322-02-3
- Synonyms: *N*-Fluorene-4-yl-acetamide; *N*-4-Fluorenylacetamide; *N*-Fluoren-4-yl-acetamide; 4-Acetylaminofluorene; 2-Acetylaminofluorene
- Chemical/Pharmaceutical/Other Class: Aromatic amine
- Chemical Structure:



Uses

Acetylaminoflourene is found as a contaminant in coal gasification processes. It has no known use.

Background Information

2-Acetylaminofluorene is used frequently by biochemists and technicians. These persons may be exposed to acetylaminofluorene. The occupations at greatest risk to acetylaminofluorene exposure are organic chemists, chemical stockroom workers, and biomedical researchers. Although neither National Institute of Occupational Safety and Health (NIOSH) nor Occupational Safety and Health Administration (OSHA) has estimated the number of US workers exposed to acetylaminofluorene, perhaps fewer than 1000 workers in 200 laboratories may come in contact with this.

Exposure Routes and Pathways

Skin contact is the most common accidental exposure pathway. Acetylaminoflourene emits toxic fumes of nitrous oxides when heated to decompose and can be toxic when inhaled.

Toxicokinetics

Acetylaminofluorene is biotransformed in the liver. 2-Acetylaminofluorene can stimulate cytochrome P450 1A1 isozyme (CYP1A1) activity, inducing both CYP1A1 and CYP1A2 proteins, whereas 4-acetylaminofluorene modestly increases CYP1A2 but does not influence CYP1A1.

Mechanism of Toxicity

4-Acetylaminofluorene is not carcinogenic; 2-acetylaminofluorene is carcinogenic. 2-Acetylaminofluorene can be metabolized to form *N*-hydroxyacetylaminofluorene and 2-aminofluorene, which may covalently bind to the DNA and macromolecules. Ring hydroxylation, however, leads to the formation and excretion of water-soluble conjugates (e.g., glucuronides) of the respective hydroxylated metabolites and detoxification.

Acute and Short-Term Toxicity (or Exposure)

Animal

A single dose of acetylaminofluorene at 0.1 mg kg^{-1} when injected into mice (DD strain) on gestation days 8–15 produced mainly skeletal defects, cleft lips, cleft palates, and cerebral hernias. Fisher 344 rats were fed 0.06% acetylaminofluorene in diet for 4 weeks and then on control diet for 1 week. This schedule was carried out for three cycles (12 weeks). A smaller group was also treated for only one cycle. Rats treated for three cycles showed high incidence of liver, testis, and zymbal gland tumors. No tumors observed in rats treated for one cycle. The LD_{50} of 4-acetylaminofluorene in mice is 364 mg kg^{-1} by the intraperitoneal route. When fed to male rats (0.05% of diet) for 3 or 4 weeks, 4-acetylaminofluorene caused proliferation of agranular endoplasmic reticulum and glycogen depletion in hepatocytes. The same treatment when continued for 10 months produces conspicuous morphological alterations in pancreatic granular endoplasmic reticulum together with mitochondrial damage and focal cytoplasmic degradation.

Human

Human exposure data are not available. 2-Acetylaminofluorene is thought to be carcinogenic to humans.

Chronic Toxicity (or Exposure)

Animal

Different species respond differently to chronic administration of 2-fluoroacetylamine. Guinea pigs and

monkeys fail to develop tumors after treatment. Bladder and liver tumors have been induced in dogs. Liver tumors (but not bladder tumors) have been induced in chickens, fish, cats, and hamsters. Bladder tumors (not liver tumors) have been induced in rabbits. There were also variations in different colonies of inbred animals.

Human

Human exposure data are not available. 2-Acetylaminofluorene is thought to be carcinogenic to humans.

In Vitro Toxicity Data

The mutagenicity of *N*-2-fluorenylacetamide (NFA) was evaluated in *Salmonella* tester strains TA98, TA100, TA1535, TA1537, and TA1538 (Ames test), both in the presence and absence of added metabolic activation by aroclor-induced rat liver S9 fraction. Based on the results of preliminary bacterial toxicity determinations, NFA, diluted with dimethyl sulfoxide (DMSO), was tested for mutagenicity at concentrations up to 1 mg per plate using the plate incorporation assay. NFA caused a positive response in strains TA1535, TA100, and TA98 following metabolic activation.

Clinical Management

In case of contact with the eyes, the eyes should be immediately flushed with running water for at least 15 min. Affected skin should be washed with soap and water. If vapor is inhaled, the victim should be moved to fresh air and emergency medical care provided. If the victim is not breathing, artificial respiration should be provided; if breathing is difficult, oxygen should be administered. Contaminated clothing and shoes should be removed and isolated at the site.

Environmental Fate

Release of 2-acetylaminofluorene to the environment from artificial sources is probably not significant since less than 20 lb $year^{-1}$ of this compound are consumed in the United States. If released to soil, 2-acetylaminofluorene is expected to have low mobility. Chemical hydrolysis, oxidation, and volatilization are not expected to be significant. If released to water, 2-acetylaminofluorene may undergo direct photolysis and is expected to strongly adsorb to suspended solids and sediments. Chemical hydrolysis, oxidation, volatilization, and bioaccumulation are not expected to be significant. If released to the atmosphere, 2-acetylaminofluorene may undergo vapor phase adsorption to airborne particulate matter, it may react with photochemically generated hydroxyl radicals (estimated vapor phase half-life = 5.92 h) or it may undergo direct photolysis.

Ecotoxicology

No aquatic toxicology data available.

Other Hazards

Acetylaminofluorene can be easily ignited by heat, sparks, or flames. Vapors may form explosive mixtures with air. Vapors may travel to source of ignition and flash back. Most vapors are heavier than air. They can spread along ground and collect in low or confined areas (sewers, basements, tanks). Vapor explosion hazard exists indoors, outdoors, or in sewers.

Exposure Standards and Guidelines

As per OSHA, workers' exposure to 2-acetylaminofluorene is to be controlled through the required use of engineering controls, work practices, and personal protective equipment, including respirators. Identified as an occupational carcinogen without establishing a permissible exposure limit. NIOSH considers 2-acetylaminofluorene to be a potential occupational carcinogen. NIOSH usually recommends that occupational exposures to carcinogens be limited to the lowest feasible concentration.

See also: Carcinogenesis; Cytochrome P-450.

Further Reading

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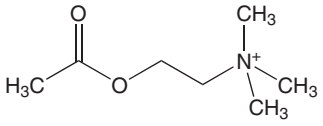
Shepard TH (1980) *Catalog of Teratogenic Agents*, 3rd edn., p. 148. Baltimore, MD: Johns Hopkins University Press.

Sigala F, Kostopanagiotou G, Andreadou I, *et al.* (2004) Histological and lipid peroxidation changes after administration of 2-acetylaminofluorene in a rat liver injury model following selective periportal and pericentral damage. *Toxicology* 196: 155–163.

Acetylcholine

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 51-84-3
- Synonyms: Acecoline; Choline acetate; Arterocholine; 2-(Acetoxy)-*N*,*N*,*N*-trimethylethanaminium; Ethanaminium; 2-(Acetyloxy)-*N*,*N*,*N*-trimethyl
- Chemical/Pharmaceutical/Other Class: Neurohumoral transmitter
- Chemical Structure:



Uses

Acetylcholine is present naturally in the body. Commercial drugs used as cholinergic agonists mimic the action of acetylcholine.

Background Information

Acetylcholine is the endogenous neurotransmitter at cholinergic synapses and neuroeffector junctions in the central and peripheral nervous systems. The actions of acetylcholine are mediated through nicotinic and muscarinic cholinergic receptors, which transduce signals via distinct mechanisms.

Exposure Routes and Pathways

Acetylcholine is present in the body as a neurohumoral transmitter. Acetylcholinesterase is the enzyme responsible for breakdown of acetylcholine in the synapse. Any drug classified as cholinergic agonist (which mimics the action of acetylcholine) or anticholinesterase agent (e.g., organophosphorus pesticides, which block the action of acetylcholinesterase and hence stop the breakdown of acetylcholine in the synapse) can increase the level of acetylcholine in the body. The most common exposure pathways for the cholinergic agonists are ingestion or contact to the eye. Acetylcholine chloride is available as an intraocular solution, methacholine chloride is available as a powder, bethanechol chloride is available as tablets, and carbachol is available as an ophthalmic solution. Common exposure pathways to anticholinesterase agents are ingestion, dermal or ocular contact, or inhalation.

Toxicokinetics

Acetylcholine is broken down by the acetylcholinesterase enzyme to choline and acetate. The time required for hydrolysis of acetylcholine is less than a millisecond. If the enzyme is depleted or inhibited, then excessive acetylcholine accumulation in the body can cause toxicity. Symptoms are salivation, lacrimation, urination, diarrhea, muscle tremor, and fasciculation.

Mechanism of Toxicity

Cholinergic agents can increase the acetylcholine level at the synaptic junction and cause rapid firing of the postsynaptic membrane. Anti-acetylcholinesterase agents block the acetylcholinesterase enzyme and thus increase the acetylcholine level in the synapse causing rapid firing of the postsynaptic membrane.

Acute and Short-Term Toxicity (or Exposure)

Animal

The clinical signs of excess acetylcholine at nerve endings mimic hyperactivity of the parasympathetic nervous system. Signs relative to the alimentary tract include excess salivation, lacrimation, abdominal pain, vomiting, intestinal hypermotility, and diarrhea. The muscarinic effects of acetylcholine cause bronchoconstriction and an increase in bronchial secretions. The nicotinic effects of acetylcholine consist of involuntary irregular, violent muscle contractions and weakness of voluntary muscles. Death occurs as a result of respiratory failure.

Human

Acetycholine agents are contraindicated in persons with asthma, hyperthyroidism, coronary insufficiency, and peptic ulcer. The bronchoconstrictor effect may precipitate asthma, hyperthyroid patients may develop atrial fibrillation, hypotension induced by these agents can reduce coronary blood flow, and gastric acid secretion caused by these agents can aggravate the symptoms of peptic ulcer. Excessive acetylcholine can also cause flushing, sweating, bradycardia, hypotension, abdominal cramps, belching, diarrhea, sensation of tightness in the urinary

bladder, involuntary defecation and urination, penile erection, difficulty in visual accommodation, headache, salivation, and lacrimation. It can also cause paralysis of the respiratory muscles. Central nervous system effects include ataxia, confusion, slurred speech, loss of reflexes, Cheyne–Stokes respiration, and finally coma. The time of death after a single acute exposure ranges from 5 min to 24 h depending on the route, dose, and agent of exposure (among other factors).

Chronic Toxicity (or Exposure)

Animal

Animals may lose weight due to the inability to feed and drink because of muscular weakness. Clinical signs in birds include goose stepping, ataxia, wing spasms, wing droop, dyspnea (difficulty in breathing), tenesmus (spasm of anal sphincter), diarrhea, salivation, lacrimation, ptosis (drooping) of the eyelids, and wing-beat convulsions. Susceptibility to organophosphate toxicity varies greatly among individuals of any species and can be increased by frequent repeated mild exposure, which results in greater susceptibility due to exhaustion of the body's store of cholinesterase. No definite postmortem changes are seen and when present are usually secondary to the symptoms and include pulmonary edema, asphyxia, gastroenteritis, and rarely kidney and liver degeneration.

Human

Chronic toxicity can cause polyneuritis, which starts with mild sensory disturbances, ataxia, weakness, and ready fatigability of legs, accompanied by fasciculation, muscle twitching, and tenderness to palpitation. In severe cases, the weakness may progress eventually to complete flaccid paralysis that, over the course of weeks or months, is often succeeded by a spastic paralysis with a concomitant exaggeration of reflexes. During these phases, muscles show marked wasting.

Clinical Management

Exposure should be terminated as soon as possible either by removal of the patient or by fitting the patient with a gas mask if the atmosphere remains contaminated. Contaminated clothing should be removed immediately; the skin and mouth should be washed with copious amounts of water. Gastric lavage should be conducted if necessary. Artificial respiration should be administered if required, and administration of oxygen may be necessary. If the convulsion persists, diazepam (5–10 mg intravenously) or sodium thiopental (2.5% intravenously) should be administered, and the patient should be treated for shock. Atropine should be administered in sufficiently large doses, but atropine is without any effect against peripheral neuromuscular activation and subsequent paralysis. Pralidoxime (1 or 2 g infused intravenously) should be administered for all the peripheral effects.

See also: A-Esterases; Anticholinergics; Cholinesterase Inhibition; Neurotoxicity; Organophosphates; Respiratory Tract.

Further Reading

Brown JH and Taylor P (1996) Muscarinic receptor agonists and antagonists. In: Hardman JG and Limbird LE (eds.) *Goodman and Gillman's Pharmacological Basis of Therapeutics*, 9th edn., pp. 141–160. New York: McGraw-Hill.

Foye WO, Lemke TL, and Williams DA (1995) *Principles of Medicinal Chemistry*, 4th edn. Baltimore and London: William & Wilkins.

Acetylcholinesterase *See* Cholinesterase Inhibition.

Acetylene

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 74-86-2
- Synonyms: Acetylene; Ethine; Ethyne; Narcylen; Welding gas
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon (C_nH_{2n-2})
- Chemical Formula: C_2H_2
- Chemical Structure: H–C≡C–H

Uses

Major producers manufacture acetylene by either the partial oxidation of natural gas or as a coproduct of the thermal cracking of ethylene; minor producers manufacture acetylene from calcium carbide. About 80% of production is used as a closed system intermediate in the manufacture of acetylene black as well as acetylenic and vinyl derivatives used in a variety of applications such as the manufacture of plastics. The remaining 20% is used primarily in oxyacetylene torches for welding and metal cutting. Although acetylene was used as an anesthetic in the early 1900s, this use has fallen into disfavor due to the explosive properties of acetylene.

Exposure Routes and Pathways

Due to its physical properties as a gas, its method of production and almost exclusive use as a closed system intermediate, potential industrial exposures are limited almost exclusively to inhalation. With the exception of torches, acetylene is not used in consumer products.

Toxicokinetics

Absorption of acetylene is driven by its partial pressure in respired air. Following inhalation, acetylene rapidly enters the blood by diffusion and is distributed to body organs in approximate proportion to their rate of perfusion. Acetylene crosses the blood–brain barrier, producing central nervous system effects characteristic of asphyxia. Diffusion is reversed upon the elimination of exposure and acetylene is excreted unchanged primarily via exhalation from the lungs, with some elimination via the urine.

Mechanism of Toxicity

Acetylene is a simple asphyxiant; a physiologically inert gas that can deplete the atmosphere of available oxygen when present in high concentrations and thereby deprive the tissues of necessary oxygen. Signs of asphyxia are noted when the atmospheric oxygen concentration is reduced to 16% or less. The tissues that are most sensitive to hypoxia are the brain and heart.

Acute and Short-Term Toxicity (or Exposure)

Animal

In dog studies, acetylene in oxygen rapidly produces anesthesia at 500 000 ppm, which becomes pronounced at 750 000–900 000 ppm. Recovery is rapid with no apparent effects. Administration of acetylene in oxygen to the cat reduces respiration and increases blood pressure at levels between 400 000 and 800 000 ppm. In the rabbit, these effects on respiration and blood pressure are noted between 600 000 and 800 000 ppm. Repeated exposures of multiple species to acetylene at concentrations $\leqslant$ 800 000 ppm acetylene did not result in cellular injury to the heart, lungs, liver, kidney, or spleen.

Human

Acetylene is not toxic below its lower explosive limit of 25 000 ppm. In the presence of adequate oxygen, acetylene causes a slight intoxication at 100 000 ppm, which leads to a staggering gait and general incoordination as the concentration increases to 300 000 ppm. Unconsciousness occurs in 5–7 min at $\sim$350 000 ppm. Full anesthesia occurs at 800 000 ppm. Anesthetic concentrations of acetylene do not affect heart, liver, or kidney function but do result in an increase in blood pressure.

Chronic Toxicity (or Exposure)

Human

Despite its long use as an industrial chemical and anesthetic, there are no epidemiological data linking acetylene exposure to deleterious health effects.

In Vitro Toxicity Data

Acetylene was not mutagenic in an Ames assay conducted both in the presence and absence of metabolic activation.

Clinical Management

The exposed individual should be removed from the toxic environment and given 100% humidified supplemental oxygen with assisted ventilation as required. If hypoxia has been prolonged, the patient should be evaluated for neurologic sequelae and supportive treatment provided. Dermal exposure to liquid acetylene should be treated as indicated for frostbite injury.

Environmental Fate

Acetylene is a gas that will partition almost exclusively to the air where it is degraded by reaction with hydroxyl radicals (13 day half-life). Although soluble in water (1230 mg l^{-1} at 25°C), acetylene is not

expected to accumulate in this medium due to its rapid volatilization to air.

Ecotoxicology

Due to its partitioning to air, exposure of aquatic receptors to acetylene does not pose a significant environmental risk and few studies have been conducted in this area. Limited experimental and modeling data indicate that the range of LC_{50} and EC_{50} values for acetylene in fish, aquatic invertebrates, and algae is 200–500 mg l^{-1}.

Other Hazards

Acetylene is a reactive material that poses a fire and explosion hazard. Its lower and upper explosive limits in air are 2.5% and 93%, respectively. Acetylene reacts with active metals (e.g., copper, silver, and mercury) to form explosive acetylide compounds. Acetylene manufactured from calcium carbide can contain impurities such as phosphine and arsine that are responsible for the ethereal to garlic-like odor of commercial acetylene and pose a greater human health risk than acetylene alone. Maintaining acetylene levels below 3000 ppm can minimize these secondary toxic effects.

Exposure Standards and Guidelines

Acetylene is recognized internationally as a simple asphyxiant. The American Conference of Governmental Industrial Hygienists recommends that atmospheres containing acetylene have a minimum oxygen concentration of 18%. The National Institute of Occupational Safety and Health has a recommended exposure limit for acetylene of 2500 ppm as a ceiling.

See also: Copper; Mercury; Silver; Toxicity Testing, Dermal; Toxicity Testing, Inhalation.

Further Reading

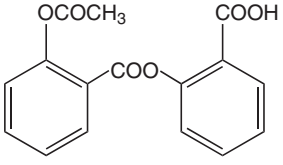
Budavari S (ed.) (1989) *The Merck Index – Encyclopedia of Chemicals, Drugs and Biologicals*, p. 14. Rahway, NJ: Merck and Co., Inc.

Acetylsalicylic Acid

Christopher P Holstege

This article is a revision of the previous print edition article by Sanjay Chanda, volume 1, pp. 21–22, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 50-78-2
- Synonyms: 2-(Acetyloxy)benzoic acid; 2-Carboxyphenyl ester; Aspirin
- Chemical/Pharmaceutical/Other Class: Salicylates
- Chemical Structure:



Uses

Aspirin is an analgesic, antipyretic, and anti-inflammatory agent.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures, but dermal and rectal exposures have also been reported.

Toxicokinetics

Absorption of aspirin occurs by passive diffusion across the gastrointestinal membrane and is influenced by gastric pH. The presence of food delays the absorption of aspirin. Aspirin-induced pylorospasm, gastric outlet obstruction, and concretions also delay the absorption. At therapeutic doses, aspirin is found within the plasma within 30 min, peak levels are obtained within 2 h, protein binding is 90%, and the volume of distribution is less than 0.2 l kg^{-1}. In overdosage, levels may not peak for over 12 h, the protein binding decreases to less than 75%, and the volume of distribution increases to over 0.3 l kg^{-1}. Aspirin exhibits Michaelis–Menton kinetics: the elimination half-life is ~15–20 min at therapeutic doses (first-order kinetics) and as long as 20 h in overdosage (zero-order kinetics). Aspirin is

metabolized primarily by the hepatic endoplasmic reticulum and mitochondria to salicyluric acid, ether glucuronide, and ester glucuronide. The metabolites are excreted in the urine. Approximately 10% of aspirin is excreted as free salicylic acid.

Mechanism of Toxicity

In salicylate toxicity, nausea, vomiting, and abdominal discomfort occur due to both local gastric irritation and stimulation of the medullary chemoreceptor trigger zone. Salicylates increase sensitivity to carbon dioxide in the medulla oblongata, thereby inducing hyperventilation, decreasing P_{CO_2}, and causing respiratory alkalosis. A compensatory increase in the renal excretion of bicarbonate leads to the loss of potassium and sodium in the urine. A metabolic acidosis may follow due to the accumulation of organic acids. As a result, aspirin may produce a mixed acid–base abnormality consisting of both respiratory alkalosis and metabolic acidosis. Salicylates uncouple oxidative phosphorylation, resulting in a failure to produce adenosine triphosphate while at the same time increasing oxygen utilization and carbon dioxide production. This results in an increase in heat production. Salicylates also interfere with glucose metabolism and gluconeogenesis. Salicylates may also profoundly decrease brain glucose concentrations despite normoglycemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may manifest toxicity to salicylates with signs and symptoms similar to those seen in humans. These may include fever, hyperpnea, seizures, respiratory alkalosis, metabolic acidosis, gastric hemorrhage, and kidney damage. Methemoglobinemia has also been seen in animals following salicylate toxicity. Activated charcoal has been used in animals. Methylene blue or ascorbic acid may be utilized for the treatment of methemoglobinemia.

Human

Nausea, vomiting, tinnitus, and hyperventilation are seen early in toxicity. As severity of toxicity increases, intractable vomiting, hyperthermia, hypotension, tachycardia, confusion, coma, seizures, pulmonary edema, acute renal failure, and death may occur. Hyperglycemia may be seen early, whereas hypoglycemia may occur later in toxicity. Acid–base disturbances such as respiratory alkalosis and/or metabolic acidosis may be noted. Signs and symptoms of salicylate toxicity may be noted as blood levels rise over $30\,mg\,dl^{-1}$.

Chronic Toxicity (or Exposure)

Animal

Daily doses of acetylsalicylic acid in cats produced toxic hepatitis, vomiting, weight loss, poor appetite in the low-dose group ($33–63\,mg\,kg^{-1}$) and anemia, gastric lesions, and death in the high-dose group ($81–130\,mg\,kg^{-1}$). High doses of aspirin given to mice on day 6 of gestation produced large incidence of lethal deformities.

Human

Chronic salicylism presents clinically in a similar fashion to the acute situation, although it is often associated with a delay in diagnosis, and a higher morbidity and mortality. Chronic salicylism is more often associated with pronounced hyperventilation, dehydration, pulmonary edema, renal failure, coma, seizures, and acidosis. Chronic salicylism can occur at serum salicylate levels as low as $15\,mg\,dl^{-1}$.

In Vitro Toxicity Data

Acetylsalicylic acid has been tested for mutagenicity using a variety of models and has not demonstrated mutagenic activity in concentrations of 0.01–50 mg per plate.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be considered in the patient with appropriate airway protection. In general, a single dose of activated charcoal should be considered in patients who have had substantial ingestions. Acetylsalicylic acid ingestions can result in substantial delays in absorption; therefore, charcoal may be given even up to 8 h postingestion and more than one dose of charcoal may be considered to prevent further drug absorption. Careful correction of fluid and electrolyte abnormalities is essential. The clinician should insure adequate urine output, but forced diuresis should be avoided. Administration of intravenous sodium bicarbonate should be considered in patients manifesting signs and symptoms of salicylate toxicity. Hemodialysis effectively increases clearance and improves fluid/electrolyte balance. This extracorporeal method of elimination should be considered in patients with acute mental status changes, renal failure, intractable acidosis, pulmonary edema, severe fluid imbalance, or acute serum salicylate levels over $100\,mg\,dl^{-1}$ or in patients with chronic salicylate overdose, who have symptoms and serum levels $>60\,mg\,dl^{-1}$.

Environmental Fate

As in humans, the environmental fate of acetylsalicylic acid is pH dependent. Above pH 5.5, acetylsalicylic acid will be the predominant form seen. Anions generally do not volatilize or undergo adsorption to the extent of their neutral counterparts. Although information is limited, it is expected that acetylsalicylic acid should biodegrade under anaerobic conditions and photodegrade in unlit soil surfaces.

See also: Ascorbic Acid; Charcoal; Salicylates.

Further Reading

Chapman BJ and Proudfoot AT (1989) Adult salicylate poisoning: Deaths and outcome in patients with high plasma salicylate concentrations. *Quarterly Journal of Medicine* 72: 699–707.

Done AK (1960) Salicylate intoxication: Significance of measurements of salicylates in blood in cases of acute ingestion. *Pediatrics* 26: 800–807.

Temple AR (1981) Acute and chronic effects of aspirin toxicity and their treatment. *Archives of Internal Medicine* 141: 364–369.

Acids

Sanjay Chanda

Uses

Acids have a wide range of uses. The specific use depends on the specific acid.

Exposure Routes and Pathways

Dermal contact, inhalation, and ingestion are the most common exposure pathways.

Toxicokinetics

The toxicokinetics depends on the specific type of acid.

Mechanism of Toxicity

Acids cause toxicity by coagulative necrosis; that is, the acid denatures all tissue protein to form an acid proteinate. As a result both structural and enzymatic proteins are denatured and cell lysis is blocked. Therefore, cell morphology is not greatly interrupted. In addition, an ester is formed which delays further corrosive damage and helps reduce systemic absorption. Thus, damage, especially with small quantities of acid, is frequently limited to local sites of injury to the skin or the gastrointestinal tract, rather than the systemic response.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acids are corrosive to skin and mucosal surfaces. Repetitive ingestion of acids may induce mucosal forestomach hyperplasia.

Human

Acids are corrosive to skin and mucosal surfaces. Repetitive ingestion of acids may induce mucosal forestomach hyperplasia.

Chronic Toxicity (or Exposure)

Animal

Acids are corrosive to skin and mucosal surfaces. Repetitive ingestion of acids may induce mucosal forestomach hyperplasia.

Human

Acids are corrosive to skin and mucosal surfaces. Repetitive ingestion of acids may induce mucosal forestomach hyperplasia.

Clinical Management

Exposure should be terminated as soon as possible by removal of the patient to fresh air. The skin, eyes, and mouth should be washed with copious amounts of water. A 15–20 min wash may be necessary to neutralize and remove all residual traces of the contaminant. Contaminated clothing and jewelry should be removed and isolated. Contact lenses should be removed from the eye to avoid prolonged contact of the acid with the area. A mild soap solution may be used for washing the skin and as an aid to neutralize the acid, but it should not be placed into the eye. No cream, ointment, or dressing should be applied to the affected area. Emesis should be avoided in case of ingestion. If a large quantity has been swallowed, then gastric lavage should be considered. Dilution with water may be effective for small quantities swallowed. Under no circumstances should carbonated beverages be used because of

large quantities of carbon dioxide gas released that distends the stomach.

Ecotoxicology

Varies depending on the type of acid.

Other Hazards

Varies depending on the type of acid.

Exposure Standards and Guidelines

Varies depending on the type of acid.

See also: Alkalies; Corrosives; Gastrointestinal System; Skin.

Further Reading

Timbrell J (2002) *Introduction to Toxicology*, 3rd edn. London: Taylor and Francis.

Aconitum Species

Christine Stork and Jeanna Marraffa

This article is a revision of the previous print edition article by Lanita B Myers, volume 1, pp. 23–24, © 1998, Elsevier Inc.

- SYNONYMS: *Aconitum napellus*; Monkshood; Wolfsbane; Helmet flower; Friar's cap; Soldier's cap; Aconite

Uses

Aconitum spp. are perennial herbs with a blackish tuberous rootstock that gives rise to several palmate or cleft leaves. Wild plants often have blue-mauve flowers. Cultivated flowers range in color from rich blue to dark purple, purple, white, or yellow. They are bilaterally symmetrical with five-membered flowers; the uppermost is shaped like a large, downward-opening hood. This feature gives the genus its name and distinguishes it from the larkspur. It grows from 1 to 6 ft high. The ripe follicles contain many seeds. *Aconitum* spp. occur naturally in the northern temperature zones of North America, Great Britain, Europe, and Asia. It usually prefers shady, moist places. Many cultivated forms and species are grown widely outdoors and in gardens.

The toxicity of any particular aconitine-containing plant varies depending on the amount of diterpenes versus the number of norditerpenes in relation to the amount of esterification of the norditerpenes. All parts of the plant contain toxic alkaloids, with the content and composition of these varying throughout the year. It is most toxic in its preflowering stage.

Exposure Routes and Pathways

The most common route of exposure is ingestion of any parts of the plant. The roots and seeds are the most toxic parts, but the whole plant is poisonous. Symptoms can occur after dermal exposure although this route is much less frequent. This is because *Aconitum* spp. can be rapidly absorbed through mucous membranes and even intact skin.

Toxicokinetics

Aconitine is rapidly absorbed after ingestion, usually within a few minutes. Absorption also occurs with dermal contact.

Mechanism of Toxicity

Aconitum spp. contain potent steroid alkaloids including aconitine, mesaconitine, and jesaconitine, the three major toxins. The main alkaloid of these plants is aconitine, a highly toxic alkaloid.

Aconitine binds to voltage-gated sodium channels, prolonging sodium current influx, slowing repolarization, and permanently activates cardiac muscle and voltage-dependent nervous tissue receptors, resulting in cardiac and neurologic toxicity. The powerful cardiac agent 'aconite' has vagal activity that causes slowing of the heart. The cardiac toxicity resembles that of cardiac glycosides with atrioventricular conduction blockade and increased ventricular automaticity inducing a variety of rapid ventricular rates, including premature ventricular contractions to ventricular fibrillation and torsades de pointes.

Acute and Short-Term Toxicity (or Exposure)

Animal

General symptoms in animals primarily include vomiting, colic, bloating, bradycardia, bradypnea, muscle weakness, paralysis, and dilated pupils. Death is usually due to cardiopulmonary failure. No specific antidote or treatment is available. The

estimated lethal dose of aconitine is 2 or 3 mg in a dog and 10 or 12 mg in a horse.

Human

Aconitine's minimal lethal dose is 3–6 mg.One gram of fresh *Aconitum napellus* may contain 2–20 mg of aconitine. Therefore, small amounts of this plant can be lethal. The effects produced by aconite poisoning are similar to that of veratrum alkaloids (veratrine) with the exception of the paresthesias being more prominent and persistent. A burning sensation and tingling of the mouth, lips, tongue, and throat occur almost instantly, within 10–20 min. This is usually followed within 2–6 h by nausea, salivation, violent emesis, generalized paresthesias, weakness, and extreme pain. Colicky diarrhea, skeletal muscle paralysis, cardiac rhythm disturbances, convulsions, and death may follow in up to 8 h. Cardiac toxicity often complicates serious aconitine poisoning with hypotension, conduction delays, and dysrythmias within 6 h. Respiratory paralysis is often the cause of death.

Chronic Toxicity (or Exposure)

Human

Chronic toxicity is not expected.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Decontamination with syrup of ipecac is essentially contraindicated due to extensive vomiting, rapid onset of symptoms, and possible respiratory paralysis. Gastric decontamination with activated charcoal may be considered for substantial recent ingestions. Fluid and electrolytes need to be frequently monitored and replaced as necessary secondary to vomiting and diarrhea.

Treatment is symptomatic and supportive after decontamination. Since toxicity is unpredictable due to alkaloid variability, observation for 2–4 h is recommended. Symptomatic patients should be hospitalized for 24 h with cardiac monitoring.

Bradycardia is usually responsive to atropine. For hypotension, intravenous fluids should be administered and if unsuccessful, vasopressor therapy should be initiated. Most arrhythmias are refractory to drug management; however, treatment should be guided by electrocardiographic changes. Sodium bicarbonate has theoretical disadvantages because of the sodium channel opening. There is no specific antidote. No specific laboratory tests are available.

See also: Atropine; Plants, Poisonous.

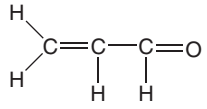
Further Reading

Agarwal B, Agarwal R, and Misra D (1977) Malignant arrhythmias induced by accidental aconite poisoning. *Indian Heart Journal* 29: 246–248.

Acrolein

James M Garrison

- Chemical Abstracts Service Registry Number: CAS 107-02-8
- Synonyms: Acrylaldehyde; Acrylic aldehyde; Allyl aldehyde; Aqualin; Ethylene aldehyde; 2-Propenal
- Chemical/Pharmaceutical/Other Class: Aldehydes
- Chemical Formula: C_3H_4O
- Chemical Structure:



Uses

Acrolein is used as an intermediate in the manufacture of glycerol, polyurethane, polyester resins, methionine, pharmaceuticals, and herbicides, and has been used in military poison gas mixtures.

Exposure Routes and Pathways

Acrolein is found in low levels in many foods. In addition, it is produced as a by-product of combustion of organic compounds, being present in smoke of all kinds, including cigarette smoke and combustion products from petrochemical fuels. Significant exposures to acrolein are most likely to occur by inhalation with potential for skin and eye contact. Ingestion is also possible.

Toxicokinetics

Following inhalation exposure, acrolein can be deposited in the nasal cavity and respiratory tract, where the majority is retained irreversibly due to its high tissue reactivity. Eventually, some traces can be absorbed into the blood and be distributed throughout the body. The uptake of acrolein in the nasal cavity is influenced by its solubility and inspiratory flow rate. Glutathione conjugation is the dominant detoxification pathway for acrolein. Further metabolism takes place in the liver resulting in glycidaldehyde and a number of metabolites that can be excreted in the urine as well as some unchanged acrolein. Most of the free acrolein is excreted in the exhaled breath.

Mechanism of Toxicity

Acrolein is soluble in the mucous membranes of the upper respiratory tract causing irritation of the sensory nerve endings. There is also depression of the mucociliary defense system. The direct action of acrolein on the skin and eyes is the result of irritation to these tissues.

Acute and Short-Term Toxicity (or Exposure)

The respiratory system is the primary target of acrolein toxicity, although dermal and ocular effects also occur. Acute exposure to acrolein results in irritation to the upper respiratory tract, skin, and eyes. Few other organ systems have been shown to exhibit significant effects from exposure.

Animal

In animal studies, the lowest oral LD_{50} for acrolein is $7\,mg\,kg^{-1}$ in rabbits. An animal inhalation exposure study at 10 ppm for 3.5 h resulted in respiratory irritation in cats. Another inhalation study on rats exposed to 8 ppm for 4 h resulted in the death of one animal while all the animals died at 16 ppm. A subchronic inhalation study with rats exposed to 4 ppm, $6\,h\,day^{-1}$, for 60 days resulted in 32/57 animal deaths due to bronchiolar necrosis and focal emphysema. The dermal LD_{50} has been reported to range from 160 to $1000\,mg\,kg^{-1}$ body weight in rabbits.

Human

Irritation to the mucous membranes occurs at as low as 0.25 ppm within 5 min and marked irritation of the eyes and nose at 1 ppm for 5 min. Fatalities have occurred at exposures to concentrations of 150 ppm for 10 min, resulting in pulmonary edema and tracheobronchitis. The lowest lethal concentration reported is 10 ppm, and the IDLH is 5 ppm. Chronic health effects, such as emphysema, can occur as a result of short-term acrolein exposure, and may last months or years.

Liquid splashes to the eye can cause corneal damage and exposures to concentrations of 0.25 ppm may cause eye irritation, lacrimation, conjunctivitis, lid edema, fibrinous or purulent discharge, and corneal injury. Splashes to the skin can result in dermal irritation, edema, and, in some cases, epidermal necrosis.

Chronic Toxicity (or Exposure)

Animal

A chronic inhalation study in rats indicates that exposure to 8 ppm acrolein for $1\,h\,day^{-1}$ for 18 months can result in emphysematous areas in the alveoli. Hamsters exposed to 4 ppm, $1\,h\,day^{-1}$, 5 days $week^{-1}$ for 12 months exhibited inflammation and epithelial metaplasia in the nasal cavity. Hamsters continued to show treatment-related effects in the nasal cavity 6 months after exposure was terminated.

There is limited evidence of acrolein carcinogenicity in animal studies, but glycidaldehyde, a potential metabolite of acrolein, is considered to be carcinogenic.

Human

Skin irritation with erythema, edema, and sensitization can occur from prolonged or repeated contact with acrolein. There is inadequate evidence in humans for chronic toxicity or carcinogenicity.

Acrolein is a weak sensitizing agent; however, the TLV – TWA of 0.1 ppm is sufficiently low to minimize irritation to most exposed individuals and a 15 min STEL of 0.3 ppm is also recommended.

In Vitro Toxicity Data

When tested in the *Salmonella* assay, acrolein was weakly positive. It was not mutagenic in the dominant lethal assay in the mouse and in the *Drosophila* sex-linked recessive lethal test, and negative for chromosome aberrations when tested in cultured Chinese hamster ovary cells; however, there was an increase in the frequency of sister-chromatid exchanges.

Clinical Management

Exposures by inhalation should be monitored for nasal and respiratory tract irritation, bronchitis, or pneumonitis. Humidified supplemental 100% oxygen should be administered. Because acrolein-induced tissue damage is slow to heal, follow-up monitoring of respiratory function may be warranted if damage to the respiratory tract is suspected.

Gastric lavage may be indicated soon after ingestion of acrolein followed by administration of activated charcoal slurry mixed with saline cathartic or sorbitol. Oxygen, in combination with intubation and mechanical ventilation, may be required in severe cases. Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. If eye irritation, pain, swelling, lacrimation, or photophobia persist, the patient should be seen in a health care facility.

Environmental Fate

Acrolein may be released to the environment from combustion processes or in effluents. Because it is a highly reactive compound, it is unstable in the environment and unlikely to persist. The half-life in air is predicted to be 15–20 h, and half-lives of dilute acrolein in water have been shown to be 1–3 days or less.

Other Hazards

Acrolein is highly reactive and is likely to polymerize violently/explosively into dimethylaniline in the presence of strong acids or bases. Care should be taken to prevent mixing with amines, sulfur dioxide, metal salts, and oxidants. In addition, acrolein is sensitive to heat, light, and air unless an inhibitor such as dimethyl sulfoxide (DMSO) is added; however, the stabilizing effects of inhibitors are usually short-lived.

Exposure Standards and Guidelines

Occupational exposure standards for acrolein include the following:

- US OSHA permissible exposure limit (PEL) time-weighted average (TWA) value of 0.1 ppm ($0.25\,mg\,m^{-3}$); short-term exposure limit (STEL) value of 0.3 ppm ($0.8\,mg\,m^{-3}$), not to exceed 15 min.
- US ACGIH threshold limit value (TLV) – TWA of 0.1 ppm; STEL of 0.3 ppm.

Miscellaneous

Acrolein is a colorless to yellowish liquid with a piercing, unpleasant odor.

See also: Combustion Toxicology; Respiratory Tract.

Further Reading

Beauchamp RO Jr., Andjelkovich DA, Kligerman AD, Morgan KT, and Heck HD (1985) A critical review of the literature on acrolein toxicity. *Critical Reviews in Toxicology* 14: 309–380.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Acrolein.
http://www.state.nj.us – New Jersey Hazardous Substances Fact Sheet.

Acrylamide

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 79-06-01
- Synonyms: Acrylic amide; Propenamide
- Chemical/Pharmaceutical/Other Class: Amide
- Chemical Formula: C_3H_5NO
- Chemical Structure: $H_2C=CH–CO–NH_2$

Uses

The primary use of acrylamide is in the production of polyacrylamide homopolymers and copolymers with nonionic, cationic, or anionic properties. Polyacrylamides are used as flocculants in wastewater treatment plants, as coagulants in the treatment of potable water, as fiber and pigment binders in the paper industry, as thickeners in soaps and personal grooming preparations, and as sizing agents in the permanent press fabric industry. Acrylamide monomer is used to

produce grouts and soil stabilizers for the construction of tunnels, dams, foundations, and roadways as well as acrylamide gels used in biotechnology laboratories.

Exposure Routes and Pathways

Exposures to acrylamide monomer are most likely to occur in the occupational setting via dermal contact with solutions of acrylamide monomer and via inhalation of the dry monomer or aerosols of acrylamide solutions. These exposures may occur during the manufacture of acrylamide and polyacrylamides, during grouting activities, and during laboratory preparation of polyacrylamide gels. The general public may be exposed to acrylamide monomer via drinking water if not removed by the water treatment process following use of polyacrylamide flocculants.

Toxicokinetics

Acrylamide is well absorbed via the gastrointestinal and respiratory tracts. It is also well absorbed through the skin but less rapidly than through the gastrointestinal tract; a significant portion of the dermally applied dose remains in the skin. Upon absorption into the blood, acrylamide is rapidly distributed throughout the body with an apparent volume of distribution equal to total body water. With the exception of plasma, erythrocytes, and testes, acrylamide and glycidamide do not exhibit preferential bioconcentration in any body tissue.

Acrylamide is rapidly metabolized to the epoxide, glycidamide, via cytochrome P450 oxidation. Both the parent and metabolite exhibit half-lives in the rat of $\sim$2 h. In rats, the conversion of acrylamide to glycidamide is saturable with 50% conversion at low doses and 13% conversion at 100 mg kg^{-1}. Both substances are detoxified by conjugation with glutathione; glycidamide is also detoxified by hydrolysis, presumably via epoxide hydrolase. While it appears that metabolism is qualitatively similar among species, quantitative differences exist depending on species and dose. Limited data indicate that the conversion of acrylamide to glycidamide is about twofold greater in the mouse than rat and that humans convert at about a twofold lower rate than the rat does.

Acrylamide is excreted primarily via the kidneys. About 60% of the administered dose appears in the urine within the first 24 h of exposure. Metabolites of acrylamide constitute the majority of the dose excreted in the urine; only $\sim$2% of the dose is excreted as the parent compound. Acrylamide and/or its metabolites are subject to enterohepatic circulation; $\sim$6% of the applied dose is eliminated in the feces. About 5% of the dose is expired as CO_2.

Mechanism of Toxicity

Although the mechanism of acrylamide toxicity is unknown, glycidamide may mediate the genotoxicity associated with acrylamide exposure. While both acrylamide and glycidamide bind to hemoglobin *in vivo*, only glycidamide forms adducts with DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Studies in several animal species indicate that acute exposures to acrylamide cause dose-related neurotoxic effects. Acrylamide has been observed to produce testicular lesions at high dose levels that also result in neurotoxicity. The oral LD_{50} for acrylamide in mice, rats, rabbits, and guinea pigs ranges between 107 and 203 mg kg^{-1}; the dermal LD_{50} in rabbits is $\sim$1150 mg kg^{-1}. Acrylamide produces equivocal responses in skin irritation tests but is irritating to the eye. Acrylamide is also a skin sensitizer. *In vivo* genotoxicity studies of somatic cells indicate that acrylamide produces clastogenic effects rather than gene mutations. In germ cells, acrylamide is both mutagenic and clastogenic and is capable of causing heritable translocations in mice.

Human

Upon chronic exposure, acrylamide produces a motor and sensory polyneuropathy in which the distal regions of the longest and largest axons appear to be preferentially affected. These effects may be manifested by weakness, paresthesias, fatigue, as well as decreased pinprick sensation and reflexes. Recovery generally occurs within a year following cessation of exposure although severe exposures may result in permanent peripheral nerve damage. Acrylamide can irritate the skin and may also cause allergic contact dermatitis.

Chronic Toxicity (or Exposure)

Animal

In a lifetime drinking water study, rats were exposed to acrylamide in drinking water at doses of 0, 0.01, 0.1, 0.5, or 2 mg kg^{-1} day^{-1}. At the two highest doses, acrylamide produced increased incidences of benign and malignant tumors in a variety of organs (e.g., thyroid and adrenal glands, testicular tunica, uterus, and mammary glands). The principal noncarcinogenic effect associated with these chronic exposures was degenerative changes in peripheral and optic nerves as well as the lateral geniculate

nucleus; peripheral nerve lesions were observed histopathologically at $2\,mg\,kg^{-1}$ but not at $0.5\,mg\,kg^{-1}$. Degenerative changes in the testes have been described at neurotoxic doses. No effects on fertility were observed in a two-generation rat reproduction study in which parental animals of each generation were exposed to $5\,mg\,kg^{-1}\,day^{-1}$ acrylamide for 10–11 weeks while in another study the fertility of male rats was impaired by exposure to $\geqslant 15\,mg\,kg^{-1}$ for 5 days. There is no evidence that acrylamide produces selective developmental toxicity in rodents as such effects were associated with maternal toxicity.

Human

Available epidemiological studies have not provided a significant link between acrylamide exposures and increases in the incidence of cancer.

In Vitro Toxicity Data

Acrylamide is not mutagenic in standard bacterial assays either in the presence or absence of metabolic activation; in contrast, glycidamide causes mutations in bacteria without addition of an exogenous metabolic system. Acrylamide is clastogenic in mammalian cells both with and without metabolic activation. These results suggest that acrylamide is a direct acting mutagen, probably causing clastogenic effects rather than gene mutations.

Clinical Management

Clinical management involves removal from exposure and treatment of symptoms.

Environmental Fate

At room temperature, acrylamide is a crystalline solid that slowly sublimes. When released into the environment, acrylamide is expected to partition almost exclusively to water (>99.9%) with only trace amounts to air (<0.1%), soil (<0.1%) and sediment (<0.05%). Acrylamide is very soluble in water ($2155\,g\,l^{-1}$ at 30 C) with little propensity to volatilize to air (Henry's law constant of $3 \times 10^{-5}\,Pa\,m^3\,mol^{-1}$). In water, acrylamide is removed primarily by biodegradation. At 15 days postapplication, 75% ($2\,mg\,l^{-1}$ acrylamide) to 100% ($0.5\,mg\,l^{-1}$ acrylamide) is degraded. In air, acrylamide will be removed by reaction with photochemically produced hydroxyl radicals (8.3 h half-life). In soil, acrylamide is biodegraded with an estimated half-life of 30 days. Based on its relatively low octanol–water partition coefficient ($\log K_{ow}$ of ~ -1.0) and measured bioconcentration factor in aquatic organisms (<1), acrylamide does not pose a significant bioaccumulation hazard.

Ecotoxicology

Acrylamide is moderately toxic to aquatic organisms. In a series of studies, acrylamide exhibited a 96 h LC_{50} value in four freshwater fish of 100–$180\,mg\,l^{-1}$, a 48 h LC_{50} (immobilization) value of $98\,mg\,l^{-1}$ in an aquatic invertebrate (*Daphnia magna*), and a 72 h EC_{50} (growth inhibition) value of $33.8\,mg\,l^{-1}$ in freshwater algae (*Selenastrum capricornutum*).

Other Hazards

Acrylamide is reactive but stable at room temperature. It can polymerize violently when heated to its melting point (84.5°C) or under ultraviolet light.

Exposure Standards and Guidelines

International occupational exposure limits (OEL) for acrylamide generally range from 0.03 to 0.3 ppm as an 8 h time-weighted average (TWA), with 0.03 ppm being the predominant value and the TWA OEL established by the American Conference of Governmental Industrial Hygienists (ACGIH). The US Occupational Safety and Health Administration lists a permissible exposure limit of 1 ppm for acrylamide. The National Institute of Occupational Safety and Health has a recommended exposure limit of 0.1 ppm as a 10 h TWA. Acrylamide is classified as *possibly carcinogenic to humans* (Group 2B) by the International Agency for Research on Cancer and as *reasonably anticipated to be a human carcinogen* by the US National Toxicology Program.

See also: Neurotoxicity; Pollution, Water; Polymers.

Further Reading

Ellenhorn MJ, Schonwald S, Ordog G, and Wasserberger J (1997) *Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning*, 2nd edn., pp. 1672–1673. Baltimore, MD: Williams and Wilkins.

LoPachin RM (2004) The changing view of acrylamide neurotoxicity. *Neurotoxicology* 25(4): 617–630.

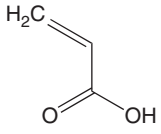
Ruden C (2004) Acrylamide and cancer risk: Expert risk assessments and the public debate. *Food and Chemical Toxicology* 42(3): 335–349.

Acrylates *See* Acrylic Acid; Ethyl Acrylate; Methyl Acrylate.

Acrylic Acid

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 79-10-7
- Synonyms: Acroleic acid; Ethylenecarboxylic acid; Propene acid; Propenoic acid; Vinylformic acid; 2-Propenoic acid; RCRA waste number U008; UN 2218 (DOT)
- Chemical/Pharmaceutical/Other Class: Copolymer
- Chemical Structure:



Uses

Acrylic acid derivatives treated with heparin are used to coat surfaces of clinical equipment. Acrylic acid is also used as a copolymer component in aerosol hair spray, in plastics, in molding powder for signs, in paint formulations, in leather finishing, in paper coatings, and in latex applications to prevent premature coagulation. It is also used in the production of hydrogels used for contact lenses.

Background Information

For more than decades, acrylic acid has served as an essential building block in the production of some of our most commonly used industrial and consumer products. Approximately two-thirds of the acrylic acid manufactured in the United States is used to produce acrylic esters – methyl acrylate, butyl acrylate, ethyl acrylate, and 2-ethylhexyl acrylate – which, when polymerized, are ingredients in paints, coatings, textiles, adhesives, plastics, and many other applications. The remaining one-third of the acrylic acid is used to produce polyacrylic acid, or cross-linked polyacrylic acid compounds, which have been successfully used in the manufacture of hygienic products, detergents, and waste water treatment chemicals.

Exposure Routes and Pathways

Inhalation, skin and eye contact, and ingestion are the most common exposure pathways. Acrylic acid is available as a colorless liquid.

Toxicokinetics

The excretion half-life of acrylic acid has been found to be 40 min. Both *in vivo* and *in vitro* studies of acrylic acid metabolism have produced strong evidence that the metabolism proceeds by a mitochondrial biochemical pathway for propionic acid metabolism that normally functions in the body at the final stages of breakdown of fatty acids and the production of intermediates for the tricarboxylic acid cycle. It is primarily excreted as carbon dioxide through the lungs. 3-Hydroxypropionate has been found to be a major metabolite. Part of acrylic acid also binds to glutathione and is excreted as the cysteine conjugate in the urine. Some part of acrylic acid can also be converted to acrylyl-CoA and reacts with glutathione to be excreted as cysteine conjugate.

Mechanism of Toxicity

Acrylic acid causes toxicity by rapid polymerization in the presence of light, heat, and oxygen and thereby interfering with the incorporation of thymidine into DNA and uracil into RNA and inhibits protein synthesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acrylic acid has been tested on mice, rats, and rabbits. The toxicity of acrylic acid in animals is similar to that found in humans.

Human

Acrylic acid is corrosive to skin. Acrylic acid vapor can cause moderate to severe skin and eye irritation.

It can also cause forestomach edema. Acute exposure can be corrosive to the skin, eyes, nose, and mucous membranes of the upper respiratory and gastrointestinal tracts. Inhalation of vapors may produce burning sensation, cough, nasal discharge, sore throat, labored breathing, headache, nausea, vomiting, confusion, dizziness, and unconsciousness.

Chronic Toxicity (or Exposure)

Animal

Animals exposed via chronic inhalation developed lethargy, weight loss, kidney abnormalities, embryotoxicity, and inflammation to the upper respiratory tract and gastric mucosa.

Human

Repetitive exposure to acrylic acid may induce mucosal forestomach hyperplasia.

Clinical Management

Exposure should be terminated as soon as possible by moving the victim to fresh air. The skin, eyes, and mouth should be washed with copious amounts of water. Contaminated clothing should be removed and isolated. The victim should be kept calm and normal body temperature should be maintained. Artificial respiration should be provided if the breathing has stopped. Treatment is usually symptomatic.

Environmental Fate

Acrylic acid's production and use in the manufacture of plastics, paint formulations, leather finishings, paper coatings, and in medicine and dentistry for dental plates, artificial teeth, and orthopedic cement may result in its release to the environment through various waste streams. Acrylic acid has also been identified in nine species of chlorophyceae algae, 10 species of rhodophyceae algae, and in the rumen fluid of sheep. If released to air, a vapor pressure of 3.97 mmHg at 25 °C indicates acrylic acid will exist solely as a vapor in the ambient atmosphere. Vapor-phase acrylic acid will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 2 days. If released to soil, acrylic acid is expected to have very high mobility. Volatilization from moist soil surfaces is expected to be slow. Acrylic acid may potentially volatilize from dry soil surfaces based upon its vapor pressure. If released into water, acrylic acid is not expected to adsorb to suspended solids and sediment in the water column. Biodegradation under both aerobic and anaerobic conditions is expected to occur.

Exposure Standards and Guidelines

Occupational Safety and Health Administration: 8 h time-weighted average (TWA) is 2 ppm. Worker exposure levels may exceed three times the threshold limit value (TLV) – TWA for no more than a total of 30 min during a work day, and under no circumstances should they exceed five times the TLV – TWA, provided that the TLV – TWA is not exceeded. National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit – 10 h TWA: 2 ppm ($6\,mg\,m^{-3}$).

See also: Polymers.

Relevant Websites

http://www.epa.gov – Acrylic Acid (from the US EPA's Technology Transfer Network Air Toxics Website).

http://www.inchem.org – Acrylic Acid (Environmental Health Criteria 191) from the International Programme on Chemical Safety, 1997.

Acrylonitrile

Raja S Mangipudy and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 107-13-1
- Synonyms: Acritet; Carbacryl; Propenenitrile; Ventox; Vinyl cyanide; TL 314
- Chemical/Pharmaceutical/Other Class: Industrial chemical; Solvent
- Chemical Formula: C_3H_3N

Uses

Acrylonitrile is used in the manufacture of acrylic fibers and in the plastic surface coatings and adhesive industries. It is also used as a pesticide/fumigant. It is a chemical intermediate in the synthesis of antioxidants, pharmaceutical dyes, surface-active agents, and in reactions requiring the cyanoethyl group.

Exposure Routes and Pathways

Accidental exposure can occur via dermal contact, ingestion, or inhalation. Acrylonitrile is found in cigarette smoke. It does not occur naturally.

Toxicokinetics

Acrylonitrile is absorbed by way of inhalation, ingestion, and percutaneously. Rats treated with [^{14}C]acrylonitrile via oral or intravenous route produced radioactivity in the blood, liver, kidneys, lungs, adrenal cortex, and stomach mucosa. Significant amounts are retained in the plasma. Acrylonitrile is metabolized to a lesser extent in humans than in rodents. Acrylonitrile metabolism in humans follows first-order kinetics and acrylonitrile has a half-life of $\sim$8 h. The elimination of acrylonitrile from the plasma of rats is biphasic, with a half-life of 3.5–5.8 and 50–77 h in the a and b phases, respectively.

There are four major pathways of metabolism for acrylonitrile: formation of glucuronides, direct reaction with glutathione to form cyanoethyl mercapturic acid, direct reaction with the thiol groups of proteins, and epoxidation to 2-cyanoethylene oxide. *N*-Acetyl-*S*-(2-cyanoethyl)-L-cysteine is a major urinary metabolite in human volunteers exposed to 5–10 mg.

Mechanism of Toxicity

Acrylonitrile owes some of its toxicity to cyanide generation, which inhibits cellular respiration. Preinduction of microsomal mixed function oxidase (MFO) with Arochlor 1254 greatly enhanced the toxicity of acrylonitrile and caused a threefold increase in cyanide levels in rats. Therefore, metabolic activation appears to be necessary in the toxicity of acrylonitrile. The direct reaction of acrylonitrile with the SH groups of proteins and its epoxide metabolite are also expected to be responsible for its effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute animal tests in rats, mice, rabbits, and guinea pigs have demonstrated acrylonitrile to have high acute toxicity from inhalation and high to extreme acute toxicity from oral or dermal exposure. No information is available on the reproductive or developmental effects of acrylonitrile in humans.

Fetal malformations (including short tail, missing vertebrae, short trunk, omphalocele, and hemivertebra) have been reported in rats exposed to acrylonitrile by inhalation. In mice orally exposed to acrylonitrile, degenerative changes in testicular tubules and decreased sperm count were observed.

Human

Workers exposed via inhalation to high levels of acrylonitrile for less than an hour experienced mucous membrane irritation, headaches, nausea, feelings of apprehension and nervous irritability. Low-grade anemia, leukocytosis, kidney irritation, and mild jaundice were also observed in the workers, with these effects subsiding with the ending of exposure. Symptoms associated with acrylonitrile poisoning include limb weakness, labored and irregular breathing, dizziness and impaired judgment, cyanosis, nausea, collapse, and convulsions. A child died after being exposed to acrylonitrile by inhalation, suffering from respiratory malfunction, lip cyanosis, and tachycardia before death. Several adults exposed to the same concentration of acrylonitrile exhibited eye irritation, but no toxic effects. Acute dermal exposure may cause severe burns to the skin in humans.

Chronic Toxicity (or Exposure)

Animal

In rats chronically exposed by inhalation, degenerative and inflammatory changes in the respiratory epithelium of the nasal turbinates and effects on brain cells have been observed. In several studies, an increased incidence of tumors has been observed in rats exposed by inhalation, drinking water, and gavage. Astrocytomas in the brain and spinal cord and tumors of the Zymbal gland (in the ear canal) have been most frequently reported, as well as tumors of the stomach, tongue, small intestine in males and females, and mammary gland in females. The reference concentration (RfC) for acrylonitrile is 0.002 mg m^{-3} based on degeneration and inflammation of nasal respiratory epithelium in rats. The Environmental Protection Agency (EPA) has calculated a provisional reference dose (RfD) of 0.001 milligrams per kilogram body weight per day (mg kg^{-1} day^{-1}) for acrylonitrile based on decreased sperm counts in mice.

Human

Headaches, fatigue, nausea, and weakness have been frequently reported in chronically (long-term) exposed workers. A statistically significant increase in the incidence of lung cancer has been reported in several studies of chronically exposed workers. However, some of these studies contain deficiencies such as lack of exposure information, short

follow-up, and confounding factors. EPA has classified acrylonitrile as a Group B1, probable human carcinogen (cancer-causing agent).

Clinical Management

In oral exposure, gastric lavage may be performed soon after ingestion or in patients who are comatose or at risk of convulsing. The volume of lavage return should approximate the volume given. Charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, may be administered. The usual charcoal dose is 30–100 g in adults and 15–30 g in children (1 or 2 g kg^{-1} in infants). In case of inhalation exposure, the patient must be moved to fresh air for respiratory distress. If cough or difficulty in breathing develops, evaluation for respiratory tract irritation, bronchitis, or pneumonitis must be performed. For eye exposure, eyes must be washed with copious amounts of tepid water for at least 15 min. If irritation, pain, lacrimation, or photophobia persists, the patient should be removed to a health care facility. For dermal exposure, the exposed area must be washed thoroughly with soap and water.

Environmental Fate

Acrylonitrile is both readily volatile in air and highly soluble in water. These characteristics determine the behavior of acrylonitrile in the environment. The principal pathway leading to the degradation of acrylonitrile in air is photooxidation, mainly by reaction with hydroxyl radicals (OH). Acrylonitrile may also be oxidized by other atmospheric components such as ozone and oxygen. Very little is known about the nonbiologically mediated transformation of acrylonitirle in water. It is oxidized by strong oxidants such as chlorine used to disinfect water. Acrylonitrile is readily degraded by aerobic microorganisms in water.

Other Hazards

Acrylonitrile is a reactive chemical that polymerizes spontaneously, when heated, or in the presence of a strong alkali unless it is inhibited, usually with ethylhydroquinone. It can explode when exposed to flame. It attacks copper. It is incompatible and reactive with strong oxidizers, acids and alkalis; bromine; and amines.

Exposure Standards and Guidelines

- Immediately dangerous to life or health (IDLH): Ca (85 ppm)
- Threshold limit value time-weighted average (TLV TWA): 2 ppm confirmed animal carcinogen (skin)
- Emergency Response Planning Guideline (ERPG)-1: 25 ppm
  - ERPG-2: 35 ppm
  - ERPG-3: 75 ppm
- National Institute for Occupational Safety and Health recommended exposure limit (NIOSH REL): Ca TWA 1 ppm C 10 ppm (15 min) (skin)

See also: Combustion Toxicology; Cyanide; Polymers; Respiratory Tract.

Further Reading

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Sakurai H (2000) Carcinogenicity and other health effects of acrylonitrile with reference to occupational exposure limit. *Industrial Health* 38(2): 165–180.

Starr TB, Gause C, Youk AO, Stone R, Marsh GM, and Collins JJ (2004) A risk assessment for occupational acrylonitrile exposure using epidemiology data. *Risk Analysis* 24(3): 587–601.

Swaen GM, Bloemen LJ, Twisk J, *et al.* (2004) Mortality update of workers exposed to acrylonitrile in The Netherlands. *Journal of Occupational and Environmental Medicine* 46(7): 691–698.

Thier R, Lewalter J, and Bolt HM (2000) Species differences in acrylonitrile metabolism and toxicity between experimental animals and humans based on observations in human accidental poisonings. *Archives of Toxicology* 74(4/5): 184–189.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Acrylonitrile.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Acrylonitrile.

Acute Toxicity *See* Toxicity, Acute.

Adiponitrile

Shashi Ramaiah and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 111-69-3
- Synonyms: Adipic acid dinitrile; Adipic acid nitrile; 1,4-Dicyanobutane; Hexanedinitrile; Tetramethylene cyanide
- Chemical Formula: $C_6H_8N_2$

Uses

Adiponitrile is a starting chemical intended for synthesis of hexamethylenediamine to make nylon, corrosion inhibitors, and rubber accelerators; it is also used for the synthesis of adipoguanamine, which is used as an extractant for aromatic hydrocarbons.

Background Information

Adiponitrile is an odorless, oily colorless liquid, which decomposes on heating and reacts violently with strong oxidants. Upon burning, adiponitrile produces highly toxic hydrogen cyanide.

Exposure Routes and Pathways

Adiponitrile may be inhaled, swallowed, or absorbed through skin.

Adiponitrile could potentially be released to the environment in the effluent or emissions from plants manufacturing adiponitrile, hexamethyelenediamine, or nylon-66. If released to soil, aerobic biodegradation may be an important removal mechanism. Although adiponitrile has the potential to undergo extensive leaching, biodegradation should limit movement through soil. Volatilization from soil surfaces is not expected to be significant. If released to water, aerobic biodegradation may again be an important removal mechanism.

Toxicokinetics

Seventy percent of the dose ($\sim 50\,mg\,kg^{-1}$) administered subcutaneously to guinea pigs was eliminated as thiocyanate in urine. After application of adiponitrile to depilated skin, skin penetration was suggested by increased thiocyanate in urine. Greater quantities were absorbed when skin was abraded. Based on the ratio between administered adiponitrile dose and quantity of cyanide detected, it was shown that a greater part of the dose was metabolized to cyanide. Cyanide thus released is the principle cause of toxicity.

Mechanism of Toxicity

Adiponitrile's mechanism of toxicity is similar to cyanide because it can potentially liberate cyanide in the body spontaneously. It forms a stable complex with ferric iron in the cytochrome oxidase enzymes, thereby inhibiting cellular respiration. Cyanide affects primarily the central nervous system (CNS), producing early stimulation followed by depression. It initially stimulates the peripheral chemoreceptors (causing increased respiration) and the carotid bodies (thereby slowing the heart). Early CNS, respiratory, and myocardial depression result in decreased oxygenation of the blood and decreased cardiac output. These effects produce both stagnation and hypoxemic hypoxia in addition to cytotoxic hypoxia from inhibition of mitochondrial cytochrome oxidase.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} is $155\,mg\,kg^{-1}$ in rats, $172\,mg\,kg^{-1}$ in mice, and $22\,mg\,kg^{-1}$ in rabbits. The subcutaneous LD_{50} in the guinea pig is $50\,mg\,kg^{-1}$.

Human

Vapors are irritating to the eyes and respiratory system at higher concentrations. Humans may experience tightness in the chest, headache, and weakness with difficulty in standing, and vertigo, cyanotic, rapid respirations, low blood pressures, and tachycardia. Mental confusion, tonic clonic contractions of limbs and facial muscles, irregular heartbeat, coma, and death may occur after exposure to higher

concentrations. Contact with skin and eyes may cause burns. Adiponitrile may be fatal if absorbed through skin, inhaled, or swallowed. The American Conference of Governmental Industrial Hygienists' threshold limit value is 2 ppm (with a skin designation indicating the potential significant contribution to the overall exposure by the cutaneous route). Short-term inhalation limits are not available.

Chronic Toxicity (or Exposure)

Animal

Adiponitrile was negative for mutagenicity in *Salmonella* with or without bioactivation. Adiponitrile has not been tested for its ability to cause cancer in animals.

Human

Upon repeated or chronic exposure, adiponitrile may have effects on the blood and adrenals, resulting in anemia and tissue lesions. Adiponitrile is not classifiable as a human carcinogen because no human data are available.

In Vitro Toxicity Data

There are no *in vitro* toxicity data available for adiponitrile.

Clinical Management

Emergency Treatment

The affected person should be removed from exposure to adiponitrile immediately. Contaminated clothes should be removed and the patient sponged to avoid any absorption through skin. Immediate cardiopulmonary resuscitation should be administered. If the victim breathes with difficulty, oxygen should be given. In case of ocular contact, the eyes should be flushed with copious amounts of water for at least 20 min. In cases of ingestion, vomiting should be induced. Mouth-to-mouth resuscitation should be avoided in order to prevent self-poisoning.

Medical Treatment

The goal of medical treatment is to eliminate the cyanide formed in the body. Sodium nitrate, amylnitrate, and thiosulfate should be administered. Sodium nitrite should be administered intravenously very slowly. Amylnitrite can also be inhaled from ampoules. Later, sodium thiosulfate should be administered.

Sodium nitrate reacts with hemoglobin in the red blood cells forming methemoglobin, which in turn can react with the free cyanide ion forming cyanmethemoglobin, thereby binding free cyanide and preventing its reaction with cytochrome oxidase enzymes in the cells. Cyanmethemoglobin dissociates slowly into free cyanide plus methemoglobin. The cyanide released by dissociation of cyanmethemoglobin then reacts with the thiosulfate ion forming thiocyanate, a relatively nontoxic compound that is excreted in the urine.

Ecotoxicology

There is no aquatic toxicity information available for adiponitrile.

Miscellaneous

Adiponitrile must be stored to avoid contact with oxidizing agents such as perchlorates, peroxides, permanganates, and fluorine, since violent reactions can occur. Adiponitrile is not compatible with strong acids and reducing agents. Adiponitrile should be stored in tightly closed containers in a cool, well-ventilated area.

Adiponitrile is produced as an intermediate or final product by a process covered under regulatory performance standards that have been promulgated to protect the atmosphere from equipment leaks of volatile organic compounds (VOCs) in the synthetic organic chemical manufacturing industry (SOCMI). The intended effect of these standards is to require all newly constructed, modified, and reconstructed SOCMI process units to use the best demonstrated system of continuous emission reduction for equipment leaks of VOCs, considering costs, non-air quality health, and environment impact and energy requirements.

See also: Cyanide; Volatile Organic Compounds (VOC).

Further Reading

Smith LW and Kennedy GL Jr. (1982) Inhalation toxicity of adiponitrile in rats. *Toxicology and Applied Pharmacology* 65: 257–263.

Relevant Website

http://www.state.nj.us – New Jersey Hazardous Substances Fact Sheet.

Aerosols

Raja S Mangipudy

- DESCRIPTION: Aerosols consist of very finely subdivided liquid or solid particles dispersed in and surrounded by a gas
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aerosols are systems ranging from those of colloidal nature to systems consisting of 'therapeutic packages'. Aerosols are classified as follows:

  (A) Liquified-gas systems
  Two-phase: space-spray; surface-coating; dispersion or suspension
  Three-phase: two-layer; foam; stabilized; quick-breaking
  (B) Compressed-gas systems
  Solid-stream dispensing
  Foam dispensing
  Spray dispensing
  (C) Separation of propellant from concentrate systems
  Piston type
  Flexible type
  Atomizer type
  Mechanical systems
  Latex diaphragm

Uses

Many therapeutically active ingredients are administered or applied to the body by means of the aerosol dosage form, including agents such as epinephrine, isoproterenol, antibiotics, antiseptics, steroids, and ergotamine. Oral aerosols have been used for the symptomatic treatment of asthma as well as for the treatment of migraine headaches, whereas topical aerosols find use in numerous dermatological manifestations.

Human Toxicity

The following pertains to the general evaluation and treatment of individuals exposed to potentially toxic chemicals via aerosols.

A. General evaluation

1. Exposed individuals should have a careful, thorough medical history and physical examination performed, looking for any abnormalities. Exposure to chemicals with a strong odor often results in such nonspecific symptoms as headache, dizziness, weakness, and nausea.

B. Irritation

1. Many chemicals cause irritation of the eyes, skin, and respiratory tract. In severe cases respiratory tract irritation can progress to acute respiratory distress syndrome (ARDS)/acute lung injury, which may be delayed in onset for up to 24–72 h in some cases.
2. Irritation or burns of the esophagus or gastrointestinal tract are also possible if caustic or irritant chemicals are ingested.

C. Hypersensitivity

1. A number of chemical agents produce an allergic hypersensitivity dermatitis or asthma with bronchospasm and wheezing with chronic exposure.

The inflammability and toxicity of the propellant needs to be considered. Additionally, the topical effects of the propellants must be determined.

Clinical Management

1. Supportive care must be instituted for patients accidentally exposed to aerosol contents via topical, inhalation, or oral routes. A number of chemicals produce abnormalities of the hematopoietic system, liver, and kidneys. Monitoring complete blood count, urinalysis, and liver and kidney function tests is suggested for patients with significant exposure.
2. If respiratory tract irritation or respiratory depression is evident, monitor arterial blood gases, chest X-ray, and pulmonary function tests.

See also: Corticosteroids.

A-Esterases

Lester Grant Sultatos

The phosphorothioate insecticides, such as chlorpyrifos and methyl parathion (**Figure 1**), are some of the most commonly used organophosphorus insecticides in the United States. Interestingly, these compounds have little capacity to inhibit the enzyme acetylcholinesterase and are, therefore, not highly toxic themselves. However, they are converted by the liver to potent acetylcholinesterase inhibitors termed oxons (such as chlorpyrifos oxon and methyl paraoxon; **Figure 1**), which are responsible for the toxicities observed following exposure to phosphorothioate insecticides. Once the oxons have been produced from the parent insecticides, one of the ways in which these highly toxic compounds can be metabolized by a variety of species is through their hydrolysis by an enzyme(s) termed A-esterase(s) (**Figure 2**). Since the products of these hydrolysis reactions are usually of low toxicity, A-esterase(s) catalyzes the detoxification of these oxons. Consequently, A-esterase(s) likely plays an important role in the protection of mammals against phosphorothioate insecticide toxicity. For example, although paraoxon and chlorpyrifos oxon have about the same capacity to inhibit acetylcholinesterase, the insecticide chlorpyrifos is about 10 times less toxic to laboratory mice and rats than is parathion, probably because chlorpyrifos oxon is detoxified much more avidly by A-esterase(s).

The term A-esterase originally referred to an enzyme(s) in the serum that metabolized carboxylic esters and was insensitive to inhibition by organophosphates (in contrast to the B-esterases, which are inhibited by organophosphates). Later this activity was shown to be associated with the detoxification of paraoxon, leading to the use of the term A-esterase to refer to enzymes that hydrolyze organophosphates. Since the original discovery of A-esterase, several enzymes have been identified, which can hydrolyze certain organophosphates, and hence have been referred to as A-esterases, even though it is currently not known if they also hydrolyze carboxylic esters.

Currently, there is much confusion regarding the nomenclature of A-esterase(s), and the term A-esterase is by no means universally endorsed. This enzyme(s) has been referred to by many different names, including paraoxonase, aryl-ester hydrolase, arylesterase, organophosphate hydrolase, organophosphorus compound hydrolase, and organophosphorus acid anhydrolase. Part of the confusion in the classification of this enzyme(s) appears to result from the presence of different forms (which may or may not be related) within an organism, as well as different forms within different species. For example, an enzyme that can detoxify paraoxon has been isolated from the bacteria *Pseudomonas diminuta*. This enzyme requires zinc for activity and can also detoxify the parent phosphorothioate insecticide parathion. In contrast, mammals seem to have at least two kinds of enzymes that could be called A-esterase, neither of which can detoxify parathion. The first detoxifies the compound diisopropylfluorophosphate and requires magnesium, manganese, or cobalt for activity. The second detoxifies oxons, such as chlorpyrifos oxon and paraoxon, and requires calcium for activity.

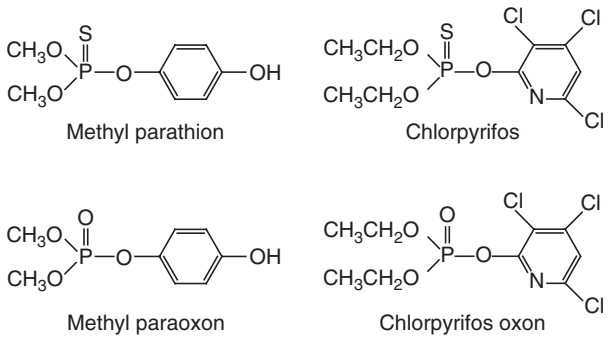

Figure 1 Structures of the phosphorothioate insecticides chlorpyrifos and methyl parathion and their corresponding oxygen analogs.

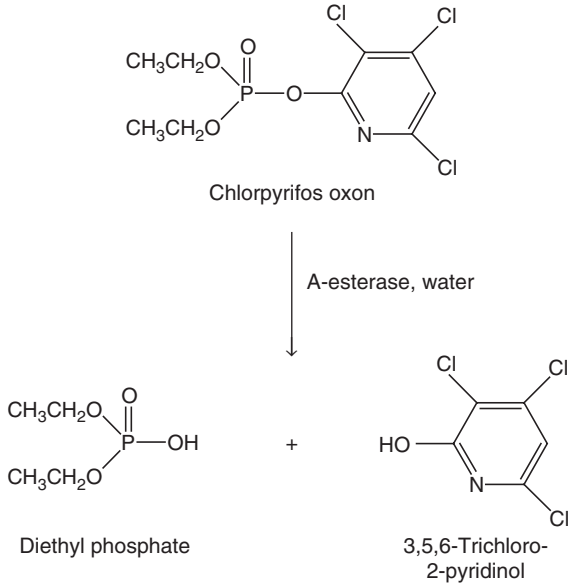

Figure 2 Hydrolysis of chlorpyrifos oxon by A-esterase. Although water is also involved in the reaction, it is usually ignored because it is present at an extremely high and constant concentration.

Although the term A-esterase has been sometimes applied to all these enzymes, it is more often used to refer to the enzyme(s) that requires calcium and detoxifies the oxygen analogs of phosphorothioate insecticides.

Considerable species differences in A-esterase activity exist, ranging from very low or nonexistent in certain birds and fish, to very high in rabbits. Species differences in A-esterase activity could account, at least in part, for species differences in the relative sensitivity to certain phosphorothioate insecticides. For example, birds are much more susceptible to the toxicity of pirimiphos methyl than are mammals.

In mammals, A-esterase(s) has been identified in several tissues, with the highest activity usually found in the blood and liver. It is now known that the liver synthesizes A-esterase(s) and secretes it into the blood. Within the past decade, the A-esterase that has been most characterized has been referred to as paraoxonase (named for its capacity to hydrolyze paraoxon). Interest in human paraoxonase has been fueled by the documentation of the existence of genetic polymorphisms that control its catalytic activity as well as its expression levels. While human paraoxonase has been shown to be encoded by at least three genes, designated *PON1*, *PON2*, and *PON3*; *PON1*, encoding for the protein PON1, has been the most studied. For example, the PON1 Q/R polymorphism of position 192 has been shown to affect catalytic activity in a substrate-dependent manner. The PON_{R192} isoform hydrolyzes paraoxon at a greater rate than does the PON_{Q192} isoform, while the opposite relationship has been observed for the subtrates diazonon, sarin, and soman. Other polymorphisms in the noncoding region of *PON1* have been shown to alter PON1 expression levels.

The identification of paraoxonase genetic polymorphisms has led to much discussion in the literature regarding the role that these polymorphisms might play in determination of individual susceptibility to organophosphorus insecticide toxicity. The issue of the toxicological significance of PON1 polymorphisms is confounded by evidence that suggests this enzyme might not play much of a role in the detoxification of oxons following exposure to the parent insecticide. Although administration of purified enzyme to animals can protect against insecticide toxicity, various lines of evidence suggest that endogenous enzyme plays a limited or negligible role following organophosphorus insecticide exposures and that other detoxification pathways are more significant. Moreover, the limited epidemiological studies that have examined possible relationships between PON1 activity and susceptibility to organophosphorus insecticide toxicity have reported mixed results and are, therefore, at present, inconclusive. Consequently, the toxicological significance of the human PON1 genetic polymorphisms is currently not known.

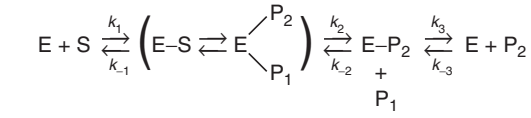

Figure 3 Kinetic mechanism for the interaction of paraoxon (S) with A-esterase (E) or acetylcholinesterase (E). *p*-Nitrophenol (P_1) is the first product released, whereas diethyl phosphate (P_2) is the second.

As outlined in **Figure 3**, the hydrolysis of paraoxon by human serum A-esterase(s) is very similar to the phosphorylation of B-esterases, such as acetylcholinesterase, by paraoxon. Both reactions involve an initial binding of paraoxon to the enzyme, followed by a rapid conformational change that produces diethyl phosphate and *p*-nitrophenol from paraoxon. *p*-Nitrophenol is quickly released from the enzyme, leaving diethyl phosphate covalently bound to enzyme. At this point, A-esterase quickly releases diethyl phosphate as a result of interacting with a water molecule. However, B-esterases, such as acetylcholinesterase, retain the diethyl phosphate for a much longer period of time, thereby resulting in inhibition of the enzyme.

While A-esterase(s) and B-esterases interact kinetically with paraoxon in a similar fashion (**Figure 3**), the molecular events occurring at their active sites during catalysis are probably very different. The active site of B-esterases such as acetylcholinesterase has been well characterized and contains a serine residue that is phosphorylated by paraoxon at the hydroxyl group. In contrast, the active site of A-esterase(s) has not been studied as extensively, but it likely does not contain a serine residue that participates in the hydrolysis of paraoxon. Additionally, A-esterase(s) requires a divalent cation like calcium for activity, whereas B-esterases do not.

For many decades, the function of mammalian A-esterase(s) was unknown. More recent studies, however, are perhaps uncovering a physiological role for this enzyme. Serum PON1 has been shown to be closely associated with high-density lipoproteins, and it might contribute to the antioxidant protection for low-density lipoprotein oxidation. Interestingly, when fed a high fat diet $PON1^{-/-}$ mice (knockout mice without PON1) exhibited larger atherosclerotic lesions compared to wild type mice. Furthermore, high-density lipoproteins from these knockout mice did not protect against low-density lipoprotein

oxidation *in vitro*. While some human studies have linked certain PON1 polymorphisms with an increased incidence of cardiovascular disease, others have reported the opposite. Further studies are required in order to better characterize possible roles for PON1 (and other isoforms) in cardiovascular disease.

See also: Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates.

Further Reading

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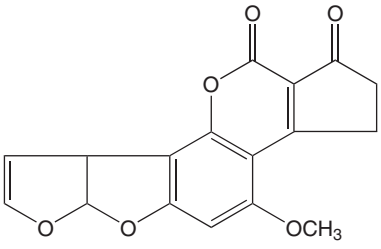
Reiner E, Aldridge WN, and Hoskin FCG (eds.) (1989) *Enzymes Hydrolyzing Organophosphorus Compounds*. West Sussex, UK: Ellis Horwood.

Aflatoxin

Raja S Mangipudy and Harihara M Mehendale

- Synonyms: Aflatoxins B1; B2; B3; B4; G1; G2; M1; M2
- Chemical Structure:



Background Information

Aflatoxins are naturally occurring bisfuranocoumarin compounds produced from the molds *Aspergillus flavus* and *Aspergillus parasiticus*. The aflatoxins are highly fluorescent. The 'B' refers to blue, the 'G' signifies green fluorescence. 'M' aflatoxins are fungal metabolites present in milk. Aflatoxin B1 is the most potent. Aflatoxins are contaminants in corn, peanuts, tree nuts, cotton seed, and certain meats. They have also been found in hypoallergenic milk.

Exposure Routes and Pathways

Ingestion and dermal contact are possible routes of exposure.

Toxicokinetics

Aflatoxins are well absorbed orally. Exposure to human skin results in slow absorption. Aflatoxins are rapidly cleared from blood. Sixty-five percent of an initial dose of aflatoxin B1 is removed from the blood within 90 min and excreted primarily in the bile. The plasma half-life of aflatoxin is short, and it is excreted slowly as multiple moieties as a result of extensive metabolism. When estimated in human liver homogenates, the parent compound had an estimated half-life of 13 min.

Aflatoxins are metabolized by the NADPH-dependent enzyme system using cytochrome P450. *In vitro* liver metabolism studies have shown five different types of metabolic pathways for aflatoxin B1: reduction, hydroxylation, hydration, *O*-demethylation, and epoxidation. All of these products contain hydroxide groups that allow them to be conjugated with glucuronic acid and sulfate, thus becoming detoxified.

Mechanism of Toxicity

Aflatoxins combine with DNA, suppressing DNA and RNA synthesis. This leads to structural changes in cell nucleoli and reduction of protein synthesis. Formation of reactive DNA adducts causes cancer.

Acute and Short-Term Toxicity (or Exposure)

Animal

Aflatoxins are carcinogenic in animals. The carcinogenic potential seems to be increased in malnutrition, especially pyridoxine deficiency. It has been proposed that aflatoxin B1-2,3-oxide (metabolite of aflatoxin B1) is the actual carcinogen.

The *in vitro* exposure of rat embryos to aflatoxin B1 induced neural tube defects. Aflatoxins have also

been shown to be teratogenic in hamsters and mice, causing neural tube closure defects, microcephaly, umbilical hernia, and cleft palate. Although negative teratogenicity studies exist for rats, mice, and commercial livestock, the negative studies involve long-term feeding exposures while the positive studies involve acute exposure, suggesting high doses and maternal toxicity may play a role in adverse effects on the offspring. Aflatoxin may be a transplacental carcinogen in the rat. Analysis of aflatoxins in maternal and cord blood samples has also demonstrated the transplacental transport of aflatoxins in humans. Although suspected of playing a role in the early onset of liver cancer in some populations, prenatal exposure has not been demonstrated to be a significant route of exposure to the aflatoxins. Aflatoxins and active carcinogenic metabolites are excreted in breast milk. An estimate of the percentage of the oral dose excreted in milk ranged from 0.09% to 0.43%.

A small number of studies have reported that male rats fed aflatoxins developed testicular degeneration and impaired spermatogenesis, although no clear association with aflatoxins and clinical infertility was uncovered in one of these studies.

Chronic Toxicity (or Exposure)

Human

Aflatoxin poisoning is difficult to diagnose early in humans. The first clinical symptoms are anorexia and weight loss. Aflatoxins are associated with hepatocellular damage and necrosis, cholestasis, hepatomas, acute hepatitis, periportal fibrosis, hemorrhage, jaundice, fatty liver changes, cirrhosis in malnourished children, and Kwashiorkor. There is evidence of transplacental transport of aflatoxin by the fetoplacental unit. Aflatoxins are proven human carcinogens.

In Vitro Toxicity Data

The *in vitro* exposure of rat embryos to aflatoxin B1 induced neural tube defects.

Clinical Management

Acute aflatoxin toxicity should be treated with decontamination procedures and good supportive care. With chronic ingestions, the primary treatment remains supportive in nature. Elevation of serum alkaline phosphatase is a good indicator of aflatoxin toxicity.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Immune System; Mycotoxins; Toxicity Testing, Mutagenicity; Veterinary Toxicology.

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Aflatoxin.

Aggregate Exposures

Jeffrey H Driver

Chemicals and other agents (biological, physical), both natural and man-made, may be released into the environment as a result of their production and use and, through dispersion and transport processes, may be present in food, water, soil, indoor and outdoor air, and other media (e.g., residential turf, indoor carpet, clothing). Thus, humans may be exposed to these agents by one or more routes (ingestion, inhalation, dermal absorption) and from one or more sources. Aggregate exposure assessments involve estimating the magnitude and frequency of exposure to a given agent by ingestion, inhalation, and dermal absorption for a defined population, taking into account reliable information on occurrence of the agent in all relevant media (**Figure 1**).

Exposure assessment methodologies in the past have often focused on a single source and/or route of exposure. However, it has become increasingly apparent in recent years that, for some chemicals, significant exposures may occur by more than one route and from more than one source. Therefore, integrated aggregate assessment methods have been

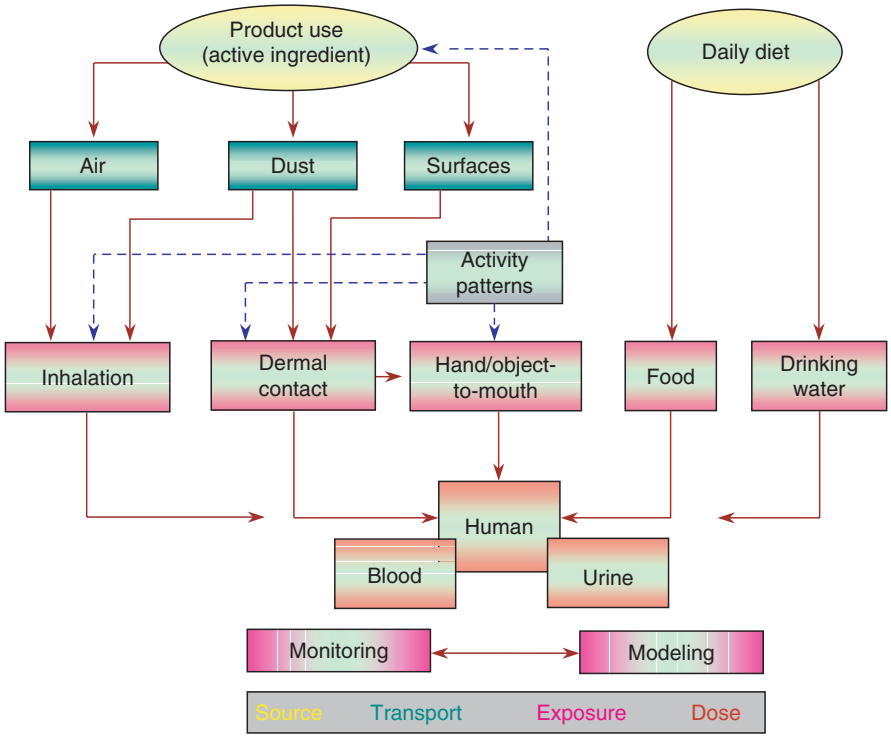

Figure 1 Components of multi-pathway, multi-route aggregate exposure assessment.

developed, and continue to be refined, to facilitate aggregate exposure assessments for these chemicals. Additional impetus has been provided by regulatory mandates, e.g., the US Food Quality Protection Act (FQPA) of 1996. FQPA requires a standard of safety for pesticide food tolerances, that is, reasonable certainty of no harm, resulting from aggregate exposure to the pesticide chemical residue, including anticipated dietary exposures and other relevant exposures for which there is reliable information (e.g., pesticide products applied in and around residences).

Aggregate exposure assessment has two complementary objectives. First, it seeks to estimate the dose that an individual will receive from exposures by all relevant routes and from all sources and pathways under given circumstances. Such an estimate of individual dose may vary as a function of space and time (e.g., at different locations, on different days). Second, it seeks to portray the range of individual doses that may be received in a well-defined population of such individuals as a distribution, reflecting the influence of varying individual characteristics (e.g., age, sex, ethnicity, place of residence, occupation). The process is based on an aggregate exposure model, which is generally a methodology incorporating mathematical algorithms for combining exposure input data from various sources to derive an exposure estimate. Exposure models often include procedures for estimating data that are not or cannot be measured directly. As with any model, exposure models are only as valid as the input parameters, data, and assumptions. Their accuracy and precision ultimately must be evaluated, at least, in part, by comparison with whatever data from measurements are available. In an aggregate exposure model, the exposure from each source is described by a set of equations. Many of the components of these equations have values that are variable (from individual to individual, from day to day or season to season, from sample to sample) and/or uncertain. These components of the exposure equations can be described by probability distributions that reflect the relative frequency of the different values for the variable components and the relative likelihood of the different possible values for the uncertain components. Exposure values from the various sources and/or routes need not be additive; whatever mathematical function is physiologically and toxicologically appropriate should be used to aggregate these values for an individual. Also, aggregate modeling should provide, where appropriate, for correlation among variables and ensuring that the component values (and the aggregate exposure estimate) for an individual are internally consistent. Input variable distributions for aggregate exposure assessments can be presented not only for a single day but also for

individuals or populations over time. In fact, the dimension of time may play a particularly important role in aggregate exposure assessments for many agents, such as chemical pesticides, where exposures may be seasonal based on pest pressures.

Aggregate assessments require consideration of indirect exposure measurements (e.g., dermal exposure by measurement of residues that are transferable from a surface such as residential turf to clothing, skin, or relevant surrogate media; inhalation exposure by measurement of air concentration in the breathing zones of individuals; ingestion exposure by measurement of concentrations in water, food, etc., ingested by an individual), and where available, direct exposure measurements (e.g., measurement of the concentration of the chemical or its biotransformation products in biological tissues or fluids).

Therefore, to estimate or measure aggregate exposures, relevant and reliable data are required. Further, aggregate exposure assessment methods and modeling tools are needed that more accurately reflect real-life situations (in contrast to methods and models that are based on very conservative assumptions and may lead to less realistic and sometimes gross overestimates of exposure).

Estimating aggregate exposure risk for a single agent, such as a chemical pesticide, brings to the forefront the need for both input data quality objectives and exposure estimate (model output) interpretation. Interpretation can be facilitated by comparing estimates of aggregate exposure to population-based or situation-specific biological monitoring data, which reflect aggregate measure of total absorbed dose. Aggregate exposure assessments typically require the use of data from indirect measurements and modeling at some level.

There is an emerging body of evidence that suggests person-to-person differences in exposure play an important role in the variability and uncertainty associated with risk assessments for chemicals (and other agents). The traditional or standard default approaches used in human health risk assessment often do not effectively evaluate interindividual variation and may underestimate the impact of chemical exposures on particular groups of individuals. Traditional approaches must be refined to adequately account for temporal variation in factors that contribute to complex aggregate exposure patterns (e.g., chemical-specific exposure media concentrations and time–activity interactions by humans) involving multiple, intermittent exposures.

Longitudinal exposure assessment methods and measurements have emerged to address temporal and spatial aspects of aggregate exposures. Longitudinal studies are being implemented to address the following:

- Temporal human time–activity patterns.
- Temporal dietary (food consumption) surveys.
- Temporal consumer and professional product use in and around the home (which addresses the potential co-occurrence of two or more exposures to a given chemical during the same toxicologically relevant time period).
- Population-based and situation-specific (e.g., reentry of residents onto pesticide-treated lawns) biological monitoring surveys.
- Integrated aggregate exposure monitoring programs (e.g., concurrent product use surveys, dietary surveys, and exposure media measurements).
- The development of assessments of exposure that include refined evaluations of the variability and uncertainty associated with aggregate exposure estimates.

See also: Exposure; Exposure Assessment; Exposure Criteria; Food Quality Protection Act, US; Mixtures, Toxicology and Risk Assessment.

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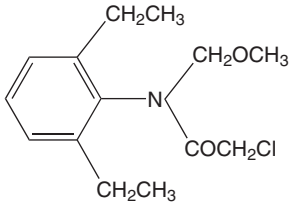
Air Pollution *See* Pollution, Air.

Alachlor

Raja S Mangipudy and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 15972-60-8
- Synonyms: Alachlore; Alanex; Alanox; Alatox 480; Lasso; Lasagrin
- Chemical/Pharmaceutical/Other Class: Herbicide
- Chemical Structure:



Uses

Alachlor is a pre-emergence herbicide registered by Monsanto in 1969. It is used as an herbicide for grasses, broadleaf seeds, corn, sorghum, soybeans, peanuts, cotton, vegetables, and forage crops.

Exposure Routes and Pathways

Dermal exposure is most common, although exposure via oral/parenteral route and ocular contact are also possible.

Toxicokinetics

Alachlor is absorbed orally. Dermal absorption may be linear over time for the duration of exposure. Excretion via kidneys is the major route of elimination.

Mechanism of Toxicity

This agent is a mucous membrane irritant. The exact mechanism of potential teratogenic changes is still being investigated. In mammals, alachlor appears to form conjugates with glucuronic acid, sulfate, and mercapturic acid. Sister chromatid exchanges have been demonstrated in human lymphocytes *in vivo* as well as dose-dependent chromosomal aberrations *in vitro* in human lymphocytes.

Acute and Short-Term Toxicity (or Exposure)

Animal

Alachlor is a slightly toxic herbicide. The LD_{50} of alachlor in rats is between 930 and 1350 mg kg^{-1}. In the mouse, the LD_{50} is between 1910 and 2310 mg kg^{-1}. The dermal LD_{50} in rabbits is 13 300 mg kg^{-1}, but some of the formulated materials can be more toxic, with dermal LD_{50} values ranging from 7800 to 16 000 mg kg^{-1}. Skin irritation is slight to moderate. The inhalation LC_{50} in rats is reportedly greater than 23.4 mg l^{-1} for 6 h of exposure. High oral doses (150 or 400 mg kg^{-1} day^{-1}) fed to rats during gestation resulted in maternal and fetal toxicity, but there was no indication that reproduction was affected. Alachlor does not appear to cause reproductive effects. Doses of up to 150 mg kg^{-1} day^{-1} fed to rabbits on days 7 through 19 of pregnancy did not result in any birth defects. Similar studies in rats at doses up to 400 mg kg^{-1} day^{-1} did not result in birth defects, but toxic effects in the mothers and offspring were seen at the highest dose. These data indicate that alachlor is not likely to cause birth defects.

Human

No specific information on the acute toxicity of alachlor in humans is available.

Chronic Toxicity (or Exposure)

Animal

A 90-day study on rats and dogs given diets containing low to moderate amounts of alachlor (1–100 $mg\,kg^{-1}\,day^{-1}$) showed no adverse effects. However, a 6-month dog study showed liver toxicity at all doses above 5 $mg\,kg^{-1}\,day^{-1}$, and a 1-year study established that above 1 $mg\,kg^{-1}\,day^{-1}$, alachlor causes effects in the liver, spleen, and kidney. In 2-year rat studies, doses above 2.5 $mg\,kg^{-1}\,day^{-1}$ caused irreversible degeneration of the iris and related eye structures.

Rats given high doses of alachlor developed stomach, thyroid, and nasal turbinate tumors. An 18-month mouse study with doses from 26 to 260 $mg\,kg^{-1}\,day^{-1}$ showed an increase of lung tumors at the highest dose for females but not males. Because of inconsistencies in these studies, the oncogenic potential of alachlor is uncertain.

Human

No specific information on the chronic toxicity of alachlor in humans is available.

In Vitro Toxicity Data

Alachlor does not appear to be mutagenic. Mutagenicity assays with a variety of microbial strains at numerous concentrations of alachlor were all negative.

Clinical Management

There are few acute symptoms. Treatment is symptomatic and supportive. There are no specific antidotes. In cases of oral exposure, measures to decrease absorption may be useful. Emesis may be induced after careful consideration. For dermal exposure, decontamination by washing the exposed area thoroughly with soap and water is recommended. In cases of inhalation exposure, the victim must be moved to fresh air and monitored for respiratory distress. In cases of eye exposure, the eyes should be irrigated with copious amounts of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the person should be seen in a health care facility.

Environmental Fate

Alachlor has a low persistence in soil, with a half-life of ~8 days. The main means of degradation is by soil microbes. It has moderate mobility in sandy and silty soils, and thus can migrate to groundwater. The largest groundwater testing program for a pesticide, the National Alachlor Well Water Survey, was conducted throughout the last half of the 1980s. Over 6 million private and domestic wells were tested for the presence of alachlor. Less than 1% of all of the wells had detectable levels of alachlor. In the wells where the compound was detected, concentrations ranged from 0.1 to 1.0 $\mu g\,l^{-1}$, with the majority having concentrations ~0.2 $\mu g\,l^{-1}$. Alachlor breaks down rapidly in natural water, primarily due to the action of microorganisms. The breakdown rate is much slower in water with no oxygen. Absorption is primarily by germinating shoots and it is readily translocated throughout the plant. Higher concentrations appear in the vegetative parts than in the reproductive parts of the plant. Alachlor is rapidly metabolized to water-soluble products in plants. It is almost completely metabolized within 10 days.

Other Hazards

Alachlor is moderately toxic to fish. The bioaccumulation factor in the channel catfish is 5.8 times the ambient water concentration, indicating that alachlor is not expected to accumulate appreciably in aquatic organisms. It is phytotoxic to sugar beet and cucurbits. The Material Safety Data Sheet should always be referred to for detailed information on handling and disposal.

Exposure Standards and Guidelines

- Acceptable daily intake is 0.0025 $mg\,kg^{-1}\,day^{-1}$.
- Maximum contaminant level is 0.002 $mg\,l^{-1}$.
- Reference dose is 0.01 $mg\,kg^{-1}\,day^{-1}$.

See also: Pesticides.

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Alar

Raja S Mangipudy

- SYNONYMS: Aminozide; Daminozide; DMSA; B-995; Kylar; Aminocide
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organic acid
- CHEMICAL STRUCTURE: $HOOCCH_2CH_2CONHN(CH_3)_2$

Uses

Alar is used as a translocated plant growth regulator. It reduces internode elongation; induces heat, drought, and frost resistance; and produces darker foliage and stronger stems. It also produces earlier and multiple flowers and fruits. A spray is often applied at the rate of 1500–10 000 ppm. It is systemic (i.e., it is taken up by the fruit). Its residues cannot be washed off or removed by peeling. Use of alar in apples caused environmental concern a few years ago; it has now been banned in the United States.

Exposure Routes and Pathways

Dermal contact and ingestion are routes of exposure.

Toxicokinetics

A breakdown product of alar is an asymmetrical 1,1-dimethylhydrazine and is excreted renally.

Mechanism of Toxicity

The growth retardant action has been attributed to formation of 1,1-dimethylhydrazine, which inhibits tryptamine oxidation by pea epicotyl homogenates.

Acute and Short-Term Toxicity (or Exposure)

Animal

The primary toxic effects seen in animals include ptosis, central nervous system (CNS) depression, gastrointestinal irritation, and possibly liver functional abnormalities.

Human

There are little data on mammalian toxicity. No human case reports are available. Based on animal data, alar should be low in toxicity. The US Environmental Protection Agency has determined that alar does not represent an imminent health hazard.

Clinical Management

No human cases have been reported so treatment recommendations are speculative. Dermal contamination probably requires no treatment other than decontamination. For gastric contamination caused by swallowing, treatment by emesis, gastric lavage, and/or activated charcoal may be indicated. Patients should be monitored for CNS depression, ptosis (drooping eyelid), and liver functional abnormalities if significant amounts (>8 g) have been ingested.

See also: Acids; Pesticides.

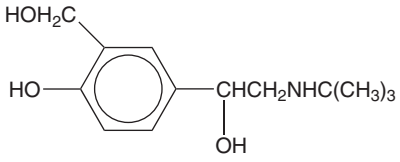
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Alar.

Albuterol

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 18559-94-9
- Synonyms: Salbutamol; Ventolin; Proventil; Apo-Salvent; Novo-Salmol; Albuterol sulfate; Volmax
- Chemical/Pharmaceutical/Other Class: Selective β_2-adrenergic agonist
- Chemical Formula: $C_{13}H_{21}O_3$
- Chemical Structure:



Uses

Albuterol is used as a bronchodilator in the treatment of asthma. Albuterol is also used in the prevention of premature labor. Off label uses include treatment of hyperkalemia.

Mechanism of Action

β-Adrenergic receptors mediate the effects of the sympathetic nervous system throughout the body. β_2-Receptors are found on vascular, bronchial, gastrointestinal, and uterine smooth muscle as well as skeletal muscle, hepatocytes, and also the myocardium. Albuteral stimulates adenyl cyclase which catalyzes cyclic adenosine monophosphate (AMP) from adenosine triphosphate (ATP). This mediates bronchdilation and smooth muscle relaxation through activation of protein kinases, leading to phosphorylastion of proteins, which in turn increases bound intracellular calcium. The reduced availability of intracellular ionized calcium inhibits actin-myosin linkage, leading to the relaxation of smooth muscle. β_2-Adrenergic receptors in the lung also inhibit secretions and decrease histamine release. Stimulation of β_2-adrenergic receptors found on the uterine smooth muscle inhibits the onset of labor.

Routes of Administration

Albuterol is available as tablets and as syrup for oral use, as solution and as sulfate for inhalation, as solution for injection, and as solution for intravenous infusion for parenteral use. Inhaled albuterol has been found to be more effective and less toxic than alternative forms.

Toxicokinetics

Nebulized albuterol has been found more effective than systemic administration. Oral albuterol is readily absorbed from the gut. There is significant first-pass conjugation with 50% bioavailability of an ingested (oral) dose. From 21% to 30% of an inhaled dose is available for absorption. Only $\sim$3% of an oral inhaled dose reaches the lungs. With a nebulizer, $\sim$10–20% is absorbed. Parenteral absorption is 100%. Sulfate conjugation is the primary metabolic pathway; it is transformed in the liver. There appears to be no direct biotransformation of albuterol in the lungs. Most of an inhaled dose is deposited on the pharynx after inhalation and then swallowed.

The volume of distribution is 156 ± 38 l and the plasma protein binding is 8%. Albuterol, as both the sulfate and sulfate conjugates (metabolite and unchanged drug), is eliminated via the kidneys. With oral dosing, 28% of albuterol is excreted unchanged in the urine and 64–80% unchanged with intravenous dosing. Albuterol follows first-order kinetics. Total plasma clearance is $0.41\,\mathrm{l\,h^{-1}\,kg^{-1}}$ and renal clearance is $0.28\,\mathrm{l\,h^{-1}\,kg^{-1}}$. The half-life is 3–5 h with oral dosing, 2–7 h with inhalation, and 5.5–6.9 h with intravenous dosing. Maximum brochodilation occurs within 15–30 min.

Side Effects

Tachycardia occurs as a reflex to the drop in mean arterial pressure (MAP) or as a result of β-1 stimulus. β-Adrenergic receptors in the locus ceruleus also regulate norepnephrine-induced inhibitory effects, resulting in agitation, restlessness, and hand tremor. Stimulation of nonpulmonary β_2 receptors may lead to an increase in heart rate, QT_c interval prolongation, nonspecific T-wave changes, skeletal muscle tremor, and slight increases in blood glucose and nonesterified fatty acids. Hypokalemia is more pronounced in patients receiving intravenous albuterol. Hypotension is also known to occur mostly in overdose. The buildup of cyclic AMP in the liver stimulates glycogenolysis and an increase in serum glucose. In skeletal muscle, this process results in increased lactate production. Direct stimulus of sodium/potassium ATPase in skeletal muscle produces a shift

of potassium from the extracellular space to the intracellular space. Relaxation of smooth muscle produces a dilation of the vasculature supplying skeletal muscle, which results in a drop in diastolic and MAP. Myocardial ischemia and infarction have been associated with excessive tachycardia in elderly patients. The skin may be warm and pink with evidence of diaphoresis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Albuterol appears to be relatively benign in animals, similar to human. Agitation, vomiting, and lethargy may be seen. In rats the oral LD_{50} was more than 2000 mg kg^{-1}; and inhalation LC_{50} could not be determined.

Human

A review of albuterol overdoses revealed that up to 20 times the oral daily dose produced no deaths. The effects of albuterol overdose are usually mild and benign, although they can be prolonged. Cardiovascular effects are usually limited to a sinus tachycardia and widened pulse pressure. Although there may be a drop in diastolic pressure, the systolic pressure is maintained by increased cardiac output from the trachycardia. Transient hypokalemia can result, caused by a shift of extracellular potassium to the intracellular space with total body stores of potassium generally remaining normal. A transient metabolic acidosis can be seen due to increased lactate production. Restlessness, agitation, tremors apprehension, dizzines, nausea, vomiting, and dilated pupils are common in albuterol overdose.

Chronic Toxicity (or Exposure)

Human

Continued dependence of salbutamol tablets taken in high doses (30–40 tablets daily and 48–64 mg day^{-1}) has lead to symptoms of toxic psychosis in one elderly woman and paranoid psychosis in a 52-year-old man. Up to 60–90 100 μg inhalations of salbutamol daily has been used by asthmatics who increased doses because they 'needed it' and wanted to 'feel good'. Long-term tolerance develops to bronchodilator action, tremor, tachycardia, prolongation of QT_c interval, hyperglycemia, hypokalemia, and the vasodilator response.

Clinical Management of Overdose

Albuterol overdoses rarely require treatment beyond gastrointestinal decontamination. Children have survived overdoses as large as 100 mg and adults have survived doses up to 240 mg without serious complications. Activated charcoal effectively adsorbs albuterol. The hypokalemia produced reflects a transient shift in potassium location rather than a true deficit of potassium; external replacement therapy is rarely necessary but can be added to intravenous fluids to support the heart if electrocardiographic changes are noted. A conservative approach to tachycardia is recommended since arrhythmias beyond an increase in rate have not occurred with overdose. Support of blood pressure and control of tachycardia are major therapeutic interventions.

See also: Kidney.

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Alcoholic Beverages and Alcoholism

Kartik Shankar and Harihara M Mehendale

Alcohol abuse may be the number one health issue in the United States with annual cost estimates as high as US$ 185 billion a year. Forty four percent of the adult US population are current drinkers having consumed at least 12 drinks in the past year. Although most people who drink do it safely, ~14 million Americans (7.4% of the population) meet the diagnostic criteria for alcohol abuse or alcoholism. More than one-half of American adults have a close family member who has or has had alcoholism. Almost 2.7 million violent crimes and 16 000 traffic crashes can be directly linked to alcohol. Alcohol consumption has consequences for the health and well-being of those who drink and, by extension, the lives of those around them. Because alcoholism

affects many aspects of our society, clearly alcoholism has enormous social implications including burden on social and health services.

In the United States, a 'drink' is considered to be 0.5 ounces (oz) or 15 g of alcohol, which is equivalent to 12 oz of beer, 5 oz of wine, or 1.5 oz of 80 proof distilled spirits. According to the Dietary Guidelines for Americans, jointly issued by the US Department of Agriculture and the US Department of Health and Human Services, moderate drinking is no more than two standard drinks per day for men and no more than one per day for women. Moderate drinking may be defined as drinking that does not generally cause problems, either for the drinker or for society. The term is often confused with 'social drinking', which refers to drinking patterns that are accepted by the society in which they occur. However, social drinking is not necessarily free of problems. The National Institute on Alcohol Abuse and Alcoholism further recommends that people aged 65 and older limit their consumption of alcohol to one drink per day. Alcoholism, also known as 'alcohol dependence', is a disease that includes four symptoms: (1) *Craving*: a strong need, or compulsion, to drink; (2) *Loss of control*: the inability to limit one's drinking on any given occasion; (3) *Physical dependence*: withdrawal symptoms, such as nausea, sweating, shakiness, and anxiety, occur when alcohol use is stopped after a period of heavy drinking; and (4) *Tolerance*: the need to drink greater amounts of alcohol in order to 'get high'.

Health Effects of Alcohol Abuse

Effects of Alcohol on the Liver

There is no question that alcohol abuse contributes significantly to liver-related morbidity and mortality in the United States. Long-term alcohol use is the leading cause of illness and death from liver disease. There are three phases of alcohol-induced liver damage, alcoholic fatty liver, which is usually reversible with abstinence; alcoholic hepatitis or inflammation; and alcoholic cirrhosis or scarring of the liver. Patients with both alcoholic cirrhosis and hepatitis have a death rate of more than 60% over a 4-year period. The prognosis is bleaker than the outlook for many types of cancers. As many as 900 000 people in the United States suffer from cirrhosis and some 26 000 of these die each year. The risk for liver disease is related to how much a person drinks: the risk is low at levels of alcohol consumption but steeply increases with higher levels of consumption. Because effects of alcohol are dose-related and because of the steepness at which the adverse effects are observed, moderation is emphasized in social or occasional drinking. Gender also plays a role in the development of alcohol-induced liver damage. Some evidence indicates that women are more susceptible to the cumulative effects of alcohol on the liver.

Cancer and Alcohol Abuse

Alcohol has been linked to a number of cancers, including cancers of the head and neck, digestive tract, and breast. Alcohol is clearly established as a cause of cancer of various tissues in the airway and digestive tract, including the mouth, pharynx, larynx, and esophagus. Research suggests that the risk of cancers is associated with both the concentration of alcohol and number of drinks consumed. Alcohol acts synergistically with tobacco to dramatically increase the risk of cancers that is above that of alcohol or tobacco alone. An increased risk of stomach cancer among alcohol drinkers has been identified in several but not the majority of studies. The link between alcohol use and chronic gastritis is clear, although the progression from chronic gastritis to neoplasia is less well understood and involves factors in addition to alcohol. Only weak positive association between alcohol use and cancers of the colon, rectum, and breast exists.

Cardiovascular Health and Alcohol Use

Cardiovascular diseases account for more deaths among Americans than any other group of diseases. Of all causes of death, coronary heart disease (CHD) is the leading cause of death among Americans. Several large prospective studies throughout the world suggest a reduced risk of CHD with alcohol use over a wide range of consumption levels. However, in these studies the apparent protective effects of alcohol against CHD were realized at low to moderate levels of alcohol (ranging from one to two drinks per week to one to two drinks per day). However, the risk increased at drinking levels above five drinks a day for men and two drinks a day for women. Both the type of alcoholic beverage consumed and the pattern of drinking (small amounts everyday versus large amounts on only one or two days a week) influence protection against CHD. The relationship between alcohol consumption and stroke risk suggests that heavy drinking increases the risk of stroke, especially in women. However, evidence suggesting that moderate level of alcohol consumption protects from stroke is at best equivocal. In addition, it appears that a high level of alcohol consumption increases blood pressure, a critical risk factor for stroke.

Alcohol and the Skeleton

An association between alcohol intake and accidental injury is well established. The risk of falling is tripled in those having a blood alcohol concentration (BAC) of 0.1–0.15% and 60 times higher in those with a BAC of 0.16% or higher, compared with those whose BAC is 0.1% or lower. Beyond the risks of falling, however, emerging evidence suggests alcoholics may also suffer from a generalized skeletal fragility, leading to alcohol-induced osteopenia. Although the degree to which alcohol contributes to the osteopenia in the general population is not clear, but data from experimental animal studies suggest that alcohol can disrupt the tightly coupled processes of bone formation and resorption.

Fetal Alcohol Syndrome

Fetal alcohol syndrome (FAS) is a set of birth defects caused by maternal consumption of alcohol during pregnancy. FAS is considered the most common preventable cause of mental retardation. The annual cost of FAS according to the 10th Special Report to the US Congress on Alcohol and Health estimated the annual cost of FAS in 1998 to be $2.8 billion.

See also: Ethanol; Fetal Alcohol Syndrome.

Further Reading

Mann RE, Smart RG, and Govoni R (2003) The epidemiology of alcoholic liver disease. *Alcohol Research & Health* 27(3): 209–219.

McIntosh C and Chick J (2004) Alcohol and the nervous system. *Journal of Neurology, Neurosurgery & Psychiatry* Sep: 75 Suppl 3(iii): 16–21.

Room R, Babor T, and Rehm J (2005) Alcohol and public health. *Lancet* Feb 5: 365(9458): 519–530.

Relevant Website

http://www.niaaa.nih.gov – *10th Special Report to the US Congress on Alcohol and Health*. US Department of Health and Human Services.

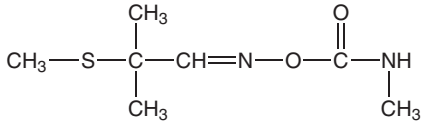
Alcoholism *See* Alcoholic Beverages and Alcoholism.

Alcohols *See* Alcoholic Beverages and Alcoholism; Allyl Alcohol; Benzyl Alcohol; Ethanol.

Aldicarb

Paul R Harp

- Chemical Abstracts Service Registry Number: CAS 116-06-3
- Synonyms: 2-Methyl-2(methylthio)-propionaldehyde *O*-(methylcarbamoyl)oxime; Aldecarb; Aldicarbe; Temik; AI3-27093; ENT 27093; OMS 771; NCI 08640; SHA 098301; UC 21149; RCRA Waste Number P070
- Chemical/Pharmaceutical/Other Class: *N*-Methylcarbamate insecticide
- Chemical Structure:



Uses

Aldicarb is a soil-applied systemic insecticide used to control a wide variety of insects, mites, and nematodes. Major uses include ornamentals, cotton, and some fruit and vegetable crops. Aldicarb is the most acutely toxic insecticide currently registered for use in the United States and is classified as a restricted-use agent that can only be applied by or under supervision of a certified applicator. Certain uses have been voluntarily canceled due to potential for groundwater contamination. Aldicarb is only available as a granular mix to help reduce the hazards associated with handling the product.

Exposure Routes and Pathways

The most common exposure routes are dermal (during processing, packaging, or application) and oral (through consumption of products containing aldicarb residues). Illegal applications to melons and cucumbers have resulted in consumer poisonings. Exposure may also occur through unprotected handling of treated plants or soil. Although inhalation of fine particles and dusts of aldicarb has been reduced through improvements in applicator design, inhalation can still represent a significant route of exposure if equipment that grinds the granules is used during application.

Toxicokinetics

Aldicarb is readily absorbed from all routes of exposure. Oxidation reactions rapidly convert aldicarb to aldicarb sulfoxide, of which a small portion may then be slowly oxidized to aldicarb sulfone. Both the parent compound and its oxidized metabolites can be converted to their respective oximes and nitriles, which may ultimately be converted to aldehydes, acids, and alcohols. Animal studies have indicated aldicarb and its metabolites are distributed to many different tissues but no evidence of accumulation has been found. In the various tissues examined, aldicarb residues were not detected more than 5 days after exposure. The presence of aldicarb in fetal tissue indicates placental transfer in pregnant rats. Various aldicarb metabolites have been found in the milk of cows acutely treated with aldicarb.

Animal studies have indicated the major route of excretion to be urinary with at least 80% of the original dose generally eliminated within 24 h. Aldicarb is excreted primarily as aldicarb sulfoxide and sulfoxide oxime; the parent compound is excreted only in trace amounts. Biliary metabolites have been shown to undergo resorption and urinary excretion.

Mechanism of Toxicity

Aldicarb binds and inhibits acetylcholinesterase, the enzyme responsible for metabolizing the neurotransmitter acetylcholine and terminating its action at cholinergic synapses. Exposure to aldicarb results in synaptic accumulation of acetylcholine in both the central and peripheral nervous systems and hyperstimulation of muscarinic and nicotinic receptors leading to 'cholinergic crisis'. Aldicarb sulfoxide is a more potent inhibitor of acetylcholinesterase than the parent compound. In contrast to the organophosphate anticholinesterases, acetylcholinesterase inhibition by *N*-methylcarbamates is reversible with fairly rapid reactivation occurring through spontaneous decarbamoylation or via hydrolysis of the carbamate.

Acute and Short-Term Toxicity (or Exposure)

Animal

Signs of acute exposure in laboratory animals are similar to those described for humans and recovery from nonlethal exposures occurs rapidly. LD_{50} values for acute exposure in rats are 0.46–1.23 mg kg^{-1} (oral) and 3.2 to >10 mg kg^{-1} (dermal).

Human

The acute effects of aldicarb exposure are due to cholinergic overstimulation and may include the SLUDGE syndrome (salivation, lacrimation, urination, diarrhea, gastrointestinal cramping, and emesis), respiratory depression, bronchospasms, increased bronchial secretions, pulmonary edema, blurred vision, miosis, headache, tremors, muscle fasciculations, convulsions, mental confusion, coma, and death due to respiratory failure. Recovery from nonlethal exposures occurs very rapidly, usually within a few hours.

Chronic Toxicity (or Exposure)

Animal/Human

Researchers have examined the possible effects of aldicarb on the induction of peripheral neuropathies. Currently, insufficient evidence exists to indicate any significant long-term health risk associated with aldicarb exposure. The US Environmental Protection Agency's (EPA) Office of Pesticide Programs has classified aldicarb as group E – evidence of noncarcinogenicity for humans.

Clinical Management

Persons providing medical assistance should avoid contact with contaminated clothing. Contaminated clothing should be removed, bagged, and discarded. Exposed dermal areas should be cleaned thoroughly with soap and water. Exposed eyes should be flushed with generous amounts of clean water for at least 15 min. If necessary, use an endotracheal tube to maintain a clear airway, aspirate any secretions, and provide oxygen via mechanical ventilation.

If the patient is asymptomatic and can be treated soon after exposure, activated charcoal may be used to reduce absorption from the gastrointestinal tract. If potentially life-threatening quantities have been ingested, gastric lavage should be considered if it can be conducted within ~1 h of exposure. Charcoal and/or catharsis are contraindicated in presence of severe vomiting or diarrhea. Muscarinic effects (i.e., SLUDGE) may be reduced by intravenous or intramuscular administration of atropine. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenobarbital may be helpful for recurrent seizures. Pralidoxime is indicated in cases of mixed exposure to both carbamates and organophosphorus compounds but is contraindicated in cases of carbamate-only exposure. Furosemide may be useful for pulmonary edema that continues after full atropinization. Metabolite analysis of a urine sample may allow confirmation of the intoxicating agent.

Ecotoxicology

Aldicarb is highly water soluble and soil application of this insecticide has the potential to result in runoff or leaching of the insecticide or active metabolites and contamination of surface or groundwater. Aldicarb is acutely toxic to bees, birds, and fish. Species-specific rates of bioactivation may influence the sensitivity of a particular organism to this insecticide.

Exposure Standards and Guidelines

The acceptable daily intake for aldicarb is $0.003\,mg\,kg^{-1}\,day^{-1}$. The reference dose for aldicarb is $0.001\,mg\,kg^{-1}\,day^{-1}$, but is currently being reassessed by the US EPA.

See also: Carbamate Pesticides; Cholinesterase Inhibition; Neurotoxicity; Pesticides; Pollution, Water.

Relevant Websites

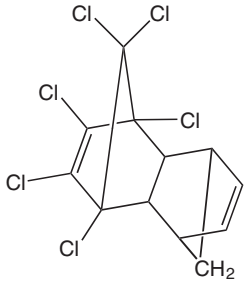
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.
http://www.inchem.org – Environmental Health Criteria 121: Aldicarb–International Programme on Chemical Safety.

Aldosterone *See* Corticosteroids.

Aldrin

Benny L Blaylock

- Chemical Abstracts Service Registry Number: CAS 309-00-2
- Synonyms: 1,2,3,4,10,10-Hexachloro-1,4,4α,5,8,8α-hexahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene; Aldrec; Aldrex; Drinox; Octalene; Seedrin; Compound 118
- Chemical/Pharmaceutical/Other Class: Organochlorine insecticide
- Chemical Formula: $C_{12}H_8Cl_6$
- Chemical Structure:



Uses

Although not currently manufactured for use in the United States, aldrin is used as an insecticide.

Exposure Routes and Pathways

Aldrin is absorbed from the gastrointestinal tract, the respiratory tract, and through the skin.

Toxicokinetics

The most important exposure routes are oral and dermal. Aldrin is readily absorbed through the gastrointestinal tract via the hepatic portal vein and through the skin.

Epoxidation by cytochromes P450 of aldrin to dieldrin occurs in the liver and, to a lesser extent, in the lungs. In mammals, two major metabolism routes of dieldrin seem to be predominant: (1) direct oxidation by cytochrome oxidases, resulting in 9-hydroxydieldrin, and (2) the opening of the epoxide ring by epoxide hydrases, resulting in 6,7-*trans*-dihydroxydihydroaldrin. Like other organochlorine insecticides, adipose tissue is the major storage tissue followed by liver, brain, and blood. The water-soluble metabolites of aldrin detoxification are excreted primarily in the feces via the bile and, to a lesser extent, in the urine. Dieldrin is also found in mothers' milk and can be excreted via that mechanism.

Mechanism of Toxicity

Aldrin and dieldrin characteristically stimulate the central nervous system (CNS) causing hyperexcitation and generalized seizures. Both *in vitro* experiments using rat brain membranes and intravenous or intraperitoneal administration of aldrin and dieldrin to rats have shown that these agents are capable of blocking the activity of GABA by blocking the influx of chloride through the $GABA_A$ receptor–ionophore complex.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal toxicity with aldrin is similar to dieldrin. Convulsion and incoordination are frequently observed. Acute symptoms observed in ducks, pheasants, and bobwhite quail following acute oral LD_{50} exposure were ataxia, low carriage, nictitating membrane closed for long periods, fluffed feathers, tremors, phonation, violent wing-beat convulsions, seizures, and opisthotonos. Death occurred 0.5 h to 10 days posttreatment. Weight losses occurred among survivors of higher levels. Gross autopsies revealed occasional liver adhesions to parietal peritoneum.

Human

The toxicity of aldrin is essentially that of dieldrin and similar to other cyclodiene insecticides. The CNS is the primary target with convulsions as the major symptom. Patients may also experience nausea, vomiting, hyperexcitability, and coma. Onset of symptoms may be between 20 min and 12 h after ingestion and include malaise, headache, nausea, vomiting, dizziness, and tremors. This may progress to clonic and tonic convulsions, sometimes without premonitory symptoms. Convulsive episodes may alternate with periods of severe central nervous depression. A 3-year-old girl who was stricken 5 min after eating a cooked meal contaminated with aldrin and who died 12 h later was thought to have consumed 120 mg of aldrin, resulting in a dosage of $\sim 8.2\,mg\,kg^{-1}$. A 23-year-old man who intentionally drank aldrin at a dose of $25.6\,mg\,kg^{-1}$ was very seriously poisoned, although eventually he recovered completely.

Chronic Toxicity (or Exposure)

Animal

Aldrin administered daily to 30- and 90-day-old Wistar rats at 8 or 11 ppm in diet showed an alteration in antibody production when exposed to *Escherichia coli* at 60 days. Functional disorders of thymus and adrenal glands and in protein synthesis were also noted. Younger rats were more strongly affected than older rats. Cats fed aldrin at $1\,mg\,kg^{-1}\,day^{-1}$ or made to inhale $0.1\,\mu g\,l^{-1}$ of air had marked lowering of conditioned reflexes and of unconditioned food and orientation reflexes, which required up to 8 days to return to normal. Groups of 12 male and 12 female Osborne–Mendel rats were fed diets containing 0, 0.5, 2, 10, 50, 100, or 150 ppm recrystallized aldrin for 2 years. Considering together groups given 0.5, 2, or 10 ppm (i.e., the groups showing survival rates at 2 years comparable to those of controls), the number of tumor-bearing animals was 25/60 compared with 3/17 controls. Among treated animals, 12 developed lymphomas, 13 had mammary tumors (malignant in four rats), two had fibrosarcomas, and three had tumors at other sites. The three tumor-bearing control rats had, respectively, a pulmonary lymphoma, a benign mammary tumor, and a tumor at another site.

Human

Among workers who have been engaged in the manufacture, handling, and spraying of aldrin, only acute effects such as eye, skin, or respiratory irritation were reported, particularly following exposures to dusty formulations of the compound. High worker exposure was associated with induction of liver microsomal enzymes and the ability of some highly exposed workers to increase their drug metabolizing capacity. Frank liver injury or injury to other human organs has not been reported in the United States, Canadian, and western European literature. Aldrin is not classifiable as to its carcinogenicity to humans by International Agency for Research on Cancer.

Clinical Management

Treatment is symptomatic. Activated charcoal as a slurry has been reported to absorb aldrin and increase its rate of excretion after oral exposure. Emesis is not recommended due to potential CNS depression or seizures. Diazepam or phenobarbital is used when anticonvulsant therapy is necessary.

Environmental Fate

Aldrin binds strongly to soil particles and is very resistant to leaching into groundwater. Volatilization is an important mechanism of loss from the soil. Due to its persistent nature and hydrophobicity, aldrin is known to bioconcentrate, mainly as its conversion products. As aldrin is readily and rapidly converted to dieldrin in the environment, its fate is closely linked to that of dieldrin. Aldrin is readily metabolized to dieldrin in both animals and plants, and therefore aldrin residues are rarely present in animals and then only in very small amounts. Residues of aldrin have been detected in fish in Egypt; the average concentration was $8.8\,\mu g\,kg^{-1}$, and a maximum concentration of $54.27\,\mu g\,kg^{-1}$. Aldrin has

low phytotoxicity, with plants affected only by extremely high application rates.

Ecotoxicology

The acute toxicity of aldrin to avian species varies in the range of $6.6\,mg\,kg^{-1}$ for bobwhite quail to $520\,mg\,kg^{-1}$ for mallard ducks. Aldrin-treated rice is thought to have been the cause of deaths of waterfowl, shorebirds, and passerines along the Texas Gulf Coast, both by direct poisoning by ingestion of aldrin-treated rice and indirectly by consuming organisms contaminated with aldrin. Residues of aldrin were detected in all samples of bird casualties, eggs, scavengers, predators, fish, frogs, invertebrates, and soil.

The toxicity of aldrin to aquatic organisms is quite variable, with aquatic insects being the most sensitive group of invertebrates. The 96 h LC_{50} values range from 1 to $200\,\mu g\,l^{-1}$ for insects and from 2.2 to $53\,\mu g\,l^{-1}$ for fish.

Other Hazards

Aldrin is corrosive to metals, owing to the slow formation of hydrogen chloride during storage. It is also noncombustible as the substance itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.0001\,mg\,kg^{-1}\,day^{-1}$.
- Reference dose is $0.03\,mg\,kg^{-1}\,day^{-1}$.
- Permissible exposure limit is $0.25\,mg\,m^{-3}$ (8 h).

See also: Charcoal; Cyclodienes; Diazepam; Dieldrin.

Further Reading

Jorgenson JL (2001) Aldrin and dieldrin: A review of research on their production, environmental deposition and fate, bioaccumulation, toxicology, and epidemiology in the United States. *Envionmental Health Perspectives* 109(suppl 1): 113–139.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Aldrin.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Aldrin.
http://www.osha-slc.gov – US Department of Labor, Occupational Safety and Health Administration.

Algae

Keiko Okamoto and Lora E Fleming

Toxins discussed in this section are produced by microscopic, simple aquatic organisms classified as algae. Algae are photoautotrophic, which means they obtain energy and nourishment from light. Included in algae are organisms also commonly referred to as phytoplankton, dinoflagellates, and diatoms. Some of these tiny organisms produce very potent toxins. This section focuses on the following algal toxins that are fairly well characterized in terms of adverse effects known to occur in humans and laboratory animals: azaspiracids, brevetoxins, ciguatoxins, maitotoxins, domoic acid, okadaic (or okadeic) acid, saxitoxins and other cyanobacterial toxins. Azaspiracids, brevetoxins, ciguatoxins, and okadaic acid are all classified chemically as polyether toxins. Domoic acid is an excitatory neurotoxic amino acid.

Commonly used synonyms for these sources and types of toxicity include: azaspiracid shellfish poisoning (AZP) caused by five azaspiracid analogs; neurotoxic shellfish poisoning (NSP) caused by 10 known brevetoxins; ciguatera fish poisoning (CFP or Ciguatera) caused by more than 10 ciguatoxin congeners and maitotoxins; amnesic shellfish poisoning (ASP) caused by one or more of three domoic acid derivatives; paralytic shellfish poisoning (PSP) caused by at least 20 derivatives of saxitoxins; diarrheic shellfish poisoning (DSP) caused by okadaic acid, and other toxins too numerous to detail in this brief overview (e.g., six derivatives of dinophysistoxin, four pectenotoxins (polyether lactones), and yessotoxins (including two sulfate esters which resemble brevetoxins); and finally, red tides; harmful algal blooms (HAB), dinoflagellate blooms, and phycotoxins.

Exposure Routes and Pathways

A major route of human exposure to algal toxins is through the consumption of contaminated seafood products. The consumption of contaminated clams, mussels, scallops, oysters, and other shellfish causes shellfish-associated diseases (ASP, AZP, DSP, NSP,

PSP). Consuming contaminated large reef fish, like barracuda and grouper, causes CFP. Consumption of puffer fish with saxitoxin through shellfish feeding has resulted in cases of PSP.

Inhalation exposure of airborne toxins is also known to occur. For example, the *Karenia brevis* organism that produces brevetoxin is relatively fragile and easily broken apart, particularly in wave action along beaches, thus releasing the toxin. During an active nearshore red tide, the water and aerosols of contaminated salt spray will contain the toxins and organism fragments both in the droplets and attached to salt particles. These airborne particulates can cause respiratory irritation in humans on or near beach areas, and also be carried inland depending on wind and other environmental conditions. The use of particle filter masks or retreat to an air-conditioned environment may provide protection from toxicity.

Ciguatera, caused by ingested ciguatoxins and maitotoxins, can reportedly be sexually transmitted. There are also reports of acute health effects of ciguatera toxin in the fetus and newborn child exposed through placental and breast milk transmission from the mother.

Humans can also be exposed to cyanobacteria and their toxins through direct skin contact or by drinking contaminated water. Other possible routes of exposure include inhalation of contaminated aerosols, consumption of contaminated food, and even through dialysis. Therefore, occupational exposures for fisherman, watermen, and scientists, as well as recreational exposures for the general public, are all possible.

Toxicokinetics

The fate and metabolism of algal toxins is unclear and understudied; however, it is known that the absorption of both lipophilic and hydrophilic algal toxins occurs rapidly from the gastrointestinal and respiratory tracts. For example, to evaluate brevetoxin toxicokinetics from acute inhalation exposure up to 7 days, 12-week-old male F344/Crl BR rats were exposed to a single dose of 6.6 $\mu g\,kg^{-1}$ the brevetoxin PbTx-3 through intratracheal instillation. More than 80% of PbTx-3 was rapidly cleared from the lung and distributed by the blood throughout the body, particularly the skeletal muscle, intestines, and liver with low but constant amounts present in blood, brain, and fat. Approximately 20% of the toxin was retained in the lung, liver, and kidneys for up to 7 days. Absorption of many of the cyanobacterial toxins occurs rapidly from the gastrointestinal tract. The greatest concentrations are found in the liver; some are found in the kidney and remain detectable for up to 24 h.

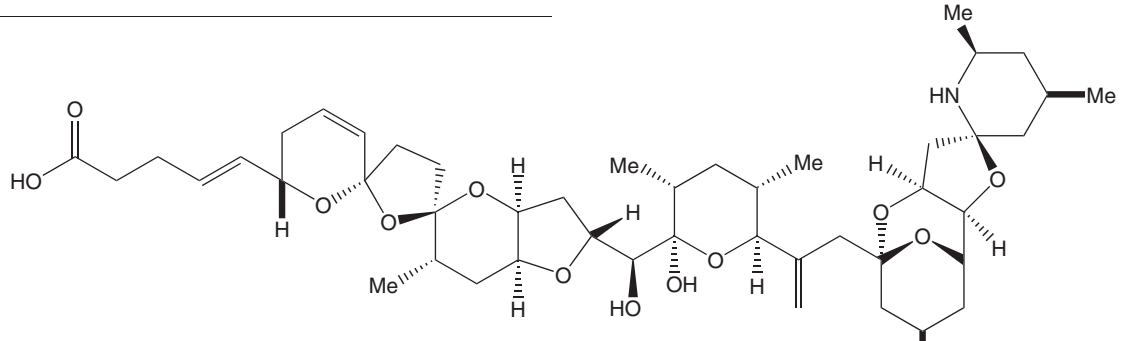
Acute and Chronic Toxicity and Mechanisms of Action

In general terms, people suffering from signs and symptoms of illnesses associated with eating seafood contaminated with algal toxins typically present the acute onset of gastrointestinal symptoms within minutes to 24 h. Victims may also exhibit a wide range of signs and symptoms involving many organ systems, including respiratory (difficulty breathing), peripheral nervous system (numbness and tingling), central nervous system (hallucinations and memory loss), and cardiovascular system (fluctuating blood pressure and cardiac arrythmias). These signs and symptoms, depending on the particular disease, may last from hours to months.

Chronic algal toxin exposure remains mostly unstudied, although some limited information about specific toxins is included in the descriptions that follow. On the other hand, exactly how some of these toxins affect cells and tissues (mechanism of action) has received considerable attention from researchers.

Azaspiracids

Azaspiracids (produced by *Protoperidinium*) are a relatively new class of polyether toxins. Consequently, little information is known yet about the AZP mechanism of toxicity except that it appears to be hepatotoxic.



Azaspiracid

The first human acute intoxications attributed to newly described azaspiracid poisoning (AZP) occurred in the Netherlands after consumption of contaminated shellfish. The symptoms were similar to the nausea, vomiting, severe diarrhea, and stomach cramps of DSP. AZP in humans has been reported throughout Europe since 1995, and azaspiracids have been found in shellfish harvested in Spain, France, and northern Europe.

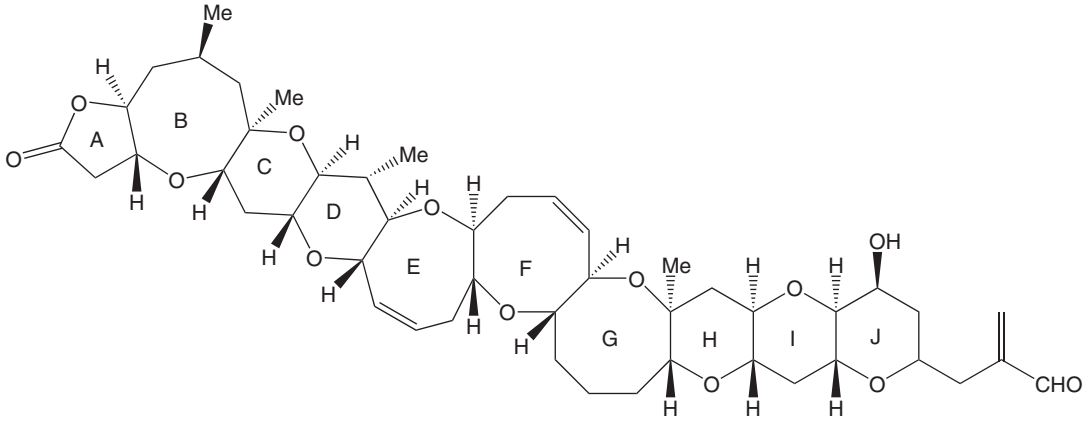
Chronic effects observed in mice after oral administration of azaspiracid were interstitial pneumonia, shortened villi in the stomach and small intestine, fatty changes in the liver, and necrosis of lymphocytes in the thymus and spleen.

Brevetoxins

Several brevetoxins (synonyms: PbTx-1, PbTx-2, PbTx-3, PbTx-4, PbTx-5, PbTx-6, PbTx-7, PbTx-8, PbTx-9) are produced by *Karenia brevis,* formerly known as *Gymnodinium breve* and *Ptychodiscus brevis*. Both brevetoxins and ciguatoxins (see below) open voltage-dependent sodium channels in cell membranes, leading to uncontrolled sodium influx into the nerve cells and striated muscle cells. Brevetoxins and ciguatoxins cause biphasic cardiovascular response with hypotension and bradycardia followed by hypertension and tachycardia. The respiratory arrest induced by a lethal dose results mainly from depression of the central respiratory center. Although evidence suggests that brevetoxins affect mammalian cortical synaptosomes and neuromuscular preparations, the majority of toxic effects associated with brevetoxins predominantly appear to result from the substantial and persistent depolarization of nerve membranes. In the lung, brevetoxin appears to be a potent respiratory toxin involving both cholinergic and histamine-related mechanisms.

Fish, birds, and mammals are all susceptible to brevetoxins. In Japanese medaka fish (*Oryzias latipes*), brevetoxins induce embryonic toxicity and developmental abnormalities. The fish are killed apparently through lack of muscle coordination and paralysis, convulsions, and death by respiratory failure. In the mosquito fish (*Gambusia affinis*) bioassay, the lethal dose (LD_{50}) is reported at $0.011\,\mu g\,l^{-1}$. Exposed birds die acutely with neurologic and hematologic effects. Brevetoxins were implicated in the deaths of manatees in Florida during a widespread bloom of *G. breve*. At necropsy, the animals did not appear to be unhealthy, and they had recently fed. High levels of brevetoxin were found by histochemical stain in cells throughout the body, particularly macrophages. The mouse LD_{50} is $0.17\,mg\,kg^{-1}$ body weight intraperitoneally, $0.094\,mg\,kg^{-1}$ body weight intravenously, and $0.520\,mg\,kg^{-1}$ body weight orally.

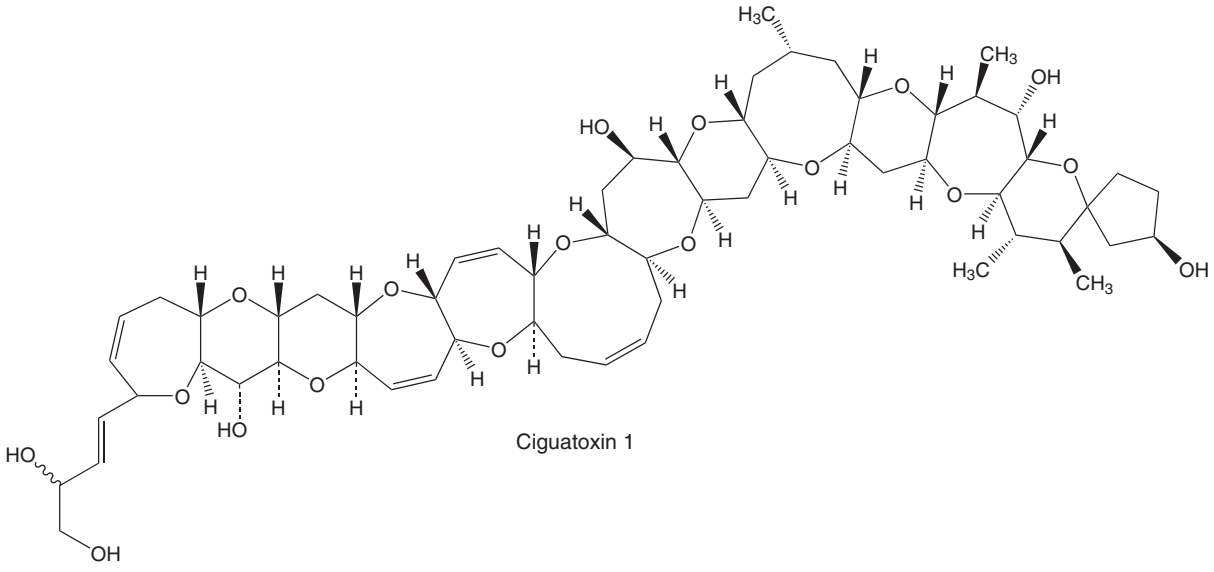
The two forms of brevetoxin-associated clinical effects first characterized in Florida are (1) an acute gastroenteritis with neurologic symptoms following ingestion of contaminated shellfish (aka neurotoxic shellfish poisoning (NSP)), and (2) an apparently reversible upper respiratory syndrome (conjunctival irritation, copious catarrhal exudates, rhinorrhea, nonproductive cough, and bronchoconstriction) following inhalation of contaminated aerosols. Recovery is reportedly complete in few days, although persons with chronic pulmonary disease such as asthma may experience more severe and prolonged respiratory effects. In addition, skin and eye irritation by environmental exposures among people living or visiting Florida during *K. brevis* bloom has been reported. NSP and the respiratory irritation associated with aerosolized brevetoxins have both been reported along the Gulf of Mexico as well as far north as North Carolina; similar brevetoxin-associated syndromes have been reported in New Zealand.



Brevetoxin A

Ciguatoxins

Ciguatoxins are produced by *Gambierdiscus toxicus*. Lipid-soluble ciguatoxins and brevetoxins have immunologic cross reactivity, and thus have similar epitopic sites and mechanisms of action, as described in the previous section.



The LD_{50} in mice for ciguatoxin CTX-1 is $0.25\,mg\,kg^{-1}$ body weight when injected intraperitoneally. Ciguatoxins are reported to induce developmental toxicity in Japanese medaka fish (*O. latipes)*.

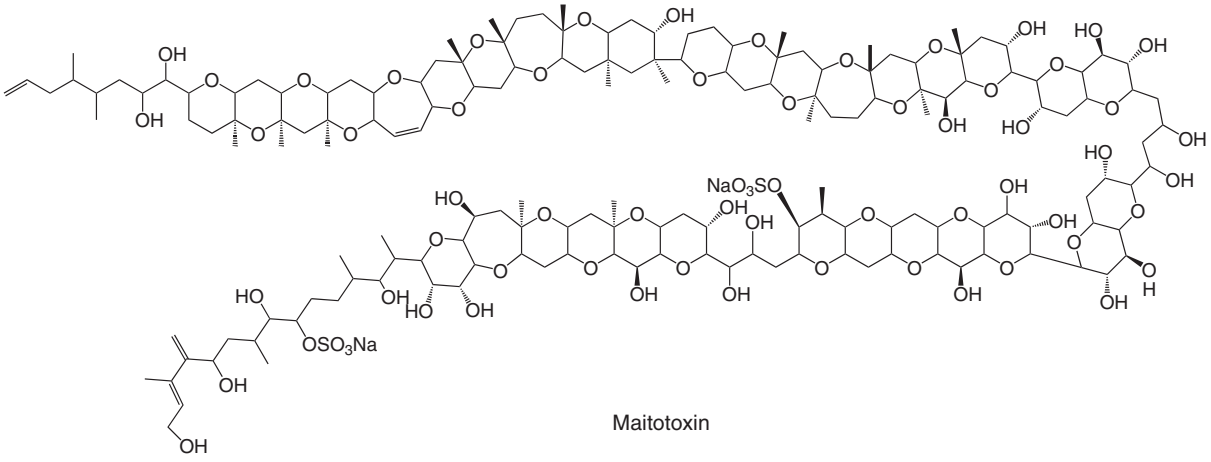
Ciguatoxin fish poisoning caused by ciguatoxins (and also by maitotoxins, see below) is the most commonly reported marine toxin disease in the world. It presents primarily as an acute neurologic disease manifested by a constellation of gastrointestinal (diarrhea, abdominal cramps, vomiting), and cardiovascular (arrhythmias, heart block) signs and symptoms within a few hours of contaminated fish ingestion, followed by neurologic (paresthesias, pain in the teeth, pain on urination, blurred vision, temperature reversal) within hours to days. Reportedly neurologic symptoms may precede the gastrointestinal symptoms in Pacific CFP. Acute fatality, usually due to respiratory failure, circulatory collapse or arrhythmias is reported. Lethality is usually seen with ingestion of the most toxic parts of fish (liver, viscera, roe). The minimal lethal dose for a person weighing 165 lbs is less than $1\,\mu g\,kg^{-1}$. Those surviving ciguatera intoxication, especially in the Caribbean, suffer for weeks to months with debilitating neurologic symptoms, including profound weakness, temperature sensation changes, pain, and numbness in the extremities. CFP outbreaks typically occur in a circumglobal belt extending approximately from latitude 35°N to 34°S, which includes Hawaii, the South Pacific including Australia, the Caribbean, and the Indo-Pacific, although the transport of contaminated fish and tourism have led to cases of CFP in both North American and Northern Europe.

Chronic ciguatera can present as a psychiatric disorder of general malaise, depression, headaches, muscular aches, and peculiar feelings in extremities for several weeks to months. This may be due to prolonged debilitating paresthesias ranging from extreme fatigue to pain in the joints and changes in temperature sensation that can last from weeks to months, and possibly to years. It is reported anecdotally that those with chronic symptoms seem to have recurrences of their symptoms with the ingestion of fish (regardless of type), ethanol, caffeine, and nuts up to 3–6 months from initial ingestion of ciguatera.

Maitotoxins

Maitotoxin precursors are also produced by *G. toxicus*, *Prorocentrum* spp., *Ostereopsis* spp., *Coolia monotis*, *Thecadinium* sp., and *Amphidinium carterae*.

In smooth muscle and skeletal muscle exposed *in vitro*, maitotoxins cause calcium ion-dependent contraction. Water-soluble maitotoxins increase the calcium ion influx through the excitable membrane. These toxins possess a specific calcium-dependent action, which causes a release of norepinephrine from rat pheochromocytoma cells. This action occurs in



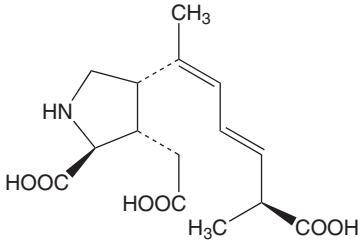
Maitotoxin

the absence of sodium ions and in the presence of tetrodotoxin, precluding the participation of sodium channels. Maitotoxin appears to exert its effects on endogenous membrane calcium channels.

Maitotoxin is lethal in mice at a dose of $0.15\,\mu g\,kg^{-1}$ body weight intraperitoneally.

Domoic Acid

Domoic acid (CAS 14277-97-5, $C_{15}H_{21}NO_6$) is produced by *Nitzchia pungens*. The toxin accumulates in the hepatopancreas of mussels, scallops, and other filter-feeding shellfish. Heat-stable neurotoxic domoic acid is similar in structure to the excitatory dicarboxylic amino acid, kainic acid, and has an antagonistic effect at the glutamate receptor. Domoic acid acts as a potent excitatory neurotransmitter, and it binds to excitatory amino acid receptors in the central nervous system in invertebrates.



Domoic acid

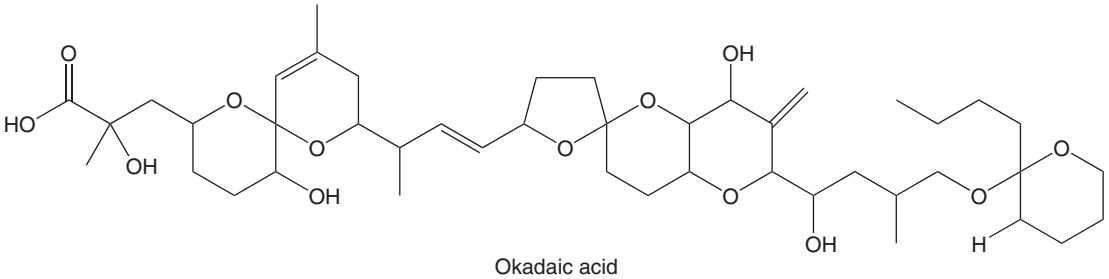
In 1998, domoic acid toxicity was reported in California sea lions. Predominantly neurological signs were observed, which included severe seizures that resulted in opisthotonus (spasm in which the head, neck and back are arched backward), then death. Domoic acid has also been implicated in the deaths of marine mammals and birds in the Pacific Northwest of the US coast. The mouse LD_{50} of domoic acid is $3.6\,mg\,kg^{-1}$ when injected intraperitoneally.

In humans, the acute symptoms of ASP caused by domoic acid include vomiting, abdominal cramps, diarrhea, severe headache, and loss of short-term memory. In some cases, confusion, memory loss, disorientation, and even coma are reported. In addition, seizures and myoclonus are observed acutely. Permanent neurologic sequelae, especially cognitive dysfunction, were reportedly most likely in persons who developed neurologic illness within 48 h, males, in older patients (>60 years), and in younger persons with pre-existing illnesses such as diabetes, chronic renal disease, and hypertension with a history of transient ischemic attacks. The first human cases of ASP were identified after an outbreak in Prince Edward Island, Canada; since then, there have been cases of ASP in marine mammals and birds in the Pacific Northwest of the United States and Canada.

Although no long-term follow-up has been done with ASP victims, the short-term memory loss associated with ASP appeared to be permanent.

Okadaic Acid

Okadaic acid (or okadeic acid, CAS 78111-17-8, $C_{44}H_{68}O_{13}$) is produced by dinoflagelates *Dinophysis* sp. and *Prorocentrum lima*. Lipophilic okadaic acid is a potent inhibitor of protein phosphorylase phosphatase-1 and -2A in the cytosol of the mammalian cells that dephosphorylates serine and threonine. Okadaic acid is thought to induce diarrhea by stimulating the phosphorylation of proteins that controls sodium secretion by intestinal cells, or by increasing phosphorylation of elements that regulate permeability to solutes, resulting in a passive loss of fluids. Okadaic acid also acts through the variation in cellular concentration of the calcium second messenger; it strongly increases the L-type inward calcium current in isolated guinea pig cardiac myocytes.



Okadaic acid

Diarrheic shellfish poisoning caused by okadaic acid is a gastrointestinal illness without chronic sequellae. There is no evidence of neurotoxicity and no fatal cases have ever been reported. Diarrhea is the most commonly reported symptom, closely followed by nausea and vomiting with onset 30 min to 12 h from ingestion of contaminated shellfish. Complete clinical recovery is seen even in severe cases within 3 days. DSP has been reported predominantly in Japan and Europe. The toxin has also been isolated from *P. lima* cultures from the Gulf of California, Mexico. The incidence of DSP in this location as a result of this is unknown.

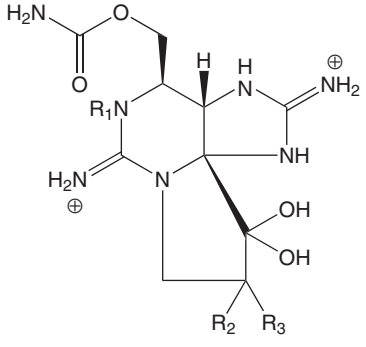
Okadaic acid is a promoter for skin tumors in mice. It also increases gonadal tumors in shellfish and is hepatoxic in other animals. In humans, okadaic acid induces apoptosis and has been linked to gastrointestinal cancer, both the evidence for this is not very strong.

Saxitoxins

Saxitoxins (see structure for Saxitoxin or SXT, CAS 35523-89-8, $C_{10}H_{17}N_7O_4$) are produced by *Alexandrium* spp. (*Gonyaulax*) and several of the cyanobacteria. Heat-stable neurotoxic saxitoxins have a relaxant action on vascular smooth muscle. They inhibit the temporary permeability of sodium ion influx by binding 1:1 with high affinity to a specific receptor site on the outside surface of the membrane in close proximity to the external orifice of the sodium channel. In fact, neurophysiologic studies using saxitoxin as a probe helped to show that sodium and potassium act independently with separate membrane channels. By preventing sodium ions from passing through the membranes of nerve cells, saxitoxins interfere with the transmission of signals along the nerves. A widespread blockage of the sodium ion channels prevents impulse generation in peripheral nerves and skeletal muscles. Saxitoxins have a direct effect on skeletal muscle by blocking the muscle action potential without depolarizing cells; they abolish peripheral nerve conduction with no curare-like action at the neuromuscular junction.

Typical neurologic effects induced by saxitoxins are nervousness, ataxia, convulsions, and paralysis. The paralysis of respiratory muscles can lead to the death of mice within a few minutes. The mouse LD_{50} is 3–9 $\mu g\,kg^{-1}$ intraperitoneally, and 263 $\mu g\,kg^{-1}$ orally. Death occurs within minutes as a result of respiratory failure.

Paralytic shellfish poisoning caused by saxitoxins presents with both gastrointestinal and neurologic symptoms. Five to 30 min after consumption of contaminated mollusks, there is a slight perioral tingling progressing to numbness that spreads to the face and neck. Other symptoms include headache, dizziness, nausea, vomiting, rapid pain, and anuria. In severe cases, there is the onset of severe



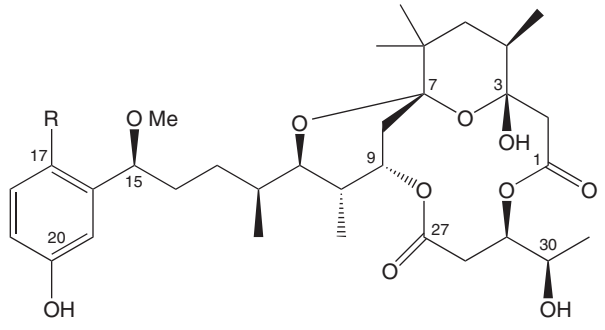
STX	R_1	R_2	R_3
STX	H	H	H
GTX-II	H	H	OSO_3^-
GTX-III	H	OSO_3^-	H
NeoSTX	OH	H	H
GTX-I	OH	H	OSO_3^-
GTX-IV	OH	OSO_3^-	H

Saxitoxin = STX, where R_1 = H, R_2 = H, R_3 = H

incoordination of the extremities and respiratory difficulty. In addition, there are medullary disturbances evidenced by difficulty swallowing, sense of throat constriction, speech incoherence, or complete loss of speech, as well as brain stem dysfunction. Access to emergency medical services in acute cases is crucial to prognosis. In very severe cases, within 2–12 h, there is complete paralysis and death from respiratory failure in the absence of ventilatory support. After 12 h, regardless of severity, victims start to recover gradually and are without any residual symptoms within a few days, although long-term studies of possible chronic effects have never been performed. The oral dose in humans for death is 1–4 mg (5000–20 000 mouse units) depending upon the age and physical condition of the patient. Historically, PSP has occurred in North America (the Pacific Northwest and the Northeast) and Europe. More recently, PSP has been reported in Japan, Malaysia, the Philippines, Indonesia, Latin America, and China.

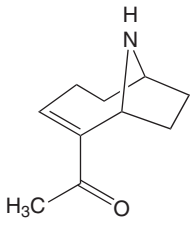
Cyanobacteria Toxins

Cyanobacteria toxins (sometimes referred to as blue green algal toxins) are represented in this entry by Aplysiatoxins, which are toxic to the skin, and anatoxin a (CAS 64285-06-9, $C_{10}H_{15}NO$) and anatoxin a (S) (very fast death factor), which are neurotoxins. Saxitoxin, discussed earlier, and neosaxitoxin are both neurotoxins that may also be classified as cyanobacterial toxins. A large variety of other toxins is produced by cyanobacteria, but is not as well documented. These include: lyngbyatoxin (dermatotoxic); cyclic peptides; predominantly microcystins, nodularins, and cylindrospermopsin (hepatotoxins); endotoxins; and other substances as yet undescribed, including additional tumor promoters.

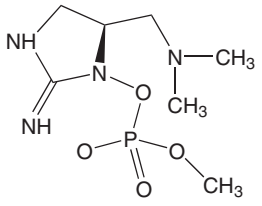


R = Br Aplysiatoxin
R = H Debromoaplysiatoxin

Aplysiatoxin a



Anatoxin a



Anatoxin a (S)

There are at least 12 different species of cyanobacteria that have been shown to produce toxins, often several different toxins per species. The main toxin-producing species include *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis raciborskii*, *Gloeotrichia*, *Hapalosiphon*, *Lyngbia*, *Nodularia*, *Nostoc*, *Oscillatoria*, *Schizothrix*, *Spirulina*, *Synechocystis*, and *Microcystis*.

Dermatotoxins associated with some cyanobacteria algal blooms are potent tumor promoters and protein kinase C activators. The neurotoxin, anatoxin a, acts like the neurotransmitter acetylcholine except that it cannot be degraded by acetylcholinesterase; anatoxin a (S) is a natural organophosphate, binding to the acetylcholinesterase enzymes. Hepatotoxins (e.g., microcystin, cylindrospermpsin and nodularins) are particularly toxic to the liver in part due to selective transport mechanisms that concentrate these toxins from the gut and blood into the liver cells; they damage the liver by deranging the cytoskeletal architecture of the hepatocytes. Microcystin is also believed to cause damage to cell DNA by the activation of endonucleases. Cylindrospermopsin is a protein synthesis inhibitor resulting in widespread necrosis of the tissues of many organs. Microcystins and nodularins are protein phosphatase inhibitors, besides being potent tumor promoters in animals (similar to the carcinogen, okadaic acid). Microcystins cause liver necrosis leading to death within hours to days.

Toxic blooms of cyanobacteria with associated animal poisonings have been reported in all continents except Antarctica. There have been frequent reports of thirsty domestic animals and wildlife consuming freshwater contaminated with toxic cyanobacterial

algal blooms, and dying within minutes to days from acute neurotoxicity and/or hepatotoxicity. Mammals and birds appear to be more susceptible to cyanobacterial algal toxins than aquatic invertebrates and fish, with some species variability. Experimentally, acute high-dose administration of microcystin can lead to death from hepatoencephalopathy within hours.

Prolonged morbidity and mortality have been reported in animals exposed to cyanobacterial algae in the wild. Chronic administration of sublethal amounts of *Microcystis* (a cyanobacterial algae which produces microcystin) extracts in drinking water to mice resulted in increased mortality with chronic active liver disease, even at fairly low doses and in relatively short time periods in the laboratory. Studies in mice have also shown that some cyanobacterial algal toxins cause precancerous damage to both the liver and the bowel. In the laboratory experimental animals, teratogenic activity has been demonstrated with oral administration of *Microcystis* extracts; ~10% of otherwise normal neonatal mice had small brains with extensive hippocampal neuronal damage.

There are individual case reports of persons exposed through swimming to cyanobacterial algal blooms with skin irritation and allergic reactions (both dermatologic and respiratory) with continued positive reaction on skin testing. In particular, urticaria (hives), blistering, and even deep desquamation of skin in sensitive areas like the lips and under swimsuits have been reported, especially with *Lyngbya majuscula* in tropical areas. Consumption of or swimming in cyanobacterial toxin-contaminated waters has also yielded increased case reports of gastrointestinal symptoms, especially diarrhea. One severe outbreak in Brazil was associated with lethality from hepatotoxicity in dialysis patients exposed to water contaminated with microcystins; another outbreak in Australia was also associated with lethality from hepatorenal syndrome in children and adults exposed to contaminated drinking water. In addition to gastrointestinal and dermatologic symptoms, eye irritation, asthma, and 'hay fever symptoms' have been reported repeatedly with exposure to contaminated recreational water exposure in the United States, Canada, UK, and Australia.

The chronic effects of exposure to small quantities of cyanobacterial algal toxins are still under study. In the mid-1980s, studies were done in China, where people were drinking untreated water contaminated with cyanobacterial algal toxins. It was found that drinking contaminated pond and ditch water was associated with high rates of liver cancer. When the quality of drinking water sources was improved in these areas, the rate of liver cancer decreased. How many cases of liver cancer can be attributed to cyanobacterial algal toxins in the United States (where drinking water is currently of higher quality) remains unknown.

Clinical Management

Very little clinical research has been conducted to determine effective treatments. Medical care is primarily supportive.

Medical treatment of CFP has been to a large extent symptomatic; a variety of agents, including vitamins, antihistamines, anticholinesterases, steroids, and tricyclic antidepressants, have been tried with limited results. If given within 3 days of exposure, i.v. mannitol (1 mg kg^{-1} given rapidly over 1 h) has been demonstrated in a single blinded control trial to resolve acute symptoms and prevent chronic symptoms, although repeated administrations may be necessary if symptoms return; a more recent clinical trial did not find an effect, however this trial included subjects treated long after the initial 3 day window. Gut emptying and decontamination with charcoal has been recommended, although often the severe ongoing vomiting and diarrhea prevents this. Atropine is indicated for bradycardia, and dopamine or calcium gluconate for shock. It is recommended that opiates and barbituarates be avoided since they may cause hypotension, and opiates may interact with maitotoxins. Amitriptyline (25–75 mg bid) and similar medications do seem to have some success in relieving the symptoms of chronic ciguatera such as fatigue and paresthesias. It is possible that nifedipine may be appropriate as a calcium channel blocker to counteract the effects of maitotoxins. Anecdotal food avoidance as mentioned above is also recommended. In addition, there is no immunity to these illnesses, and recurrences of actual ciguatera in the same individual appear to be worse than the initial illnesses. A rapid, accurate diagnosis and treatment of CFP within the first 72 h after exposure may be critical in preventing some of the neurologic symptoms that might otherwise become chronic and debilitating.

The treatment of DSP caused by okadaic acid is symptomatic and supportive. In general, hospitalization is not necessary; fluid and electrolytes can usually be replaced orally.

Supportive measures are the basis of treatment for PSP that is caused by saxitoxins, especially ventilatory support in severe cases. In animals, artificial respiration is the most effective treatment. Up to 75% of severely affected persons die within 12 h without supportive treatment. When the ingestion of contaminated food is recent, gut decontamination by the gastric lavage and administration of activated

charcoal or dilute bicarbonate solution is recommended. Care must be taken concerning aspiration with the neurologically compromised patient.

In general, the only treatment available for exposure to cyanobacterial algal toxins is supportive medical treatment after complete removal from exposure. If the exposure was oral, administration of activated carbon to decrease gut absorption may be efficacious if given within hours of exposure. Based on past outbreaks, monitoring of volume, electrolytes, liver and kidney function should all be considered in the case of acute gastroenteritis associated with some of the cyanobacterial algal toxins.

Exposure Standards and Guidelines

Global seafood safety standards have not been established. In the United States, US Food and Drug Administration (FDA) enacted the Hazard Analysis and Critical Control Points (HACCP) program of 1997. The FDA has established action levels in suspected seafood for the toxins causing some of the shellfish poisonings (see **Table 1**). When an action level is reached, the HACCP plan must be followed to prevent unsafe products from reaching the consumer.

Table 1 US FDA action levels in seafood for the toxins associated with shellfish poisonings

Shellfish poisoning	*US FDA action level*
NSP	20 mouse units $100\,g^{-1}$ shellfish brevetoxin-2 equivalent
ASP	20 ppm domoic acid; 30 ppm domoic acid in viscera of Dungeness crab (*Cancer magister*)
DSP	0.2 ppm okadaic acid plus 35-methyl okadaic acid (diphysistoxin-1)
PSP	0.8 ppm saxitoxin equivalent[a]

[a] The amount of total PSP toxins equivalent in toxicity to 0.8 ppm saxitoxin.

Source: Backer LC, Schurz-Rogers H, Fleming LE, Kirkpatrick B, and Benson J (2004) Toxins in food. In: Dabrowski W (ed.) *Phycotoxins in Marine Seafood,* Ch. 7. Boca Raton, FL: CRC Press.

See also: Ciguatoxin; Saxitoxin.

Further Reading

Backer LC, Fleming LE, Rowan A, and Baden D (2003) Epidemiology and public health of human illness associated with harmful marine phytoplankton. In: Hallegraeff GM, Anderson DM, and Cembella AD (eds.) *UNESCO Manual on Harmful Marine Algae*, pp. 725–750. Geneva, Switzerland: UNESCO/WHO.

Blythe DG, Hack E, Washington G, and Fleming LE (2001) The medical management of seafood poisoning. In: Hui YH, Kits D, and Stanfield PS (eds.) *Seafood and Environmental Toxins*, pp. 311–319. New York, NY: Dekker.

Fleming LE, Backer L, and Rowan A (2002) The epidemiology of human illnesses associated with harmful algal blooms. In: Baden D and Adams D (eds.) *Neurotoxicology Handbook*, pp. 363–381. Totowa, NJ: Humana Press Inc.

Kirkpatrick B, Fleming LE, Squicciarini D, *et al.* (2004) Literature review of Florida Red Tide: Implications for human health. *Harmful Algae* 3(2): 99–111.

Relevant Websites

http://www.mote.org – National Institution of Environmental Health Services. Red Tide Toxin, Health Effects and Exposure Study.

http://cbr-rbc.nrc-cnrc.gc.ca/issha/New_ISSHA/Images/index.htm – International Society for the Study of Harmful Algae.

http://www.rsmas.miami.edu – University of Miami. Marine and Fresh Water Biomedical Sciences Center.

http://www.whoi.edu – US Fish, Shellfish and Wildlife Affected by Toxic or Harmful Microalgal Species. Woods Hole Oceanographic Institution, The Harmful Algae Page.

Alkalies

Sanjay Chanda and Harihara M Mehendale

- Synonyms: Bases; Strong alkalies
- Chemical/Pharmaceutical/Other Class: Inorganic alkalies (e.g., sodium hydroxide, potassium hydroxide, and sodium hypochlorite)

Uses

Depends on the specific alkali. Alkalies are primarily used as cleaning agents, bleaches, and unslaked lime.

Exposure Routes and Pathways

Skin contact, ingestion, and inhalation are the most common exposure pathways.

Toxicokinetics

The toxicokinetics varies depending on the type of alkali.

Mechanism of Toxicity

Alkalies cause toxicity by liquefaction necrosis, meaning that the alkali destroys the cell membrane and cell integrity and thereby causes cell lysis.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity of alkalies in animals is the same as that in humans.

Human

Alkalies can burn skin, mucous membrane, and eyes almost immediately on contact. However, the absence of burns, irritation, erythema, or other such signs in the oral or circumoral area does not necessarily indicate that esophageal injury does not exist. Inhalation of the fumes may cause pulmonary edema or pneumonitis.

Chronic Toxicity (or Exposure)

Animal

The toxicity of alkalies in animals is the same as that in humans.

Human

Burns that at the time of injury appear to be mild can sometimes go on to cause opacification, vascularization, ulceration, or perforation.

Clinical Management

Exposure should be terminated as soon as possible by removing the victim to fresh air. The skin, eyes, and mouth should be washed with copious amounts of water. A 20–30 min wash may be necessary to neutralize and remove all residual traces of the contaminant. Contaminated clothing and jewelry should be removed and isolated. Treatment may require instillation of a local anesthetic to treat the blepharospasm (spasmodic winking from involuntary contraction of the orbicular muscle of the eyelids). Oral ingestion requires immediate dilution therapy with water or milk. Antidotes such as vinegar or lemon juice are absolutely contraindicated. Emesis should be avoided in case of ingestion.

Environmental Fate

In the case of a solid alkali spill on soil, groundwater pollution will occur if precipitation occurs prior to clean up. Precipitation will dissolve some of the solid and create an aqueous solution of that alkali which then would be able to infiltrate the soil. However, prediction of the concentration and properties of the solution produced would be difficult.

Exposure Standards and Guidelines

The guidelines given below are for sodium hydroxide (common alkali):

- Occupational Safety and Health Administration standards: permissible exposure limit: 8 h time-weighted average is $2\,mg\,m^{-3}$.
- Threshold limit values: ceiling limit is $2\,mg\,m^{-3}$.
- National Institute for Occupational Safety and Health recommendations: recommended exposure limit is $2\,mg\,m^{-3}$.

See also: Potassium; Sodium.

Further Reading

Klaassen CD, Amdur MO, and Doull J (eds.) (1995) Casarett and Doull's Toxicology. *The Basic Science of Poisons*, 5th edn. New York: McGraw-Hill.

NIOSH (1975) Criteria Document: Sodium Hydroxide. DHEW. NIOSH, pp. 76–105.

Alkyl Halides

Swarupa G Kulkarni and Harihara M Mehendale

- Representative Compounds: Methyl bromide; Methyl chloride; Methyl iodide; Dichloromethane; Tetrachloroethane; Carbon tetrachloride; Trichloroethene; Trichloroethylene; A number of fluorinated hydrocarbons (e.g., Freons)
- Synonyms: Halogenated hydrocarbons; Haloalkanes
- Chemical Formula: $R(X)_n$, where R is a hydrocarbon alkyl group and X is a halogen. One or more halogens may be present in one compound

Uses

Many halogenated hydrocarbons have important commercial applications. Alkyl halides are important intermediates in synthesis, as solvents in the laboratory and industry, and as dry cleaning fluids. They also find use as anesthetics and refrigerants. For example, trichloroethene is a common dry cleaning solvent. The fluorinated hydrocarbons (Freons) are used as refrigerants, industrial solvents, fire extinguishers, local anesthetics, and glass chillers, but mainly as propellants in aerosol products. Methyl bromide, methyl chloride, and methyl iodide are used as refrigerants in chemical synthesis and as fumigants. Methyl bromide is used with carbon tetrachloride in fire extinguishers. Methyl chloroform is used as a solvent for cleaning, degreasing, and in paint removers. Dichloromethane is used in paint removers and as an industrial solvent. Tetrachloroethane is used as a solvent in industry and occurs as a contaminant in other chlorinated hydrocarbons. It is occasionally present in household cleaners. Carbon tetrachloride is used as a solvent and intermediate in many industrial processes.

Exposure Routes and Pathways

Inhalation and dermal and ocular contact are common routes of exposure.

Toxicokinetics

Fluorocarbon compounds are lipid soluble and, thus, generally well absorbed through the lung. Absorption after ingestion is much lower than after inhalation. Most of the fluorinated hydrocarbons are immediately absorbed.

There is a significant accumulation of fluorocarbons in the brain, liver, and lungs compared to blood levels, signifying a tissue distribution of fluorocarbons similar to that of chloroform. Fluorocarbons are concentrated in body fat where they are slowly released into blood at a concentration that should not cause any risk of cardiac sensitization.

Fluorocarbons are excreted by the lungs and the parent compound is eliminated in about 15 min.

Acute and Short-Term Toxicity (or Exposure)

Animal

Deliberate ocular exposure in rabbits to liquid Freon 12 produced effects related to the duration of exposure. Severe corneal damage with opacity occurred following exposure for 30 s. In dogs, inhalation of fluorinated hydrocarbon vapors causes bradycardia followed by deterioration to ventricular fibrillation in some animals.

Human

Freons are very toxic when inhaled in high concentrations and/or for extended periods. Inhalation of fluorinated hydrocarbons such as those caused by leaking air conditioners or refrigerators usually results in transient eye, nose, and throat irritation. Palpitations and lightheadedness are also seen. Headache was a common complaint, reported in 71% of 31 workers exposed to bromotrifluoromethane in one incident. Inhalation of halides at sufficient concentrations associated with deliberate abuse, or spills or industrial use occurring in poorly ventilated areas, has been associated with ventricular arrhythmias, pulmonary edema, and sudden death. Fluorinated hydrocarbons are believed to cause arrhythmias by sensitizing the myocardium to endogenous catecholamines. Freon solvents are degreasers. Dermal contact with fluorinated hydrocarbons may result in defatting, irritation, or contact dermatitis. Severe frostbite was reported as a rare effect of severe Freon exposure. Mucosal necrosis and perforation of the stomach developed in one patient after ingesting a small amount of trichlorofluoromethane. Fluorocarbons containing bromine are more toxic than the corresponding chlorine compounds. There is a significant interpatient variation following exposure to fluorocarbons and it is difficult to predict symptoms following exposure. Compounds like dibromochloropropane, in which occupational exposure has affected male fertility, have now been removed from the market. Following acute exposure to methyl bromide, chloride, or iodide, nausea and vomiting, blurred vision, vertigo, weakness or paralysis, oliguria or anuria, drowsiness, confusion, hyperactivity, coma, convulsions, and pulmonary edema are noted. Pulmonary edema and bronchial pneumonia are most often the cause of death. Skin contact causes irritation and vesiculation.

Methyl chloroform and dichloromethane are central nervous system (CNS) depressants. Methyl chloroform sensitizes the myocardium to catecholamine-induced arrhythmias. Following exposure to tetrachloroethane, irritation of the eyes and nose, followed by headache and nausea, is observed. Cyanosis and CNS depression progressing to coma may appear after 1–4 h.

Chronic Toxicity (or Exposure)

Animal

Some of the chlorinated hydrocarbon solvents, such as methylene chloride and chloroform, have caused

cancer in several species of experimental animals and are suspect human carcinogens.

Human

A syndrome of impaired psychomotor speed, impaired memory, and learning, has been described in workers with chronic occupational exposure to fluorinated hydrocarbons. Skin irritation and defatting dermatitis upon prolonged or repeated contact with the skin to trichloromonofluoromethane have been reported. An excess of CNS symptoms was seen in a group of workers chronically exposed to trichloromonofluromethane. Repeated exposure to methyl bromide, methyl chloride, and methyl iodide will cause blurring of vision, numbness of the extremities, confusion, hallucinations, somnolence, fainting attacks, and bronchospasm. Chronic toxicity has not been reported with dichloromethane. Headache, tremor, dizziness, peripheral paresthesia, and anesthesia have been reported after chronic inhalation or skin exposure to tetrachlorethane. National Institute of Occupational Safety and Health (NIOSH) recommends that methyl chloride, methyl bromide, and methyl iodide be considered as potential occupational carcinogens and that methyl chloride be considered a potential occupational teratogen.

Clinical Management

This management is intended for use in the absence of a specific treatment protocol for a product or a chemical. Symptomatic and supportive care is the primary therapy. The general approach to a poisoned patient is to first assess the vital signs of the patient followed by assessing the route of administration for potential toxicity. Measures to prevent further absorption of the compound may be useful. Victims of inhalation exposure should be moved from the toxic environment and administered 100% humidified supplemental oxygen with assisted ventilation as required. Exposed individuals should have a careful and thorough medical examination performed to look for abnormalities. Patients with fluorohydrocarbon poisoning should not be given epinephrine or similar drugs because of the tendency of fluorohydrocarbon to induce cardiac arrhythmia, including ventricular fibrillation. Monitoring including complete blood count, urine analysis, and liver and kidney function tests is suggested for patients with significant exposure. Activated charcoal or gastric lavage may be indicated to prevent further absorption. Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persist after 15 min of irrigation, an ophthalmologic examination should be performed.

Miscellaneous

Alkyl halides are practically insoluble in water. They are miscible in all proportions with liquid hydrocarbons and are, in general, good solvents for many organic substances. Most of the common organic halides are liquids. Like alkanes, halogen compounds are insoluble in and inert to cold concentrated sulfuric acid. In a series of alkyl halides, the boiling point rises with an increase in molecular weight due to the presence of either a heavier halogen atom or a larger alkyl group. Bromides boil at temperatures distinctly higher than the corresponding chlorides, and iodides are higher boiling than the bromides. Increase in the halogen content decreases their flammability. In contact with an open flame or a very hot surface fluorocarbons may decompose into highly irritant and toxic gases such as chlorine, hydrogen fluoride, or chloride and even phosgene. Alkyl halides can be prepared by addition of the halogen or hydrogen halides to alkenes, as well as by substitution of a halogen for hydrogen in an alkane. The most important method of preparing alkyl halides is by reaction between an alcohol and a hydrogen halide.

See also: Carbon Tetrachloride; Catecholamines; Chloroform; Freons; Methyl Bromide; Methylene Chloride.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Alkyl Halides.

Allergenicity Testing *See* Toxicity Testing, Sensitization.

Allyl Alcohol

Sharmilee P Sawant and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 107-18-6
- Synonyms: 2-Propenol; 2-Propen-1-ol; Vinyl carbinol
- Chemical Structure: $CH_2 = CHCH_2OH$

Uses

Allyl alcohol is used as an industrial solvent, herbicide, and fungicide.

Background Information

Allyl alcohol is a clear liquid boiling at 96°C. It is highly toxic and hazardous to the environment. It requires special attention to handling procedures. It is synthesized by the hydrolysis of allyl chloride or isomerization of propylene oxide. It is used as a starting material in making various polymers, pharmaceuticals, pesticides, and other allyl compounds.

Exposure Routes and Pathways

The substance can be absorbed into the body by inhalation of its vapor, dermal contact, and by ingestion.

Toxicokinetics

Allyl alcohol is metabolized via two alternative oxidative pathways leading to the formation of acrolein or the epoxide 'glycidol'. The epoxide may then be converted to glycerol by epoxide hydrolase. The conversion of allyl alcohol to acrolein is mediated by alcohol dehydrogenase (ADH), which may then be further oxidized to acrylic acid by NAD- or NADP-dependent enzymes in the liver cytosol or microsomes or to glycidaldehyde by a microsomal enzyme with subsequent conversion to glyceraldehyde by epoxide hydrolase. Alternatively, acrolein may react directly both enzymatically and nonenzymatically to form stable adducts with glutathione or other low molecular weight thiol compounds prior to excretion in the urine as mercapturate. Both glycidol and glycidaldehyde are substrates for lung and liver cytosolic glutathione-*S*-transferases.

Mechanism of Toxicity

Allyl alcohol is inactive *per se* and its toxic effect is mediated by its ADH oxidation to form acrolein, which is responsible for the hepatotoxic action. The toxicity of the alcohol (or its metabolite acrolein) is dependent on the concentration of glutathione (GSH). After severe depletion of GSH, the reactive metabolite of allyl alcohol can bind to essential sulfhydryl groups in the cellular macromolecules, leading to structural and functional changes in cellular molecules, which can be responsible for cell death. In this case, the appearance of lipid peroxidation could be merely the consequence of cell death.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure to allyl alcohol causes liver and kidney damage. Allyl alcohol is classified as a periportal hepatotoxicant since it selectively damages the periportal region of the liver. Studies have shown that in adult rats, allyl alcohol produces a moderate to marked periportal necrosis with attendant inflammation, hemorrhage, and also decreases hepatic cytochrome P-450, benzphentamine *N*-demethylation, and ethoxyresorufin *O*-deethylation activities by ~30%. In immature rats, it lowered both cytochrome P-450 activity (30%) and ethoxyresorufin *O*-deethylation (75%). Benzphetamine *N*-demethylation was not significantly affected in immature rats. Intraperitoneal administration of 1.5 mmol kg^{-1} of allyl alcohol to starved Swiss albino mice causes the development of hemolysis in ~50% of the animals. Other toxic effects include renal necrosis, pulmonary edema, and central nervous system effects at higher dose levels.

Human

The most important adverse effects of occupational exposure to allyl alcohol are upper respiratory tract irritation and burning of the eyes. The substance may cause effects on the muscles, resulting in local spasm and aching. The appearance of these effects may be delayed after exposure.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to allyl alcohol can cause liver and kidney damage.

Human

Long-term exposure may lead to liver or kidney damage.

Clinical Management

Exposure should be terminated as soon as possible by removal of the patient to fresh air. Skin, eyes, and mouth should be washed with copious amounts of water. Contaminated clothings should be removed. A mild soap solution may be used for washing the skin, but should not be placed into the eye. Dilution with water may be effective if small amounts are swallowed until additional medical attention is available.

Ecotoxicology

The substance is very toxic to aquatic organisms.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration (OSHA) general industry permissible exposure limit: 2 ppm, 5 mg m^{-3} time-weighted average (TWA) (skin).

The National Institute for Occupational Health and Safety (NIOSH) recommended exposure limit: 2 ppm TWA, 4 ppm short-term exposure limit (skin).

See also: Acrolein; Allyl Formate; Liver.

Further Reading

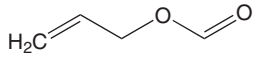
Atzori L, Dore M, and Congiu L (1989) Aspects of allyl alcohol toxicity. *Drug Metabolism and Drug Interaction* 7: 295–319.

Jaeschke H, Kleinwaechter C, and Wendel A (1987) The role of acrolein in allyl alcohol-induced lipid peroxidation and liver cell damage in mice. *Biochemical Pharmacology* 36: 51–70.

Allyl Formate

Sharmilee P Sawant and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 1838-59-1
- Synonyms: Formic acid; 2-Propenyl ester; Formic acid; Allyl ester; Allyl alcohol; Formate
- Chemical Structure:



Uses

It is used as a solvent in spray lacquers, enamels, varnishes, and latex paints and as an ingredient in paint thinners and strippers, varnish removers, and herbicides. It is also used in liquid soaps, cosmetics, industrial and household cleaners, and dry-cleaning compounds.

Exposure Routes and Pathways

The substance can be absorbed into the body by inhalation and dermal contact and by ingestion.

Toxicokinetics

Allyl formate is rapidly cleaved *in vivo* by nonspecific esterases to allyl alcohol. Allyl alcohol is metabolized via two alternative oxidative pathways leading to the formation of acrolein or the epoxide 'glycidol'. The epoxide may then be converted to glycerol by epoxide hydrolase. The conversion of allyl alcohol to acrolein is mediated by alcohol dehydrogenase, which may then be further oxidized to acrylic acid by NAD- or NADP-dependent enzymes in the liver cytosol or microsomes or to glycidaldehyde by a microsomal enzyme with subsequent conversion to glyceraldehyde by epoxide hydrolase. Alternatively, acrolein may react directly both enzymatically and nonenzymatically to form stable adducts with glutathione or other low molecular weight thiol compounds prior to excretion in the urine as mercapturate.

Mechanism of Toxicity

Allyl formate is cleaved by nonspecific esterases to allyl alcohol, which is then oxidized by alcohol dehydrogenases to the reactive acrolein, which is responsible for the hepatotoxic action. The toxicity of allyl alcohol via its metabolite acrolein is dependent on the concentration of glutathione (GSH). After depletion of GSH, the reactive metabolite of allyl alcohol can bind to essential sulfhydryl groups in the cellular macromolecules, leading to structural and functional modifications that can be responsible for hepatic injury. Appearance of lipid peroxidation signals events that follow toxication mechanisms initiated by acrolein, and subsequent and continued lipid peroxidation could be merely the consequence of cell death.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure to allyl formate causes liver and kidney damage. Allyl formate is classified as a periportal hepatotoxicant since it selectively damages the periportal region of the liver in rodents.

Human

The most important adverse effect of occupational exposure to allyl formate is upper respiratory tract irritation.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to allyl formate can cause liver and kidney damage.

Human

Long-term exposure may lead to liver or kidney damage.

Clinical Management

Exposure should be terminated as soon as possible by removal of the patient to fresh air. Skin, eyes, and mouth should be washed with copious amounts of water. Contaminated clothing should be removed. A mild soap solution may be used for washing the skin, but should not be placed into the eye. Dilution with water may be effective if small amounts are swallowed before medical attention is sought.

Ecotoxicology

The substance is very toxic to aquatic organisms.

See also: Acrolein; Allyl Alcohol.

Further Reading

Droy BF, Davis ME, and Hinton DE (1989) Mechanism of allyl formate-induced hepatotoxicity in rainbow trout. *Toxicology and Applied Pharmacology* 98: 313–324.

Rees KR and Tarlow MJ (1967) The hepatotoxic action of allyl formate. *Biochemistry Journal* 104: 757–761.

Aluminum

Abbi Heilig

- Chemical Abstracts Service Registry Number: CAS 7429-90-5
- Synonyms: Aluminum; Molten; Metana; Aluminum powder; Pyrophoric
- Chemical/Pharmaceutical/Other Class: Metals

Uses

Aluminum can be used in several different ways, either alone or compounded, and in a variety of forms, including powder. Aluminum is frequently used in food packaging, and also in utensils and electrical conductors. Aluminum compounds are widely used in industry, in the form of alums in water treatment and alumina in abrasives and furnace linings. However, aluminum is used alone very rarely since it is such a soft metal. It is often combined with other metals to create a stronger, more durable metal. These combinations are called aluminum alloys. Aluminum alloys are used extensively in aircraft. Aluminum and aluminum salts can also be found in many consumer products such as antiperspirants, food additives, antacids, astringents, and buffered aspirins. Powdered aluminum is used to make explosives and fireworks.

Background Information

Although aluminum was one of the last metals to be commercialized, it has been recognized for centuries. Aluminum was first recognized by the Romans as an astringent substance, and they called it 'alum'. By the Middle Ages it was manufactured as 'alum stone', a subsulfate of alumina and potash. In 1825, Hans C Oersted was able to isolate a few drops of the raw material, and then by 1886 it had patents from both Charles Martin Hall of the United States and Paul-Louis-Toussaint Heroult of France. Aluminum was commercialized in industry by the end of the nineteenth century.

Exposure Routes and Pathways

Aluminum is the most abundant metal, and the third most abundant element in the earth's crust. Human exposure to this metal is common and unavoidable. However, intake is relatively low because aluminum

is highly insoluble in many of its naturally occurring forms. Humans are always exposed to some form of aluminum by eating food, drinking water, ingestion of aluminum containing medicinal products, or just breathing air. The average human intake is estimated to be 30–50 mg day^{-1}. This intake comes primarily from foods, drinking water, and pharmaceuticals. Food additives can contain aluminum; due to certain additives, processed cheese and cornbread are two major contributors to high aluminum exposures in the American diet. Some common over-the-counter medications such as antacids and buffered aspirin contain aluminum, and this can increase the daily intake significantly.

There has been concern about the exposures resulting from leaching of aluminum from cookware and beverage cans; however, aluminum beverage cans are usually coated with a polymer to minimize such leaching. Leaching from aluminum cookware becomes potentially significant only when cooking highly basic or acidic foods, for example, in one study, tomato sauce cooked in aluminum pans was found to accumulate 3–6 mg aluminum per 100 g serving.

Aluminum is absorbed from the soil by many plants that humans consume. The amount that a person would inhale depends on where they reside, and aluminum levels are much higher in industrial and urban areas. Another route of exposure is through skin contact with soil, water, and with aluminum metal.

Toxicokinetics

Less than 1% of that taken into the body orally is absorbed from the gastrointestinal tract. Aluminum can increase the absorption of other chemicals such as fluoride, calcium, iron, and phosphates. Most of the aluminum absorbed into the body will eventually end up in the bones or lungs. Aluminum that is not absorbed by the bones or lungs is excreted by the kidneys.

Mechanism of Toxicity

Aluminum binds diatomic phosphates and possibly depletes phosphate, which can lead to osteomalacia. High aluminum serum values and high aluminum concentration in the bone interfere with the function of vitamin D. The incorporation of aluminum in the bone may interfere with deposition of calcium; the subsequent increase of calcium in the blood may inhibit release of parathyroid hormones by the parathyroid gland. The mechanism by which aluminum concentrates in the brain is not known; it may interfere with the blood brain barrier.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acutely, aluminum itself has minimal systemic toxicity. Overall, animals become weaker and less active due to exposure.

Human

Aluminum has not been shown to alter the immune system in humans exposed by the oral or inhalation routes. Skin sensitization may occur.

Chronic Toxicity (or Exposure)

Animal

Cats and rabbits are aluminum sensitive, and have showed neurotoxic effects from aluminum. There is no evidence that aluminum exposure will affect reproduction.

Toxicity of aluminum in animals differs from humans because animals are much more sensitive to high exposures. Monkeys on a low calcium, high aluminum diet showed neurological disease similar to those of amyotrophic lateral sclerosis and Parkinsonism. Rats and hamsters showed signs of lung damage after breathing large amounts of aluminum dust. Death often occurred after the inhalation of air highly concentrated with the chemical.

Human

Fibrosis of the lung may occur through inhalation of aluminum dust particles. Aluminum has been associated with encephalopathy, bone disease, and anemia related to dialysis. It has also been thought that aluminum may be a cofactor in the etiopathogenesis of some neurodegenerative diseases, including Alzheimer's disease (AD). Direct evidence, however, cannot link the two together. Aluminum toxicity has been well recognized in patients with renal failure. Also, an increased concentration of aluminum in infant formulas and in solutions for home parenteral nutrition has been associated with neurological consequences and metabolic bone loss.

Clinical Management

Aluminum overload has very few treatment options. Besides awareness of consumption and environment, the chelating agent, deferoxamine, is used for treatment.

Ecotoxicology

Aluminum occurs naturally in soil, water, and air. It is redistributed or moved by natural and human activities. High levels in the environment can be caused by the mining and processing of its ores and by the production of aluminum metal, alloys, and compounds. Small amounts of aluminum are released into the environment from coal-fired power plants and incinerators. Virtually all food, water, and air contain some aluminum, which nature is well adapted to handle.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA) in the United States have the following airborne exposure limits:

- *ACGIH threshold limit value*: Aluminum oxide: $10\,mg\,m^{-3}$ (time-weighted average, TWA) inhalable (total) particulate matter containing no asbestos and $<1\%$ crystalline silica, A4. Soluble salts as Al: $2\,mg\,m^{-3}$ (TWA).
- *OSHA permissible exposure limit*: Alpha alumina (aluminum oxide): $15\,mg\,m^{-3}$ total dust, $5\,mg\,m^{-3}$ respirable fraction. Aluminum as metal: $15\,mg\,m^{-3}$ total dust, $5\,mg\,m^{-3}$ respirable fraction.

See also: Metallothionein; Metals.

Further Reading

Becaria A, Campbell A, and Bondy SC (2002) Aluminum as a toxicant. *Toxicology and Industrial Health* 18(7): 309–320.

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, 5th edn., vol. 2, pp. 354–406. New York: Wiley.

Relevant Websites

http://www.inchem.org – Aluminum (Environmental Health Criteria).

http://www.aluminum.org – The (US) Aluminum Association website.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Aluminum.

Aluminum Phosphide

Christopher H Day

- Chemical Abstracts Service Registry Number: CAS 20859-73-8
- Synonyms: Al-Phos; Aluminum monophosphide; Aluminum phosphide; Celphide; Celphine; Celphos; Delicia; Delicia gastoxin; Detia; Fumitoxin; Phostoxin; Quickphos; Weevilcide
- Chemical/Pharmaceutical/Other Class: Phosphide fumigant
- Chemical Formula: AlP
- Chemical Structure: Al : P

Uses

The primary use for aluminum phosphide is as a fumigant to control insects and rodents in both nonfood and food crops in indoor environments. It is also used in the control of rodents outdoors via application to their burrows or in grain storage areas. Aluminum phosphide is formulated in solid form only, and is available for use as a tablet, pellet, or dust, and in porous bags or blister packs.

Exposure Routes and Pathways

Aluminum phosphide is usually formulated as dark gray or dark yellow crystals that have an odor similar to decaying fish or garlic. Exposure can occur to aluminum phosphide via the oral route, but because it is a solid material, dermal absorption of aluminum phosphide is unlikely. Aluminum phosphide is highly reactive with water, such that any contact with moisture will result in decomposition to phosphine gas. Phosphine gas is colorless, flammable, and explosive at room temperature. Therefore, the primary exposure route is via inhalation and absorption by the lungs. Exposure is also possible through the ingestion of commodities, such as grains and nut meats, treated with aluminum phosphide; these foods may contain residues of phosphine gas. Residues of phosphine gas in treated commodities are expected to be <0.004 ppm (limit of detection in several studies) following aeration.

Toxicokinetics

Phosphine gas is rapidly absorbed through the lungs following inhalation. Following ingestion of aluminum phosphide, phosphine gas is generated which is

then readily transferred to the bloodstream. The wide array of target organs that are affected following exposure, suggests that phosphine is effectively distributed throughout the body. However, there is a paucity of information in the literature with respect to the metabolism and elimination of phosphine. There is some evidence that unexpired phosphine may be metabolized to phosphates, hypophosphite, and phosphite.

Mechanism of Toxicity

Phosphine is known to disrupt protein synthesis and enzymatic activity, particularly in lung and heart cell mitochondria. This can lead to a blockage of the mitochondrial electron transport chain. Phosphine may cause denaturing of various enzymes involved in cellular respiration and metabolism, and may be responsible for denaturing of the oxyhemoglobin molecule.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure to elevated levels of phosphine gas by animals can result in lethargy, shallow breathing, immobility, agitation, ataxia, convulsions, seizures, and death. The 4 h inhalation LC_{50} for phosphine gas in rats has been reported at 15 mg m^{-3} (11 ppm), although a more recent study in which no mortality was noted in male and female rats exposed to a one-time 6 h exposure level of 15 mg m^{-3} suggests that the 4 h inhalation LC_{50} for rodents may exceed 15 mg m^{-3}.

Human

Acute oral exposure in humans has resulted in pulmonary edema, cardiovascular electrocardiographic abnormalities, tachycardia, hypotension, and transient atrial fibrillation following inadvertent or voluntary ingestion. These effects are likely caused by the formation and subsequent toxicity of phosphine gas liberated in the stomach following contact with water. Other adverse effects that have been noted in humans following accidental or suicide-related intake of aluminum phosphide include gastrointestinal effects such as abdominal pain and vomiting, hepatic effects such as hyperemia, hepatic dysfunction, renal effects such as profound proteinuria, anuria, renal failure, and neurological effects such as restlessness and loss of consciousness. As discussed previously, these adverse effects are likely the result of the toxicity of phosphine gas, which is produced from the decomposition of aluminum phosphide in the presence of water.

Chronic Toxicity (or Exposure)

Animal

In chronic studies with rats, exposure to aluminum phosphide fumigated chow (4.5 mg m^{-3} phosphine) resulted in decreases in food intake, body weight, hemoglobin, red blood cells, hematocrit, and in increases in platelet counts. Following a 4 week recovery period in many of the exposed rats symptoms were absent, suggesting apparent reversibility. Neither aluminum phosphide nor phosphine gas exhibit carcinogenic, reproductive, or developmental effects in animals.

Human

There is evidence that long-term phosphine exposure by individuals involved in the application of pesticides resulted in chromosome damage. Chronic exposure to very low levels of phosphine may result in altered motor, visual, and speech skills. Neither aluminum phosphide nor phosphine gas exhibit carcinogenicity in humans, or result in reproductive or developmental effects. Although definitive evidence is lacking, it is assumed that phospine is an *in vivo* inhibitor of oxidative phosphorylation.

In Vitro Toxicity Data

Phosphine has been reported as negative for induction of reverse gene mutations up to cytotoxic doses in the Ames assay (*Salmonella typhimurium*). Increased chromosomal aberrations were reported in Chinese hamster ovary (CHO) cells exposed to 2500 and 5000 ppm of phosphine without activation with the S9 fraction. Chromosomal aberrations in CHOs were also reported in cells tested with S9 activation at 2500 ppm, but not 5000 ppm.

Clinical Management

If the victim has ingested aluminum phosphide, emesis should not be induced. Phosphine gas will be produced in the stomach when aluminum phosphide contacts the resident gastric fluids. A slurry of activated charcoal may be administered at 1 g charcoal per kg body weight. Any victim who has ingested aluminum phosphide should be immediately transported to a medical facility for treatment and monitoring. Rescuers need to be aware of any solid phosphide contamination on the victim's clothing, skin, or hair which will produce phosphine following contact with water, as well as any vomitus which could off-gas phosphine.

Environmental Fate

As discussed previously, once exposed to water, even high ambient humidity, aluminum phosphide will generate phosphine gas. Therefore, atmospheric dissipation is expected to be the primary fate process for phosphine. In addition to phosphine being generated from the reaction of aluminum phosphide with water, the other reaction product is aluminum hydroxide, a common constituent of clay. If the liberated phosphine (PH_3) burns it will produce phosphorus pentoxide (P_2O_5), which when exposed to water will form orthophosphoric acid (H_3PO_4).

Ecotoxicology

Very limited ecotoxicological data are available on the effects of phosphine, while no data were found for the effects of aluminum phosphide on wildlife. One study reported that turkeys and chickens exposed to phosphine gas at concentrations of 211 and 224 mg m^{-3} for 74 and 59 min, respectively, exhibited dyspnea, organ swelling, convulsions, and death. These types of effects are unlikely in the unconfined atmospheric conditions that most birds and wildlife are exposed to in nature. However, if misapplied or disposed of incorrectly, phosphine gas liberated from the decomposition of aluminum phosphide could represent a significant hazard to nontarget wildlife exposed to the gas in burrows or other confined spaces.

Under most circumstances, exposure to aquatic organisms would be unlikely due to the limited use pattern of aluminum phosphide in terrestrial environments. Two studies of aquatic toxicity are available in the literature. Both studies are acute tests with fish, the snakehead catfish and the rainbow trout. The reported values of LC_{50} are 0.10 and 0.0041 mg l^{-1} for the snakehead catfish and rainbow trout, respectively. These results indicate that phosphine is highly toxic to these fish species.

Although there are no data for other bird or fish species, it is possible that other members of these taxa may be similarly sensitive to the effects of phosphine due to an anticipated similar mode of action.

No chronic ecotoxicity data could be located for aluminum phosphide or phosphine in the available literature.

Other Hazards

Any individual previously exposed to aluminum phosphide should check with their doctor before taking vitamins that contain phosphorus supplements.

Table 1 Summary of exposure standards and guidelines for phosphine

Agency	*Standards and guidelines (ppm)*	*Averaging time*
US EPA	RfC (0.0002)	24 h a day for a lifetime
OSHA	PEL (0.3)	8 h a day over working lifetime
ACGIH	TLV – TWA (0.3)	8 h a day over working lifetime
ACGIH	ERPG-2 (0.5)	1 h
NIOSH	IDLH (50)	NA

US EPA, United States Environmental Protection Agency; OSHA, Occupational Safety and Health Administration; NIOSH, National Institute for Occupational Safety and Health; ACGIH, American Conference of Governmental Industrial Hygienists; RfC, reference concentration; PEL, permissible exposure limit; TLV, threshold limit value; TWA, time-weighted average; ERPG-2, Emergency Response Planning Guideline; IDLH, immediately dangerous to life or health; NA, not applicable.

Exposure Standards and Guidelines

Several agencies have established exposure standards or guidelines for aluminum phosphide as phosphine (summarized in **Table 1**). Generally, a standard or guideline represents the concentration that if met, will prevent an adverse effect from occurring at low exposure doses, and will therefore necessarily prevent the occurrence of more serious effects that are known to occur at higher doses. The chronic reference concentration (RfC) of 0.0002 ppm (0.0003 mg m^{-3}) for phosphine was set to prevent decreases in body weight in the general population over a lifetime of exposure. The American Conference of Governmental Industrial Hygienists threshold limit value of 0.3 ppm was set to prevent irritation and adverse effects to the central nervous system and gastrointestinal tract in workers exposed 8 h day^{-1} throughout their working lifetime.

See also: Phosphine.

Relevant Websites

http://www.epa.gov – US Environmental Protection Agency (2004) Integrated Risk Information System (IRIS) Files: Aluminum Phosphide and Phosphine.

http://www.inchem.org – International Programme on Chemical Safety (IPCS) (1988) *Phosphine and Selected Metal Phosphides*. Environmental Health Criteria 73.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Aluminum Phosphide.

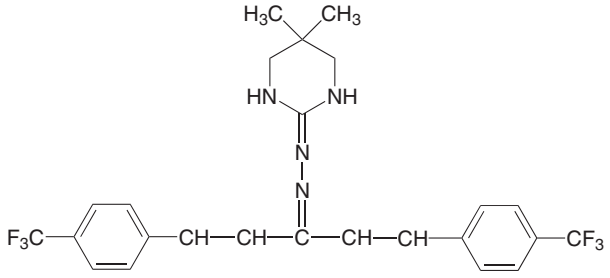
Amanitin, α- *See* Mushrooms, Cyclopeptide.

Amdro

Jamaluddin Shaikh

This article is a revision of the previous print edition article by Sushmita M Chanda, volume 1, pp. 49–50, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 67485-29-4
- Synonyms: Combat; Maxforce; Pyramdron; Aminohydrazone; Hydramethylnon
- Chemical/Pharmaceutical/Other Class: Trifluoromethyl amidinohydrazone
- Chemical Structure:



Uses

Amdro is used as an insecticide mainly for ants and cockroaches.

Exposure Routes and Pathways

Ingestion is the primary route of exposure. Dermal exposure is also possible.

Toxicokinetics

Amdro is poorly absorbed by the oral route but is well absorbed through the skin. Amdro is poorly metabolized in the body with more than 95% being excreted in the feces in the unchanged form. Elimination in rats dosed orally with amdro is 72% of the dose in 24 h and 92% of the dose by 9 days.

Mechanism of Toxicity

Amdro is a slowly activating stomach poison. The exact mechanism of toxicity is unclear.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in male and female rats was $817\,mg\,kg^{-1}$ and the acute dermal LD_{50} was $1502\,mg\,kg^{-1}$. In rabbits the LD_{50} was $>2000\,mg\,kg^{-1}$ and the acute (4 h) inhalation LC_{50} in rats was $2.9\,mg\,l^{-1}$. Amdro is not a dermal irritant or a skin sensitizer but is a mild eye irritant.

Human

Children who ingest small amounts of amdro have symptoms of diarrhea. A case study showed that one-half pound of amdro ingested by an adult diabetic patient produced no specific symptoms except diarrhea.

Chronic Toxicity (or Exposure)

Animal

A 6-month feeding study in dogs reported increased incidence of soft stools, mucoid stools, and diarrhea at a lowest-observed-effect level of $3.0\,mg\,kg^{-1}\,day^{-1}$. Based on an increase in lung adenomas and lung adenomas/carcinomas in female mice, amdro has been classified as a possible human carcinogen by the Environmental Protection Agency. Amdro is not a neurodevelopmental toxicant and is not teratogenic in either rats or rabbits. Amdro affects male reproductive function. Testicular atrophy was reported in an 18-month mouse feeding study, a two-generation reproduction study in rats, and a 91-day oral dosing study in dogs.. In a two-generation rat reproduction study, there was no evidence of systemic toxicity, nor was there any evidence of direct toxicity in the offspring. The reproductive no-observed-effect level ($1.66\,mg\,kg^{-1}\,day^{-1}$ for males) was based on histopathology in the testes. At $5.05\,mg\,kg^{-1}\,day^{-1}$, male reproductive performance (lower pregnancy,

reduced gestation weight gain, smaller litters) was decreased.

Human

Little is known regarding chronic effects of amdro in humans.

In Vitro Toxicity Data

Amdro was negative in *Salmonella typhimurium/Escherichia coli* reverse gene mutation assays, *Schizosaccharomyces pombe* P1 forward gene mutation assay, *in vitro* Chinese hamster ovary chromosome aberration, and *Saccharomyces cerevisiae* D4 mitotic gene conversion assay.

Clinical Management

The exposed area should be thoroughly washed with soap and water. If pain or irritation continues, a physician should be consulted. Eyes should be washed with copious amounts of room-temperature water for 15 min in cases of eye contamination. If irritation, pain, swelling, lacrimation, or photophobia persists after 15 min of irrigation, medical attention is necessary. Emesis is necessary only when large amounts (greater than 28 g of bait per kilogram) are ingested. In such cases, ipecac may be used for inducing emesis. Activated charcoal slurry with or without saline cathartic or sorbitol may also be administered. Basic respiratory and cardiovascular function support should be utilized.

Environmental Fate

Amdro is rapidly degraded in the environment by photolysis and more slowly by hydrolysis. The approximate half-life is 1 h in direct sunlight.

Ecotoxicology

Amdro is practically nontoxic to birds. The oral LD_{50} values in mallard duck and bobwhite quail are 2510 and 1825 mg kg^{-1}, respectively. Amdro is moderately to highly toxic to fish. The LC_{50} (4-day) values for amdro in rainbow trout, channel fish, and bluegill sunfish are 160, 100, and 1700 μg l^{-1}, respectively. Amdro is relatively nontoxic to honey bees.

Exposure Standards and Guidelines

The reference dose for amdro is 0.01 mg kg^{-1} day^{-1}.

See also: Pesticides.

Relevant Websites

http://pmep.cce.cornell.edu – Cornell University.
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Ames Test

Robin C Guy

This article is a revision of the previous print edition article by Ann D Mitchell, volume 1, pp. 56–57, © 1998, Elsevier Inc.

The Ames test is a testing system which employs a collection of genetically modified strains of bacteria in *in vitro* system to ascertain a chemical's potential to cause genetic mutations. During the mid-1960s, the test was developed by Bruce Ames of the University of California at Berkeley as a quick and inexpensive way to detect possible carcinogens and mutagens. The testing system methodology has evolved over the years and has been used extensively in regulatory and research roles, currently being the most widely performed *in vitro* test for mutagenesis. The Ames test is a part of a comprehensive battery of *in vitro* and *in vivo* studies and epidemiologic surveys employed to define the frequency or extent of genetic mutation that can be caused by a chemical and thus its potential to cause cancer, inheritable mutations or some types of degenerative diseases. The Ames test is rapid, sensitive, inexpensive, and relatively easy to conduct in a microbiology laboratory and can be used on a wide variety of materials, including volatile compounds.

Scientific Basis

The Ames test allows one to test the ability of a substance to interfere with DNA, which has the information necessary for expression of specific proteins. This information is encoded by the sequence of base pairs in the DNA molecule, with triplets of base pairs (mRNA codons) encoding for a specific amino acid in the sequence of a protein. Ames' system focuses on the fact that mutations in oncogenes and tumor suppressor genes of somatic cells can be involved in tumor formation.

Ames developed strains of bacteria that had carefully selected lethal mutations. In a test system the bacteria could survive only when its mutation had been corrected by experiencing another mutation caused by the tested material. This correction could be accomplished by causing a 'point mutation' or 'frameshift mutations'. Point mutations are base-pair substitutions, that is, a base change in DNA of at least one DNA base pair. In a reverse mutation test, this change in base pairs may occur at the site of the original mutation, or at a secondary site in the bacterial genome. Frameshift mutations are the addition or deletion of one or more base pairs in the DNA. Since amino acids are encoded by triplets of base pairs in sequence, any addition or deletion of 1 or 2 base pairs will dramatically alter the expressed protein from that point on. The Ames system employs strains of *Salmonella typhimurium* and *Escherichia coli* that require amino acids (histidine or tryptophan, respectively) to detect such reverse point and frameshift mutations. The reverse mutation allows the *S. typhimurium* or *E. coli* strains to restore the functional capability of the bacteria to be able to synthesize the specific amino acid on their own, independent of amino acid content in the medium.

Test Methodology

Testing Tools: Microbial Strains

Cultures of carefully established and karyotyped cell lines and cell strains have been developed for the Ames system. Understanding the molecular details of a strain's mutation, its sensitivity and the mechanism of repair of that mutation provides the basis to detect a mutagen and understand its mechanism of activity at the molecular level. Reliable results from the test require use of carefully developed testing strains, a disciplined testing protocol, and unbiased application of interpretative rules to the testing results. Controversy over use of the Ames test and interpretations of results from this and other microbial testing systems has sometimes resulted from use of poorly understood strains, poor testing protocols, or biased interpretative logic.

The most commonly used tester strains are *S. typhimurium* TA1535, TA1537 (or TA97 or 97a), TA98, and TA100 and either TA102 or *E. Coli* WP_2 *uvrA*. The latter two are used to detect oxidizing mutagens, cross-linking agents, and hydrazines. Other strains may be used as long as there are historical data that will support the findings. The Ames assay is considered a reverse mutation assay, as the tester strains originally used all had mutations that altered their survival in growth media: a 'reverse' mutation could enable growth by reversing the effects of the original 'forward' mutation. The *S. typhimurium* strains all have a mutation on their histidine operon as follows:

- TA1535 (hisG46),
- TA1537 (hisC3076),
- TA98 (hisD3052), and
- TA100 (hisG46).

The histidine mutation prevents the *S. typhimurium* strains from synthesizing histidine, and, therefore, prevents the growth of the cell in histidine-deficient medium. All of the *S. typhimurium* strains have additional mutations of the rfa and uvrB genes. The rfa gene has a loss of one of the enzymes responsible for the synthesis of part of the lipopolysaccharide layer of the cell wall, which increases the cell's permeability to certain chemical classes. The uvrB gene contains a deletion that causes a deficit in the DNA excision-repair system, which causes increased sensitivity to certain chemicals. Strains TA98 and TA100 also have an R-factor, in this case the pKM101 plasmid, which further causes increased sensitivity to certain chemicals. TA1537 and TA98 are reverted back to their original histidine-independent state by frameshift mutations. TA1535 is reverted back by base-pair mutations, while TA100 is reverted back by both frameshift and base-pair mutations. The tryptophan mutation prevents the *E. coli* WP_2 uvrA strain from synthesizing tryptophan. A revertant can occur by a base-pair change.

Testing Strategies

As the Ames test is an *in vitro* test, testing protocol has been developed to address the issues of metabolic changes to the test substance (activation), calibration of the response (positive and negative controls), and toxicity to the testing organism. These are critical elements of the test and can greatly influence the test results and subsequent conclusions drawn from the study. As such, it is important to give attention to the testing strategies and the documentation of study results from within a testing laboratory and between testing laboratories over time.

Activation Systems to Account for Metabolic Activity Many mutagens are activated by biotransformation pathways in the body. Therefore, testing of such chemicals in an *in vitro* system must include some metabolic activation system to mimic the biotransformation systems of *in vivo* testing. The inclusion of such a metabolic activating system, usually an exogenous source of metabolic enzymes, in the assay allows evaluation of mutagenic potential of both direct-acting mutagens and those requiring

metabolic activation. Therefore, a typical assay determines the mutagenic potential of a chemical in the absence and presence of the metabolic activating system.

The most common activating system is the S9 enzymes method, a liver preparation (supernatant from 9000*g* centrifugation) from rodents treated with enzyme-inducing agents such as Aroclor 1254. The testing is conducted with and without the enzyme preparation, each with its own control conditions, as described below. Under both conditions, a negative (solvent) and an appropriate positive control (known mutagen) should be tested concurrently.

Control Procedures The testing methodology must be calibrated to assess the degree of response of the test substance in the context of the testing methodology parameters. Substances which are known not to be active in the testing system are employed as a sentinel for false positive results. These negative controls should not elicit a positive response from the testing system being conducted simultaneously for the test substances. Likewise, positive controls are simultaneously tested and should provide a predictable positive response.

Negative (solvent) and positive controls must be utilized for a valid study. An historical database must be maintained for these results. Positive control concentrations must be documented, as different solvents and concentrations are required for different strains and metabolic activation conditions. Examples of positive control substances include:

- Absence of exogenous metabolic activation:
  - sodium azide (CAS 26628-22-8) for TAl535 and TAl00;
  - 2-nitrofluorene (CAS 607-57-8) for TA98, TA1538;
  - 9-aminoacridine or ICR 191 (CAS 90-45-9, 17070-45-0) for TA1537, TA97, and TA97a;
  - mitomycin C (CAS 50-07-7) for TA102 and WP_2 *uvrA*; and
  - 4-nitroquinoline (CAS 56-57-5) for WP_2 *uvrA*.
- Presence of exogenous metabolic activation:
  - 2-aminoanthracene (CAS 613-13-8);
  - cyclophosphamide (monohydrate) (CAS 50-18-0, 6055-19-2);
  - 9,10-dimethylanthracene (CAS #781-43-1);
  - 7,12-dimethylbenzanthracene (CAS #57-97-6); and
  - benzo(a)pyrene (CAS #50-32-8).

A third control is the 'vehicle control'. It tests the potency of the materials present in the testing cocktail in addition to the testing material which is the object of study. The test material may be solubilized in solvents such as sterile water, dimethylsulfoxide, or ethanol. Vehicle controls are tested using only the solubilizing material subjected to exactly the same protocols as those samples with the test material.

Cytotoxicity The microbes may not survive in the presence of the testing material due to nongenetic cell toxicity. If the cell does not survive and undergo cell division, it cannot express any reverse mutation, even if the test material was indeed a mutagen. In considering the meaning of the lack of response in the test system, one must consider the influence of cytotoxicity. Lack of response could mean a lack of mutagenic potency or the presence of cellular toxicity. To test for cytotoxicity, a dose range-finding study is conducted using suspensions of bacterial cells exposed to approximately five concentrations of the test material in the presence and in the absence of an exogenous metabolic activation system. Cytotoxicity is usually determined after 24–48 h of incubation. If no toxicity was observed, concentrations of up to 50 µl or 5000 µg per plate should be used. Triplicate plates per concentration per strain, with and without metabolic activation, are standard. Negative and positive controls are used as appropriate.

Methodology and Protocols (Overview) The Ames test consists of a preliminary dose range-finding phase and the final mutagenicity phase. For the dose range-finding phase each strain, with and without S9, is plated onto a single plate with nine to ten concentrations of test material.

The two most popular methods are the plate incorporation method and the preincubation method. In the plate incorporation method, tester cell suspensions are mixed with an overlay (top) agar and plated immediately onto minimal medium (bottom agar). In the preincubation method, the cell suspension mixture is incubated and then mixed with a top agar before plating onto minimal medium. For both techniques, after 2 or 3 days of incubation, normal sized, revertant colonies are counted and compared to the number of spontaneous revertant colonies on solvent control plates. The Environmental Protection Agency recommends some materials be tested using the preincubation method, namely, classes that include short-chain aliphatic nitrosamines, divalent metals, aldehydes, azo dyes and diazo compounds, pyrollizidine alkyloids, alkyl compounds and nitro compounds. Certain modifications of the methods need to be incorporated for specific types of test articles.

Interpretation and Evaluation

To ensure that the results of an assay are valid, specific criteria for interpretation of the results have evolved with experience with the tests over time among many laboratories. Both positive and negative (solvent) control values should reasonably be within the normal historical data for the laboratory. Tester strains must be identified and have a characteristic number of spontaneous revertants per plate for the vehicle controls. A minimum of three noncytotoxic concentrations needs to be evaluated.

Once the data are available for analyses, evaluation of the results follows. There are several criteria for determining a positive result, such as a concentration-related increase over the range tested and/or a reproducible increase at one or more concentrations in the number of revertant colonies per plate in at least one strain, with or without metabolic activation system. The concentration-related increase would be three times the mean concurrent vehicle control value for strains TA1535, TA1537, and TA1538 and two times the mean vehicle control value for TA98, TA100, and WP_2 uvrA. Biological relevance of the results should be considered first. Results achieved at levels of excessive cytotoxicity (50% or more) are discarded.

A negative result is when the above response criteria are not met, and the test material is thereby considered not mutagenic.

Many compounds that are positive in the Ames test are mammalian carcinogens and a huge database exists establishing that correlation. However, there is not an exact correlation between the chemical's positive response in the Ames test and carcinogenicity. Even with the addition of a metabolic activation system, this prokaryotic system cannot replicate a mammalian cell *in vivo*. Correlation may be dependent on chemical class. Care should be taken to avoid conditions that would lead to results not reflecting authentic mutagenicity. As in mammalian *in vitro* systems, positive results that do not reflect authentic mutagenicity may arise from a variety of possible changes, including pH, osmolality (very high concentrations of test article), or high levels of cytotoxicity.

The Ames test is a sensitive predictor of mutagenicity in mammals, but it should not be used in a vacuum. The Ames test should be used as part of a battery of *in vitro* and *in vivo* tests for predicting the genetic toxicity potential of test materials.

The Ames test is recommended by the International Conference on Harmonisation Guidelines as part of a standard genetic toxicology battery. The other assays include the mouse lymphoma and micronucleus tests. This bacterial mutation test may not be appropriate for the evaluation of certain classes of chemicals, for example highly bactericidal compounds (e.g., certain antibiotics), any compounds that may interfere with cell division or replication, and possibly some peptides. In such cases, mammalian mutation tests may be more appropriate.

See also: Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Genetic Toxicology; Good Laboratory Practices (GLP); International Conference on Harmonisation; Micronucleus Assay; Mitomycin C; Mouse Lymphoma Assay; Redbook; Toxicity Testing, Mutagenicity.

Further Reading

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Gatehouse D, Haworth S, Cebula T, *et al.* (1994) Recommendations for the performance of bacterial mutation assays. *Mutation Research* 312: 217–233.

Kier LD, Brusick DJ, Auletta AE, *et al.* (1986) The Salmonella typhimurium/mammalian microsomal assay: A report of the US Environmental Protection Agency Gene-Tox Program. *Mutation Research* 168: 69–240.

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Scott D, Galloway SM, Marshall RR, *et al.* (1991) Genotoxicity under extreme culture conditions. A report from ICPEMC Task Group 9. *Mutation Research* 221: 147–204.

Relevant Websites

http://www.epa.gov – US Environmental Protection Agency. The website includes Harmonized/870 Health Effects Test Guidelines.

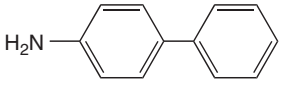
http://www.fda.gov – US Food and Drug Administration.

http://www.bruceames.com – Website of Bruce Ames.

Aminobiphenyl, 4-

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 92-67-1
- Synonyms: 4-ADP; 4-biphenylamine; 4-Aminodiphenyl; Aminobiphenyl; *p*-Phenylaniline; *p*-Xenylamine; Xenylamine
- Chemical/Pharmaceutical/Other Class: Aromatic amine
- Chemical Formula: $C_{12}H_{11}N$
- Chemical Structure:



Uses

4-Aminobiphenyl is used in research as a cancer-causing agent.

Background Information

Production and use of 4-aminobiphenyl has become very limited because of its known carcinogenic effects; however, 4-aminobiphenyl is also found in tobacco smoke.

Exposure Routes and Pathways

Because 4-aminobiphenyl is found in tobacco smoke, one of the major routes of exposure for the general population is the passive and active inhalation of tobacco smoke. Laboratory personnel working with 4-aminobiphenyl without adequate personal protection may also be exposed occupationally by the dermal or inhalation route.

Toxicokinetics

4-Aminobiphenyl is converted to its active metabolite, *N*-hydroxy-4-aminobiphenyl, in the liver and bladder. In the liver, 4-aminobiphenyl is subjected to *N*-hydroxylation and *N*-glucuronidation to produce *N*-glucuronide-4-aminobiphenyl. This metabolite accumulates in the urine in the bladder where, under acidic pH conditions, it is hydrolyzed to its active metabolite. 4-Aminobiphenyl can also be activated directly in the bladder mucosa.

Mechanism of Toxicity

4-Aminobiphenyl is one of a number of chemicals that cause methemoglobinemia, or conversion of hemoglobin to methemoglobin, which reduces the ability of the blood to carry oxygen to tissues. In addition, the active metabolite (see above) is believed to produce cancer through its reaction with cellular DNA. In animal studies, the observed incidence of 4-aminobiphenyl adducts with bladder epithelium DNA correlated well with the observed bladder tumor incidence.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} has been reported to be $500\,mg\,kg^{-1}$ in rats and $25\,mg\,kg^{-1}$ in dogs. The main target organ of toxicity is the bladder.

Human

Acute overexposure is known to produce methemoglobinemia and urinary tract damage. Signs and symptoms of overexposure include a bluish tint of the skin and mucous membranes as well as a burning sensation in the urinary tract and bloody urine.

Chronic Toxicity (or Exposure)

Animal

Chronic administration has produced tumors in bladder, mammary gland, gastrointestinal tract, and liver of exposed animals.

Human

Chronic occupational exposure has been shown to produce bladder damage and cancer. Signs and symptoms of bladder damage may include painful urination and the presence of blood and pus in the urine. The incidence of bladder tumors in workers occupationally exposed to 4-aminobiphenyl was reported to range from 11% to 17% of the exposed population.

Environmental Fate

4-Aminobiphenyl may be released into the environment during its production and use as a rubber antioxidant and dye intermediate; however, sources suggest that it was no longer in significant production since the early 1970s. It is easily oxidizable and probably also undergoes photolysis but there is little actual data on these processes. If released on land it will adsorb moderately to soil, probably binding to

humic materials and undergoing redox reactions. In water it will adsorb to sediment, and probably undergo photolysis and oxidation. Oxidation by alkoxy radicals, which are photochemically produced in eutrophic waters, has an estimated half-life of 14 days. 4-Aminobiphenyl is biodegradable and biodegradation may well occur in both soil and water but there are no rates available for soil or natural waters. It has a low potential for bioconcentration. In the atmosphere, degradation should occur due to direct photolysis, oxidation by ambient oxygen, and also photochemically produced hydroxyl radicals (estimated half-life 6.9 h in the vapor phase).

Other Hazards

Flammability is low to moderate when exposed to heat, flames (sparks), or powerful oxidizers. 4-Aminobiphenyl autoignites only at 842°F. When strongly heated, 4-aminobiphenyl emits toxic fumes.

Exposure Standards and Guidelines

The primary exposures to this compound are occupational. There is sufficient epidemiological and animal toxicological data to classify 4-aminobiphenyl as a human carcinogen. Therefore, special precautions must be taken when working with 4-aminobiphenyl. Personnel handling 4-aminobiphenyl must follow industrial hygiene and health protection requirements for handling potentially carcinogenic substances. At a minimum 4-aminobiphenyl exposures should be minimized through the use of engineering controls, work practices, and personal protective equipment, including impervious and disposable gowns and gloves as well as eye and respiratory protection. In addition, working areas and working instruments must be specially designed for handling potentially harmful substances. Although ambient air exposures are currently unlikely except for accidental releases, this compound is listed as a hazardous air pollutant by the Clean Air Act.

See also: Carcinogenesis; Clean Air Act (CAA), US; Tobacco Smoke.

Further Reading

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Sax NI and Lewis RJ (1989) *Dangerous Properties of Industrial Materials*, 7th edn. New York: Van Nostrand Reinhold.

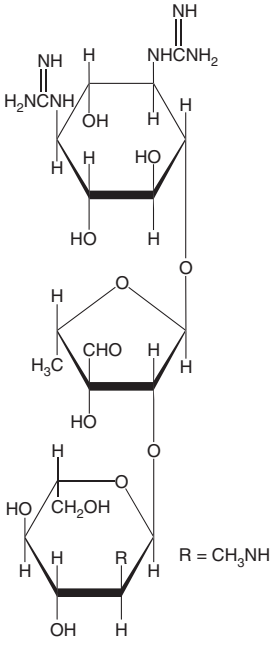
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for 4-Aminobiphenyl.

Aminoglycosides

Abraham Dalu

- Representative Compounds: Amikacin; Gentamicin; Kanamycin; Neomycin; Netilmicin; Paromomycin; Streptomycin; Tobramycin
- Chemical Abstracts Service Registry Number: CAS 57-92-1 (streptomycin)
- Chemical/Pharmaceutical/Other Class: Antimicrobial agents. These drugs contain amino sugars in glycoside linkage
- Chemical Formula: $C_{21}H_{39}N_7O_{12}$ (streptomycin)
- Chemical Structure: Aminoglycosides are antimicrobial agents with dissimilar structures; it is impossible to represent aminoglycosides with a single general structure. The following is the structure of streptomycin:



Uses

Aminoglycosides are a group of potent antibiotics primarily used to treat certain infections caused by aerobic, Gram-negative bacteria. They are used in the treatment of severe infections of the abdomen and urinary tract, complicated skin, bone, or soft tissue infection, severe pelvic inflammatory disease, bacteremia (bacteria in the blood), ocular infections (topical), inflammation of ear (topical), neonatal sepsis, and endocarditis. In general, gentamicin, tobramycin, and amikacin are used in similar circumstances, often interchangeably. Of these, gentamicin is the aminoglycoside used most often because of its low cost and reliable activity against Gram-negative aerobes. Tobramycin may be the aminoglycoside of choice for use against *Pseudomonas aeruginosa* and *Enterobacter* species because of its greater *in vitro* activity. Amikacin is used against bacteria that are resistant to other aminoglycosides, since its chemical structure makes it less susceptible to inactivating enzymes. Aminoglycosides are also effective against mycobacteria, the bacteria responsible for tuberculosis.

Aminoglycosides are ineffective against anaerobic bacteria (bacteria that cannot grow in the presence of oxygen), viruses, and fungi. Only one aminoglycoside, paromomycin, is used against parasitic infection. In addition, some of the aminoglycosides have been widely used for preparation of the bowel for surgery and as adjunct to the therapy of hepatic coma. Aminoglycosides are also used to enhance bactericidal activity of betalactam drugs for the treatment of serious infections.

Background Information

Aminoglycosides are hydrophilic sugars that possess amino and hydroxyl functionalities. They are polycationic species at physiological pH, meaning they bind to negatively charged molecules such as DNA and RNA. Since their introduction into clinical use and despite the advent of newer agents (carbapenems, monobactams, and fluoroquinolones), aminoglycoside antibiotics continue to play an important role in the treatment of severe infections, particularly those due to aerobic, Gram-negative bacilli. Several factors account for their durability and continued clinical usefulness: therapeutic efficacy, synergy with the β-lactam antibiotics, low rate of development of true resistance, and low drug cost. Their main drawback has been the occurrence of (reversible) nephrotoxicity and ototoxicity in a significant number of patients (5–25%).

The first aminoglycoside, streptomycin, was isolated from *Streptomyces griseus* in 1943; neomycin was isolated from *Streptomyces fradiae*. This antibiotic was very effective against tuberculosis. However, one of the main drawbacks to streptomycin is its toxicity, especially to cells in the inner and middle ear and the kidney. Furthermore, some strains of tuberculosis are resistant to treatment with streptomycin. Therefore, medical researchers have put considerable effort into identifying other antibiotics with streptomycin's efficacy, but without its toxicity. Gentamicin, isolated from Micromonospora in 1963, was a breakthrough in the treatment of Gram-negative bacillary infections, including those caused by *Pseudomonas aeruginosa*. Other aminoglycosides were subsequently developed, including amikacin (Amikin), netilmicin (Netromycin), and tobramycin (Nebcin), which are all currently available for systemic use in the United States.

These bacteria can be identified by their reaction to Gram's stain. In Gram's staining, a film of material containing the possible bacteria is placed on a glass slide and dried. The slide is stained with crystal violet for 1 min, cleaned off with water, and then placed into a solution of Gram's iodine solution for 1 min. The iodine solution is rinsed off and the slide is immersed in 95% ethyl alcohol. The slide is then stained again with reddish carbolfuchsin or safranine for 30 s, rinsed in water, dried, and examined. Gram-positive bacteria retain the violet purple stain. Gram-negative bacteria accept the red stain.

Exposure Routes and Pathways

Ingestion is the most common route for both accidental and intentional exposures to aminoglycosides. Dermal route of exposure is also possible, especially with some of the chronic topical applications of aminoglycosides such as 1% neomycin.

Toxicokinetics

Aminoglycosides are poorly absorbed from the gastrointestinal or respiratory tract. The extent of absorption varies with a specific agent, ranging from as low as 0.2% to as high as 9%. Protein binding of aminoglycoside is from as low as 0–3% to as high as 11% depending on the agents. The volume of distribution for aminoglycosides ranges from 0.16 to $0.34\,l\,kg^{-1}$. Greater than 90% of aminoglycosides are excreted unchanged through the kidney. After parenteral administration, aminoglycosides are primarily distributed within the extracellular fluid. Thus, the presence of disease states or iatrogenic situations that alter fluid balance may necessitate dosage

modifications. When used parenterally, adequate drug concentrations are typically found in bone, synovial fluid, and peritoneal fluid. Penetration of biologic membranes is poor because of the drug's polar structure, and intracellular concentrations are usually low, with the exception of the proximal renal tubule. Endotracheal administration results in higher bronchial levels compared with systemic administration, but differences in clinical outcome have not been consistent.

Following parenteral administration of an aminoglycoside, subtherapeutic concentrations are usually found in the cerebrospinal fluid, vitreous fluid, prostate, and brain. Aminoglycosides are rapidly excreted by glomerular filtration, resulting in a plasma half-life of therapeutic doses ranging from 1.5 to 3.2 h in a patient with 'normal' renal function to 30–60 h in patients with impaired kidney function. The half-life of aminoglycosides in the renal cortex is $\sim$100 h, so repetitive dosing may result in renal accumulation and toxicity.

Mechanism of Toxicity

The mechanism of toxicity for aminoglycosides has not been fully explained and is therefore unclear. It is known that the drug attaches to a bacterial cell wall and is drawn into the cell via channels made up of a protein, porin. Once inside the cell, the aminoglycoside attaches to the 30S bacterial ribosomes. Ribosomes are the intracellular structures responsible for manufacturing proteins. This attachment either inhibits protein biosynthesis or causes the cell to produce abnormal, ineffective proteins. The bacterial cell cannot survive with this impediment. This explanation, however, does not account for the potent bactericidal properties of these agents, since other antibiotics that inhibit the synthesis of proteins (such as tetracycline) are not bactericidal. Recent experimental studies show that the initial site of action is the outer bacterial membrane. The cationic antibiotic molecules create fissures in the outer cell membrane, resulting in leakage of intracellular contents and enhanced antibiotic uptake. This rapid action at the outer membrane probably accounts for most of the bactericidal activity.

Energy is needed for aminoglycoside uptake into the bacterial cell. Anaerobes have less energy available for this uptake, so aminoglycosides are less active against anaerobic bacteria (bacteria that cannot grow in the presence of oxygen), viruses, and fungi. And only one aminoglycoside, paromomycin, is used against parasitic infection. Like all other antibiotics, aminoglycosides are not effective against influenza, the common cold, or other viral infections.

Acute and Short-Term Toxicity (or Exposure)

Animal

Several investigators have assessed the toxic effects of high doses of aminoglycosides in animals. Studies with dogs, rabbits, rats, and guinea pigs treated with doses ranging from 7.5 to 120 $\mathrm{mg\,kg^{-1}\,day^{-1}}$ in single and divided doses for 10–29 days suggest that less frequent glycoside administration is associated with less nephrotoxicity as assessed by serum creatinine levels, the glomerular filtration rate, and histopathology. A single study in rats assessing ototoxicity based on cochlear histology reported a lack of toxicity regardless of administration frequency.

Human

Because the body does not metabolize aminoglycosides, aminoglycoside activity is unchanged by induction or inhibition of metabolic enzymes, such as those in the cytochrome P450 system. Certain medications may increase the risk of renal toxicity with aminoglycoside use (e.g., use of diuretics, radiographic contrast exposure effective circulating volume depletion, ACE inhibitors, nonsteroidal anti-inflammatory drugs, other nephrotoxic medications, concomitant use of amphotericin, and cisplatin).

All aminoglycosides have a downside: they can cause ototoxicity. In the case of systemic gentamicin, ototoxicity appears to be primarily related to the duration of treatment, especially when the treatment course exceeds 10–14 days. It is also important to realize that gentamicin-induced ototoxicity tends to be primarily vestibular, although cochleotoxicity is seen as well. Overdoses may result in renal damage or ototoxicity (deafness and vertigo), and rarely neuromuscular blockade and hypersensitivity reactions depending on the dose and duration. Nephrotoxicity receives the most attention, perhaps because of easier documentation of reduced renal function, but it is usually reversible. Nephrotoxicity results from renal cortical accumulation resulting in tubular cell degeneration and sloughing. Examination of urine sediment may reveal dark-brown, fine, or granulated casts consistent with acute tubular necrosis but not specific for aminoglycoside renal toxicity. Although serum creatinine levels are frequently monitored during aminoglycoside use, an elevation of serum creatinine is more likely to reflect glomerular damage rather than tubular damage. In most clinical trials of aminoglycosides, however, nephrotoxicity has been defined by an elevation of serum creatinine. Periodic monitoring of serum creatinine concentrations may alert the clinician to renal toxicity. Retinopathy, visual loss, and

conjunctival necrosis have been also associated with this class of antibacterial agent. Irreversible damage of the auditory and vestibular functions of the eighth cranial nerve can occur but this is thought to be related to dose and duration of treatment.

Chronic Toxicity (or Exposure)

Human

Chronic topical application of 1% neomycin to a large wound precipitated severe hearing loss in an adult within 3 weeks following application. Serious toxicity is a major limitation to the usefulness of the aminoglycosides, and all drugs in this class share the same spectrum of toxicity.

Clinical Management

With overdose of aminoglycosides, the first effort is mobilized in supporting respiratory and cardiovascular functions. For oral ingestion, treatment focuses on preventing absorption with emesis and/or activated charcoal if appropriate. Since overdoses of aminoglycosides are also associated with renal damage, maintaining urine output with intravenous fluids is recommended if necessary.

See also: Sensory Organs.

Further Reading

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Aminopyridine, 4-

David Roane

- Chemical Abstracts Service Registry Number: CAS 504-24-5
- Synonyms: 4-AP; 4-Pyridinamine; Pyridin-4-amine; Avitrol; Fampridine™
- Chemical/Pharmaceutical/Other Class: Aminopyridine pesticide; K^+ channel blocker
- Chemical Formula: $C_5H_6N_2$

Uses

4-Aminopyridine is used broadly as an avicide. It is classified as a restricted use pesticide by the US Environmental Protection Agency. The compound is also being developed for clinical human application in the treatment of certain nerve conduction disorders, and it is used in experimental laboratory settings as an *in vitro* antagonist of voltage sensitive K^+ channels.

Exposure Routes and Pathways

As an avicide, 4-aminopyridine is impregnated into grain, set out as bait, and elicits toxicity to all grain-consuming bird (and other) species. Oral exposure can occur as with misuse of impregnated grain. Any grain-consuming organism, including livestock species, is at risk of accidental ingestion. Human exposure is also possible in industrial and manufacturing settings, and in circumstances where individuals (e.g., applicators) may be exposed during avicide use. 4-Aminopyridine can be absorbed through the skin. In clinical settings, patients are deliberately exposed to the compound for its therapeutic effects.

Toxicokinetics

The parent compound is active. Approximately 90% of ingested amount is excreted unchanged in the urine. 4-Aminopyridine is not thought to accumulate in the body.

Mechanism of Toxicity

The mechanism of 4-aminopyridine toxicity is due to the direct actions of the compound as a blocker of voltage-sensitive K^+ channels. At toxic doses, the chemical disrupts normal action potential conduction and nonselectively enhances neurotransmitter release. Because 4-aminopyridine blocks voltage-sensitive K^+ channels on neurons, it has the capacity to enhance action potential conduction in demyelinated tissue with therapeutically beneficial results. Toward this end, a compound (fampridine) is in development

as a therapeutic agent in the treatment of multiple sclerosis. The compound is also being investigated for its ability to improve neuronal signaling in patients with partial spinal cord injuries.

Acute and Short-Term Toxicity (or Exposure)

Animal

4-Aminopyridine is highly toxic to mammalian species. The compound is absorbed through both the skin and gastrointestinal tract. The LD_{50} for dermal exposure in rabbits is $326\,mg\,kg^{-1}$. The oral LD_{50} in rats is $\sim 20\,mg\,kg^{-1}$. The commercially available, technical grade 4-aminopyridine contained in the avicide product, Avitrol, has a reported oral LD_{50} of $28.7\,mg\,kg^{-1}$ in rats and $3\,mg\,kg^{-1}$ in dogs. 4-Aminoyridine is an eye irritant.

Human

Reported toxicities are of an acute nature. The signs and symptoms in humans include paresthesia, sweating, dizziness, ataxia, tremors, tachycardia, hypertension, and convulsions. Because 4-aminopyridine is a nervous system stimulant, it has been suggested that individuals with a history of convulsive disorders may be at increased risk. Convulsions are reported to be responsive to benzodiazepines. 4-Aminopyridine has been reported to cause severe poisoning in adult humans at dosages of less than 60 mg. The dosages used for therapeutic purposes in human trials are $20–30\,mg\,day^{-1}$ in divided doses over extended periods. In one reported study, six patients were given 24 mg intravenously without experiencing serious side effects.

Chronic Toxicity (or Exposure)

Animal

Long-term exposure may affect liver and nervous system functions. Long-term dietary exposures increased brain weights.

Human

Scarce data exist on the long-term effects of low-dose exposure to 4-aminopyridine.

Environmental Fate

4-Aminopyridine is adsorbed to soil particles and is moderately persistent in the environment. It has been reported to be slowly metabolized by soil microorganisms. The rate of disappearance varies with the organic content of soils and the disappearance halftime has been reported to range from 3 to 32 months. Movement from upper soil layers is thought to be minimal due to strong soil adsorption and the compound is not expected to represent a significant threat to groundwater.

Ecotoxicology

Bird mortality is reported to be low because toxicant-induced behavioral responses occur at sublethal doses, that is, behavioral changes in small numbers of birds act as a repellant to the remainder of the flocks. The reported LD_{50} across species ranged from $3\,mg\,kg^{-1}$ in crows to $15\,mg\,kg^{-1}$ in Bob White quail. 4-Aminopyridine is moderately toxic to fish. The LC_{50} ranged from 2.4 to $4\,mg\,l^{-1}$ in channel catfish. The LC_{50} in bluegill was $3.2–3.4\,mg\,l^{-1}$.

Exposure Standards and Guidelines

The reference dose is $0.000\,02\,mg\,kg^{-1}\,day^{-1}$.

See also: Pesticides.

Further Reading

Smith KJ, Felts PA, and John GR (2000) Effects of 4-aminopyridine on demyelinated axons, synapses and muscle tension. *Brain* 123: 171–184.

US Environmental Protection Agency (1980) *Pesticide Registration Standard: 4-Aminopyridine: Avitrol*. Washington, DC: Office of Pesticides and Toxic Substances.

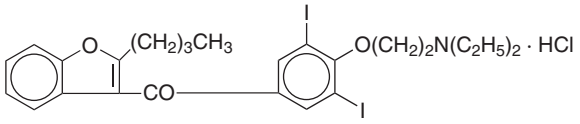
Relevant Websites

http://www.acorda.com – Acorda Therapeutics.
http://pmep.cce.cornell.edu – Cornell University.

Amiodarone

Elizabeth J Scharman

- Chemical Abstracts Service Registry Numbers: CAS 1951-25-3; CAS 19774-82-4 (hydrochloride)
- Synonyms: Amiodarone hydrochloride; Cordarone®
- Chemical/Pharmaceutical/Other Class: Class III antiarrhythmic agent, an iodinated benzofuran-derivative antiarrhythmic
- Chemical Formula: $C_{25}H_{29}I_2NO_3$
- Chemical Structure:



Uses

Amiodarone is indicated for the suppression and prevention of documented life-threatening, recurrent, ventricular tachycardia or fibrillation when other agents have failed. Amiodarone is also used in the management of supraventricular tachyarrhythmias including paroxysmal atrial fibrillation and atrial flutter, ectopic or multifocal atrial tachycardia, junctional tachycardia, and paroxysmal reentrant supraventricular tachycardia when other agents have failed to suppress or prevent their recurrence. Amiodarone has also been used to treat wide-complex tachycardia of uncertain mechanism.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to amiodarone although few cases exist in the literature. Amiodarone is available in an oral dosage form and a parenteral dosage form for intravenous administration.

Toxicokinetics

Amiodarone is slowly absorbed with an average bioavailabilty of 50% (range, 22–86%). Food has been shown to increase the rate and extent of absorption. Peak plasma concentrations are seen within 3–7 h (range, 2–12 h); however, the onset of action is not seen for at least 2–3 days and usually not until 1–3 weeks. This delay occurs even if a loading dose is given. Maximal responses may not occur until up to 5 months after starting therapy. Amiodarone is extensively metabolized to a major metabolite *N*-desethylamiodarone, which is active. The volume of distribution is $65.8\,l\,kg^{-1}$ (range $18.3–147.4\,l\,kg^{-1}$). The drug is found in adipose tissue and many organs, especially the liver, lung, spleen, and skeletal muscle. Concentrations of the drug in bile may be 50 times greater than concentrations in plasma. During chronic therapy, the metabolite appears in the same tissues with the exception of adipose tissue, which primarily contains amiodarone. Approximately 96% is protein bound. The drug can cross the placenta and is distributed into breast milk in concentrations exceeding that of maternal plasma. The therapeutic range is $1–2.5\,\mu g\,ml^{-1}$. Elimination of amiodarone is at least biphasic. With single-dose administration, the half-life is 25 days (range 9–47 days). Following chronic administration, the terminal elimination half-life in the majority of patients is 40–55 days (range 26–107 days). Amiodarone may undergo enterohepatic recirculation. Almost the entire drug is excreted in the feces.

Mechanism of Toxicity

Amiodarone is primarily a class III antiarrhythmic agent but does display activity in each of the four Vaughn–Williams antiarrhythmic classes. The drug delays repolarization via prolongation of the action potential duration and effective refractory period, decreases AV conduction, depresses sinus node and junctional automaticity, acts as a noncompetitive α- and β-adrenergic inhibitor, and slows automaticity of Purkinje fibers.

It is unknown whether thyroid dysfunction (hypothyroidism or hyperthyroidism) is a result of the amiodarone, the iodine contained in the amiodarone, or another mechanism. The production of amiodarone–phospholipid complexes within organs has been proposed as the mechanism for some of this drug's adverse effects. The mechanism of the pulmonary toxicity seen following chronic use is also uncertain but is the result of a hypersensitivity reaction in some.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in dogs is more than $3\,g\,kg^{-1}$.

Human

Determination of toxicity is based on observation as there is no milligram per kilogram toxic dose established. Symptoms may include nausea, bradycardia,

heart block, hypotension, and QT prolongation leading to torsades de pointes. The onset of toxicity may be substantially delayed for up to 3 days.

Chronic Toxicity (or Exposure)

Animal

Chronic carcinogenicity studies in rats demonstrate significant increases in thyroid tumors. The effects are dose related and have been demonstrated at doses as low as $5\,mg\,kg^{-1}$. Daily doses of $90\,mg\,kg^{-1}$ in pregnant rats showed reduced fertility. Doses of $25\,mg\,kg^{-1}\,day^{-1}$ in rabbits showed no change in fertility or adverse effects on the fetus but doses of $75\,mg\,kg^{-1}\,day^{-1}$ caused abortions in 90% of test animals.

Human

Chronic therapy with amiodarone has been associated with pulmonary interstitial pneumonitis/alveolitis, hypersensitivity pneumonitis, and pulmonary fibrosis; fatalities have resulted. Other side effects include: elevated liver function tests, worsening of arrhythmias, onset of new arrhythmias, fatigue, tremor, involuntary movements, dizziness, paresthesias, difficulty in walking, hypothyroidism, hyperthyroidism, nausea, vomiting, constipation, anorexia, corneal microdeposits, and photosensitivity. The skin may develop a blue-gray color, especially in areas exposed to the sun.

In Vitro Toxicity Data

Amiodarone has not been shown to be mutagenic in Ames, micronucleus, and lysogenic tests.

Clinical Management

Amiodarone is adsorbed to activated charcoal. Dialysis will not enhance elimination. Standard supportive therapies, such as vasopressors, should be utilized as clinically indicated. Patients may require prolonged observation due to the delay in the development of adverse effects.

See also: Iodine.

Further Reading

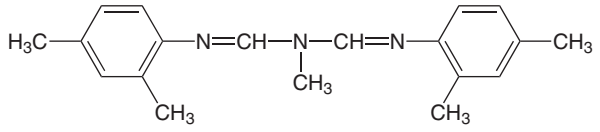
Burns KEA, Piliotis E, and Garcia BM (2000) Amiodarone pulmonary, neuromuscular and ophthalmological toxicity. *Canadian Respiratory Journal* 7: 193–197.

Olshansky B (1997) Amiodarone-induced pulmonary toxicity. *New England Journal of Medicine* 337: 1814.

Amitraz

Jamaluddin Shaikh

- Chemical Abstracts Service Registry Number: CAS 33089-61-1
- Synonyms: Aazdieno; Acadrex; Acarac; Amitraze; Ectodex; Triatox; Ovasyn; Ovidrex; Triazid
- Chemical/Pharmaceutical/Other Class: Formamidine insecticide and acaricide
- Chemical Structure:



Uses

Amitraz is used to control insects on pears, cotton, and livestock including cattle and hogs. It is used to control red spider mites, leaf miners, scale insects, and aphids. On cotton it is used to control bollworms, white fly, and leaf worms. Amitraz is effective against ticks, mites, and lice on animals.

Exposure Routes and Pathways

Amitraz is a straw-colored crystalline, odorless product. The oral route is a common exposure pathway. Amitraz is poorly absorbed by the dermal route.

Toxicokinetics

Peak plasma levels occur ~1 h after dosing. Liver, kidney, and muscle have highest residues ~0.75–1.5 h after oral dosing. Amitraz is rapidly metabolized and excreted, mainly in the urine. Metabolism of amitraz is similar among many species by hydrolysis to *N*-(2,4-dimethylphenyl)-*N*′-methyl formamide and 2,4-dimethyl formanilide, leading to production of 4-amino-3-methylbenzoic acid. This metabolite is rapidly conjugated and excreted. After repeated dosing in rats highest residues of amitraz

are found in thyroid and adrenal glands, liver, skin, spleen, and eyes.

Mechanism of Toxicity

Acute oral administration of amitraz causes central nervous system (CNS) depression. The toxic effects of amitraz are possibly from α2-adrenoreceptor agonist action. Chronic exposure of amitraz results in CNS depression, increases blood glucose levels, and produces hypothermia.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for amitraz in rats is 523–800 mg kg^{-1}. The oral LD_{50} is higher for mice (> 1600 mg kg^{-1}). The dermal LD_{50} is greater than 1600 mg kg^{-1} for rats. The inhalation LC_{50} (6 h) of amitraz for rats is 65 mg l^{-1} of air.

Human

One of the predominant signs after oral ingestion of amitraz is CNS depression. The other clinical signs reported with amitraz poisoning in humans are drowsiness, vomiting, miosis followed by mydriasis, bradycardia, hypotension, hyperglycemia, and respiratory failure. All clinical signs appear 30 min to 4 h after poisoning.

Chronic Toxicity (or Exposure)

Animal

Amitraz is not teratogenic in rats. Dogs appear most sensitive to subchronic amitraz exposures, with CNS depression being predominant. A chronic (2 year) feeding study reported that rats receiving 50 mg kg^{-1} day^{-1} and dogs receiving 0.25 mg kg^{-1} day^{-1} amitraz did not exhibit signs of overt toxicity. Chronic exposure may lead to bladder irritation, heat intolerance, and loss of muscle tone. Amitraz is not carcinogenic in rats but it does cause tumors (in the lungs, liver, and lymph nodes) in female mice.

Human

Little is known regarding chronic effects of amitraz in humans.

In Vitro Toxicity Data

Amitraz is negative in a number of mutagenesis assays and did not cause damage to DNA.

Clinical Management

With dermal exposure, areas exposed to amitraz should be washed with soap and water. Eyes should be washed with copious amounts of clean water for 15 min. Ophthalmologic consultation is required in case of persistent irritation. Gastric lavage is indicated immediately in acute oral overdose. The amitraz-poisoned patient is also treated with atropine and activated charcoal. Intubation and assisted ventilation may be required as supportive care.

Environmental Fate

Amitraz degrades in the environment to *N*-(2,4-dimethylphenyl)-*N*′-methyl formamidine. Amitraz has low potential of leaching in soils. Its half-life in soil has been reported to be less than 1 day.

Ecotoxicology

Amitraz is moderately toxic to fish. The LC_{50} (3 days) is 1.3 mg l^{-1} for bluegill sunfish and 3.2–4.2 mg l^{-1} for harlequin fish. It is slightly toxic to birds. The dietary LC_{50} (8 days) values for mallard ducks and Japanese quail are 7000 and 1800 mg kg^{-1}, respectively. Amitraz may affect reproduction in birds. It is relatively nontoxic to bees. In bees, the LD_{50} by ingestion and direct spraying are 12 μg per bee and 3.6 mg l^{-1}, respectively.

Exposure Standards and Guidelines

The oral reference dose for amitraz is 2.5 μg kg^{-1} day^{-1}.

See also: Pesticides.

Further Reading

Iyer P (2001) Developmental and reproductive toxicology of pesticides. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 375–423. San Diego, CA: Academic Press.

Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

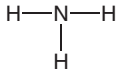
Amitroptyline *See* Tricyclic Antidepressants.

Ammonia

Ralph J Parod

This article is a revision of the previous print edition article by Edward Kerfoot, volume 1, pp. 60–61, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7664-41-7
- Synonyms: Anhydrous ammonia; Ammonia gas; Liquid ammonia; R 717
- Chemical/Pharmaceutical/Other Class: Nitrogen family
- Chemical Structure:



Uses

Commercial production of ammonia is primarily via a modified Haber–Bosch process in which atmospheric nitrogen is combined with hydrogen obtained from natural gas, a process termed nitrogen fixation. About 80% of the commercially produced ammonia is used in fertilizers with the remainder used in a variety of applications such as plastics, synthetic fibers and resins, pharmaceuticals, explosives, refrigeration, and household cleaners. Ammonia is a naturally occurring compound that is a key component of the global nitrogen cycle and all living organisms. Nitrogen fixation by current industrial processes approximates that produced naturally by biological processes and lightening strikes.

Exposure Routes and Pathways

Ammonia is a gas under normal environmental conditions; thus, human exposures typically occur via inhalation and dermal contact. Oral exposures are also possible as ammonia has high water solubility.

Toxicokinetics

Unionized ammonia can freely diffuse through tissue cells but forms ammonium hydroxide upon contact with tissue water. Dissolved ammonia and the less permeable ammonium ion exist in a dynamic equilibrium that serves to retard the absorption of ammonia into the circulation depending on the complexity of the intervening tissues. During short-term ($\leqslant$2 min) exposures to $\leqslant$500 ppm ammonia, most (83–92%) of the inspired ammonia is retained within the upper airways. This absorption process may be adaptive or saturable because most of the ammonia inspired during longer-term exposures (10–27 min) is exhaled with $\sim$4–30% being retained within the upper airways and available for systemic absorption. Ammonia or ammonium ion is well-absorbed by the gastrointestinal tract. Almost 100% of the ammonia produced endogenously in the human digestive tract ($60\,mg\,kg^{-1}\,day^{-1}$) is absorbed and metabolized in the liver to urea and glutamine. The brain can also convert ammonia to glutamine. Due to first-pass metabolism in the liver, little ammonia from the gut reaches the systemic circulation, and toxicologically significant amounts of ammonia in blood ($>1\,\mu g\,ml^{-1}$) probably occur only in severe disease states where the metabolism of ammonia by the liver and the excretion of metabolites by the kidney are compromised. It is unlikely that a significant amount of the ammonia contacting the skin is absorbed. Ammonia that reaches the circulation is distributed throughout the body where it can be used in protein synthesis or as a buffer. Most of the absorbed ammonia is excreted in the urine as urea, with minimal amounts excreted in the feces or expired air.

Mechanism of Toxicity

The primary immediate effect of ammonia exposure is burns to the skin, eyes, and respiratory tract. Ammonia dissolves in tissue water and forms ammonium hydroxide that breaks down cellular proteins, saponifies cell membrane lipids resulting in cell disruption and death, and initiates an inflammatory response that further damages surrounding tissues.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ammonia is an irritant gas that can cause severe local effects in the absence of systemic toxicity. No signs of toxicity were observed in rats continuously exposed to 58 ppm ammonia for 114 days; no abnormal changes

in the lung were noted after gross and microscopic examination. Ciliary activity in the trachea of rabbits was impaired at 100 ppm. At 300 ppm ammonia, the respiratory rate of mice and rats was depressed by 50%. Continuous exposure of four animal species to 677 ppm ammonia for 90 days resulted in extensive focal and diffuse inflammation of the pulmonary interstitium (all species), marked eye irritation with some corneal opacities (rabbit, dog), and fatalities (rat, guinea pig). The 1 h LC_{50} values in the mouse and rat were ~4500 and 9500 ppm, respectively. No genotoxicity data in laboratory animals are available.

Human

Ammonia has an odor threshold ranging from 1 to 5 ppm. Exposures between 20 and 25 ppm can cause complaints and discomfort in some workers unaccustomed to ammonia exposure but have little effect on pulmonary function or odor sensitivity. Concentrations of 100 ppm caused definite irritation of the respiratory tract and eyes, and exposures at 250 ppm ammonia are bearable for 30–60 min. Severe irritation of the respiratory tract, skin, and eyes has been observed following ammonia exposures ranging from 400 to 700 ppm. Exposure to 2500–4500 ppm ammonia can be fatal within 30 min. Immediate fatalities appear to be the result of airway obstruction, particularly laryngeal edema and glottic spasm, while infections and other secondary complications appear to be the cause of fatality among those who survive for several days to weeks.

Chronic Toxicity (or Exposure)

No data in animals and humans are available.

In Vitro Toxicity Data

Data on the *in vitro* mutagenicity and clastogenicity of ammonia are limited and equivocal.

Clinical Management

Exposures by inhalation should be monitored for respiratory tract irritation, upper airway obstruction, bronchitis, or pneumonitis. Humidified supplemental 100% oxygen should be administered to help soothe bronchial irritation. Oxygen, in combination with intubation and mechanical ventilation, may be required in severe cases. Exposed eyes and skin should be irrigated immediately with copious amounts of water; eyes should be washed for at least 30 min or until the eye reached neutral pH as tested in the conjunctival sac. If eye irritation, pain, swelling, lacrimation, or photophobia persist, the patient should be seen in a health care facility.

Environmental Fate

In the atmosphere, ammonia is estimated to have a half-life of several days. The primary fate process is reaction of ammonia with acid air pollutants and removal of the resulting ammonium compounds by dry or wet deposition. Rain washout and reaction with photochemically produced hydroxyl radicals are also expected to contribute to the atmospheric fate of vapor-phase ammonia. In water and soil, ammonia will volatilize to the atmosphere and be removed by microbial processes, by adsorption to sediment and soil matrices as well as by plant uptake.

Ecotoxicology

The 96 h LC_{50} values for a variety of fish species range between 0.1 and 5 $mg\,l^{-1}$. For a variety of aquatic invertebrates, the 48 h LC_{50} values range between 1 and 190 $mg\,l^{-1}$.

Exposure Standards and Guidelines

International occupational exposure limits (OELs) generally range between 20 and 25 ppm as an 8 h time-weighted average (TWA). The American Conference of Governmental Industrial Hygienists has established an 8 h TWA OEL for ammonia of 25 ppm with a 15 min excursion limit of 35 ppm. The National Institute of Occupational Safety and Health indicates that 300 ppm ammonia is immediately dangerous to life or health.

See also: Respiratory Tract.

Further Reading

CARB (1999) Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29.

US Environmental Protection Agency (1985) Ambient Water Quality Criteria for Ammonia (EPA 440/5-85-001), January.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Ammonia.

Ammonium Nitrate

Prathibha S Rao

- Chemical Abstracts Service Registry Number: CAS 6484-52-2
- Synonyms: Ammonium saltpeter; German saltpeter; Norway saltpeter; Nitrate d'ammonium; Nitrate of ammonium; Herco prills; Merco prills; Varioform I; AN
- Chemical/Pharmaceutical/Other Class: Nitrate
- Chemical Formula: NH_4NO_3

Uses

Ammonium nitrate is used commonly in fertilizers; in pyrotechniques, herbicides, and insecticides, as well in the manufacture of nitrous oxide. It is used as an absorbent for nitrogen oxides, an ingredient of freezing mixtures, an oxidizer in rocket propellants, and a nutrient for yeast and antibiotics. It is also used in explosives (especially as an oil mixture) for blasting rocks and in types of mining.

Nitrates and nitrites are used to cure meats and to develop the characteristic flavor and pink color, to prevent rancidity, and to prevent growth of *Clostridium botulinum* spores in or on meats.

Background Information

Ammonium nitrate is found as colorless or white to gray crystals or odorless beads with a molecular weight of 80.06 and specific gravity of $1.725\,g\,cm^{-1}$. It has a melting point of 169.5°C and boils at 210°C with evolution of nitrous oxide. It forms chloramines on chlorination and is incompatible with acetic acid; acetic anhydride, hexamethylene tetramine acetate, and nitric acid mixture; ammonia; aluminum, calcium nitrate, and formamide mixture; metals; alkali metals; and combustible agents.

Exposure Routes and Pathways

Common exposure pathways are via products in which ammonium nitrate is used. Nitrates are also found in water from soils, rocks, decomposing organic matter, and in vegetables like beets, radish, lettuce, celery, and spinach. It is also found in secretions like saliva and formed in the mouth and gut due to bacterial action.

Toxicokinetics

Nitrates are well absorbed from the gastrointestinal tract producing peak blood levels only 40 min after ingestion. They may also be absorbed through abraded or damaged skin. Nitrates are converted to nitrites by various bacteria in the stomach and intestines of animals and humans. Approximately 14–31% of nitrate is excreted via the kidneys. The mean renal clearance for nitrates is $\approx 26\,ml\,min^{-1}$. About 40% is excreted as nitrites in the urine. It is also recycled through the saliva.

Mechanism of Toxicity

Nitrate and nitrites can combine with secondarys amines to form dimethylnitrosamines, which are acutely toxic and cause centrilobular necrosis, fibrous occlusion of central veins, and pleural and peritoneal hemorrhages in animals. In the body nitrates are converted to nitrites, which can oxidize hemoglobin to methemoglobin and lead to cyanosis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methemoglobinemia, which can lead to anoxia and death in extreme cases, is the primary acute toxic effect of oral exposure to inorganic nitrates in all animals tested. Ruminant animals are most susceptible. This effect depends on a number of factors including the conversion of nitrates to nitrites, the ability of the various animals to enzymatically reduce methemoglobin, the amount of vitamins A, C, D, and E in the diet, and the nutritional state of the animal. Acute nitrate toxicity in cattle has been reported following the ingestion of water containing 500 ppm or more nitrate or feed containing 5000 ppm or more nitrates. With acute poisoning the signs of poisoning are observed and the animal is in critical condition. Nitrate is rapidly converted to nitrite in the rumen and is immediately absorbed in large amounts into the bloodstream. Animals can die within a few hours of initial ingestion of a high nitrate feed.

If cattle are fed once a day, maximum methemoglobin levels occur ~8 h after feeding. When cattle are fed twice daily, maximum levels occur 4–5 h after feeding. The once-a-day feeding program results in higher total methemoglobin levels than twice-a-day feeding. With once-a-day feeding, a

larger quantity of feed is consumed at once and a greater amount of nitrate is released from the feed in a short period of time.

Signs of acute poisoning in cattle are: increased heart rate, muscle tremors, vomiting, weakness, blue-gray mucous membranes, excess saliva and tear production, depression, labored or violent breathing, staggering gait, frequent urination, low body temperature, disorientation, and an inability to get up. Animals are often found in a lying position after a short struggle. In most cases of acute poisoning, animals are found dead before any signs of toxicity are observed.

Human

Ammonium nitrate is irritating to the eyes, nose, throat, and mucous membranes. Inhalation of this compound can cause severe lung congestion, coughing, difficulty in breathing, and increased acid urine. Exposure to large amounts can cause systemic acidosis and abnormal hemoglobin. It is considered to have low toxicity since it causes readily reversible tissue changes that disappear when exposure stops.

In the body nitrates are converted to nitrites, which can oxidize hemoglobin to methemoglobin and lead to cyanosis. They also cause unconsciousness, dizziness, fatigue, shortness of breath, nausea, and vomiting. The skin is warm and sweaty and later becomes cold due to vasodilation. It causes coronary blood vessel contraction, bradycardia, atrial fibrillation, cardiac ischemia, headache, convulsions, and diarrhea.

Nitrate transferred through breast milk causes methemoglobinemia in the infant. Infants are more predisposed to nitrate-related toxicity than adults due to decreased ability to secrete gastric acid, higher levels of fetal hemoglobin, and diminished enzymatic capability to reduce methemoglobin to hemoglobin.

No data are available on the teratogenicity or mutagenicity of ammonium nitrate.

Chronic Toxicity (or Exposure)

Animal

Chronic nitrate toxicity is a form of nitrate poisoning where the clinical signs of the disease are not observed. It is more common to see a reduction in weight gain, lower milk production, depressed appetite, and a greater susceptibility to infections. These production-related problems or losses are not often recognized and will occur when nitrate levels are at 0.5–1.0% of the daily feed consumption.

Chronic nitrate poisoning can cause abortions to occur within the first 100 days of pregnancy because nitrates interfere with the implantation of the egg in the uterus. When implantation does not occur, the fetus dies and is reabsorbed. During the first trimester of pregnancy, no obvious signs of an abortion are seen. Reproductive problems may also occur due to a nitrate or nitrite-induced hormone imbalance, but most are usually not recognized as feed related.

Calves affected by nitrate poisoning during the last three months of gestation are usually born one to four weeks premature, and most appear normal but die within 18–24 h of birth. Surviving newborn calves that are affected by nitrate poisoning may have convulsions and seizures.

Human

Chronic ingestion of $5\,mg\,kg^{-1}\,day^{-1}$ is considered unacceptable. Common findings associated with nitrate poisoning include unconsciousness, dizziness, fatigue, shortness of breath, nausea, vomiting, coma, cyanosis, dyspnea, and pallor.

Clinical Management

Absorption should be prevented by dilution with 4–8 ounces of milk or water or by gastric lavage in patients who are comatose or at a risk of convulsing. Charcoal or saline cathartic may also be given. Emesis may be induced if initiated within 30 min of ingestion. Methylene blue is used to treat methemoglobinemia. Diazepam is administered (maximum rate $5\,mg\,min^{-1}$) to control seizures. Recurrent seizures are controlled by phenytoin or phenobarbital. An EKG should be monitored while administering phenytoin. Dopamine or norepinephrine is administered to control hypotension.

Ecotoxicology

Upon decomposition ammonium nitrate will release ammonium ions. Ammonia is a toxic hazard to fish and maybe harmful to animals on direct ingestion. Ammonium nitrate is nonpersistent and noncumulative when applied using normal agriculture practices. It is not listed as a marine pollutant.

Exposure Standards and Guidelines

The nitrate limit in drinking water was established as a safeguard against infantile acquired methemoglobinemia. EPA's maximum contaminant level (MCL) for nitrates is 10 ppm. The MCL for nitrites is 1 ppm.

See also: Nitrous Oxide.

Further Reading

Heindel JJ, Chapin RE, Gulati DK, *et al.* (1994) Assessment of the reproductive and developmental toxicity of pesticide/fertilizer mixtures based on confirmed pesticide contamination in California and Iowa groundwater. *Fundamental and Applied Toxicology* 22: 605–621.

National Academy of Sciences (1981) The health effects of nitrite, nitrate and *N*-nitroso compounds. Washington: National Academy Press.

US Environmental Protection Agency (1985) Health effects criteria document for nitrate/nitrite. Washington: US Environmental Protection Agency, Office of Drinking Water, Criteria and Standards Division.

US Environmental Protection Agency (1987) Nitrate/nitrite health advisory. Washington: US Environmental Protection Agency, Office of Drinking Water.

US Environmental Protection Agency (1990) National pesticide survey: Summary results of pesticides in drinking water wells. Washington: US Environmental Protection Agency, Office of Pesticides and Toxic Substances.

Yang R (1993). NTP technical report on the toxicity studies of pesticide/fertilizer mixtures administered in drinking water to F344/N rats and B6C3F1 mice. Toxicity Report Series 36: 1-G3.

Relevant Websites

http://risk.lsd.ornl.gov – The Risk Assessment Information System.

http://www.1.agric.gov.ab.ca – Agriculture, Food and Rural Development, Government of Alberta, Canada.

Ammonium Perchlorate

Joan Strawson

This article is a previous print edition
article by Prathiba S Rao and Harihara M Mehendale,
volume 1, pp. 62–63, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7790-98-9
- Synonyms: Ammonium perchlorate; Perchloric acid; Ammonium salt; UN 0402; UN 1442
- Chemical Formula: NH_4ClO_4
- Chemical Structure: ClO_4–H_4N

Uses

Ammonium perchlorate is an explosive agent used as a component of fireworks, flash powders, explosives, smokeless jet, and rocket propellants. It is also used in oxidizing, engraving, or etching compounds, and as a reagent in analytical chemistry. Historically, perchlorate salts have been used therapeutically to treat hyperthyroid disorders, including Graves' disease. More recently, perchlorate has been used (alone or in combination with other antithyroid drugs) to treat amiodarone-induced thyrotoxicosis, a condition in which thyroid abnormality results from excess iodine when the iodine-containing drug amiodarone is given to control cardiac arrhythmia. Perchlorate also occurs naturally in nitrate-rich mineral deposits used in fertilizers.

Exposure Routes and Pathways

Perchlorate salts dissolve readily in water and are easily absorbed from the gastrointestinal tract. However, because of its high charge, the perchlorate anion does not penetrate the skin readily. Uptake of inorganic ions such as perchlorate through the skin is typically less than 10%, and frequently less than 1%. Exposure via inhalation of fumes or vapors is considered negligible because the vapor pressure of perchlorate salts and acids is low at room temperatures. The risk from exposure to particles would depend on the particle size distribution. Thus, the ingestion route is the major concern for the risk posed by the perchlorate contamination and is the focus of this characterization.

Toxicokinetics

Perchlorate appears to be well absorbed from the gastrointestinal tract. Perchlorate appears to be eliminated rapidly, primarily in the urine ($>90\%$), and virtually unchanged from both rats and humans. Half-lives have been reported for the rat ranging from <8 to 20 h. Perchlorate has been detected in the urine within 10–15 min of oral dosing and peak plasma levels occur within 3 h.

Mechanism of Toxicity

The perchlorate anion is a tetrahedron with four oxygen molecules at the corners and a chlorine molecule at the center. Perchlorate, with a partial molal ionic volume of 44.5, has a similar ionic size as iodide with a partial molal ionic volume of 36.7 at 25°C. Because of its chemical properties, perchlorate is a competitive inhibitor of the process by which iodide circulating in the blood is actively transported into thyroid follicular cells. The site of this inhibition is

the sodium–iodide symporter, a membrane protein located adjacent to the capillaries supplying blood to the thyroid. Inhibition of iodine uptake is the basis for the current and former pharmacological uses of perchlorate, and the likely precursor of potentially adverse effects. Subsequent events include decreases in serum thyroid hormone T4 (and thyroid hormone T3), leading to the potential for altered neurodevelopment in either dams or fetuses/neonates, and increases in serum thyroid stimulating hormone (TSH), leading to the potential for thyroid hyperplasia and tumors. The repeated observation of thyroid effects such as alterations of hormones, increased thyroid weight, and alterations of thyroid histopathology (including tumors) from a large number of rat studies on perchlorate provide supporting evidence for the proposed mode of action, and confirms that the perturbation of thyroid hormone level is the primary biological effect of perchlorate.

Acute and Short-Term Toxicity (or Exposure)

The systemic toxicity of perchlorate appears to be directly related to its action as a competitive inhibitor of iodide, and the symptoms tend to be similar to those of iodine deficiency.

Animal

Oral LD_{50} values for ammonium perchlorate range from 750 to 1900 mg kg^{-1} for rabbits, from 1900 to 2000 mg kg^{-1} for mice, and from 3500 to 4200 mg kg^{-1} for rats. Drinking water studies in rodents demonstrate that the thyroid is the only target organ for perchlorate toxicity. Following drinking water exposure of 4 and 14 days, decreased serum levels of the T3 and T4 as well as increased levels of TSH were observed. Following 14 days of exposure, increases in thyroid weight and histopathological changes in thyroid including hypertrophy and hyperplasia were observed in rats at doses greater than 1 mg kg^{-1} day^{-1}. No developmental effects were observed in either rats or rabbits exposed to ammonium perchlorate in drinking water during gestation.

Human

No case reports of acute poisoning in humans have been reported. Two clinical studies have evaluated the effects of ammonium perchlorate in drinking water at doses up to 0.5 mg kg^{-1} day^{-1} in healthy adult volunteers. The threshold for inhibition of iodine uptake in humans appears to be about 0.006 mg kg^{-1} day^{-1}. Doses of 0.5 mg kg^{-1} day^{-1} resulted in iodine uptake inhibition of ~70%. However, at this dose, no effect was observed on thyroid hormone levels or on any other parameter evaluated.

Chronic Toxicity (or Exposure)

Animal

In 90 day toxicity studies, only thyroid effects were observed in rats and mice following exposure to ammonium perchlorate in drinking water. As with shorter-term studies, changes in the serum levels of T3, T4, and TSH were observed, followed by increased thyroid weight and thyroid histopathological changes at doses greater than 1 mg kg^{-1} day^{-1}. These changes were reversible following cessation of exposure. No effects were observed on any other target organ. No reproductive effects in either males or females were observed in a two-generation study of rats exposed to ammonium perchlorate in drinking water. Two-year carcinogenicity bioassays demonstrated thyroid tumors in rats.

Human

Since perchlorate has become a public health issue, several human studies have been published, including several epidemiological studies and two occupational studies. The epidemiology studies have examined thyroid endpoints, including congenital hypothyroidism and T4 and TSH levels, in neonates born in areas known to have perchlorate in the public water supply, compared with infants born in areas without perchlorate in the public water supply. Another study in the United States has compared the prevalence of thyroid disease in Medicaid users in counties with perchlorate exposure through drinking water compared to Medicaid users in counties without perchlorate exposure. All studies, except one, showed that perchlorate had no effect on thyroid parameters. The remaining study found that infants in counties with perchlorate in drinking water had elevated TSH levels when measured by an analysis of variance on the log-transformed TSH values ($P = 0.017$), but not when measured by *t*-tests for each day of birth separately. Another study tested the hypothesis that perchlorate in drinking water suppresses thyroid function in 9784 newborns and 162 school-aged children. The study was conducted in Northern Chile, which has naturally occurring perchlorate in the drinking water, and compared populations in three different cities that had perchlorate concentrations of 100–120 μg l^{-1}, 5–7 μg l^{-1}, and nondetectable (<4 μg l^{-1}), respectively. The thyroid parameters measured in the newborn or school-age children were comparable among the three cities.

The occupational studies evaluated the thyroid function of workers in perchlorate production facilities. No effect on thyroid function was observed in workers after a single shift, or after a working lifetime. Lifetime exposures were up to 0.5 mg kg^{-1} day^{-1}.

In Vitro Toxicity Data

Ammonium perchlorate was tested in a battery of genotoxicity tests and found to be negative in all tests, including a reverse mutation assay in *Salmonella typhimurium* and the L5178Y/$TK^{+/-}$ mouse lymphoma assay. In addition, ammonium perchlorate was tested *in vivo* in a mouse micronucleus assay at doses up to 1000 mg kg^{-1} for 3 days, and found to be negative. Based on these data, perchlorate does not appear to be mutagenic or clastogenic, and genotoxicity does not appear to be a mode of action for perchlorate.

Clinical Management

The victim should be removed to fresh air and monitored for respiratory distress. Early intravenous administration of corticosteroids is recommended to prevent or treat noncardiogenic pulmonary edema. Inhalation of sympathomimetic agents is used to treat bronchospasm and wheezing. Absorption can be prevented by dilution with 4–8 oz ($\sim$118–237 ml) of milk or water. Absorption can also be prevented by gastric lavage in patients who are comatose or at the risk of convulsing. Charcoal, saline, or other cathartics can also be used. Cathartics should be avoided in patients with ileus or impaired renal function.

Environmental Fate

Ammonium perchlorate's manufacture and use in a variety of explosives, as well as its presence in some nitrate fertilizers, has resulted in its release to the environment. Perchlorate dust can be suspended in the air and inhaled by individuals working in perchlorate manufacturing facilities. In addition, open detonation of explosive materials or open burning of perchlorate-containing materials can result in the release of perchlorate in air. Perchlorate may also be found in soil, particularly where perchlorate-containing fertilizer has been applied, or where perchlorate-containing water is used for irrigation. Because perchlorate is highly soluble, it is not expected to concentrate in soil. Due in part to improved analytical methods, perchlorate has been detected in surface water and groundwater near various facilities that have manufactured and tested solid rocket fuels, most notably in California, Nevada, and Utah. Perchlorate has been measured in the public drinking water supply in several areas in California and in Lake Mead in Nevada.

Ecotoxicology

Perchlorate is concentrated by plants, especially in the leaves, that are irrigated with water-containing perchlorate. Perchlorate has been detected in vegetation and wildlife (aquatic insects, fish, frogs, mammals) near an ammunition plant historically associated with perchlorate-containing rocket propellants. There has been limited testing of perchlorate in aquatic species, soil invertebrates, and amphibians. Results in aquatic species are inconclusive because of the lack of cation controls. Perchlorate appears to inhibit thyroid activity and alter gonadal differentiation in amphibians at concentrations found in surface water.

Exposure Standards and Guidelines

Currently, there are no US federal or international occupational or environmental standards for ammonium perchlorate. California has a public health goal (PHG) for perchlorate of 6 ppb.

See also: Iodine; Levothyroxine; Perchlorate.

Further Reading

Strawson J, Zhao Q, and Dourson M (2004) Reference dose for perchlorate based on thyroid hormone change in pregnant women as the critical effect. *Regulatory Toxicology and Pharmacology* 39: 44–65.

Relevant Websites

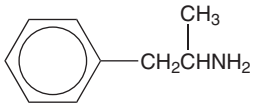
http://www.oehha.org – California Office of Environmental Health Hazard Assessment.
http://cfpub.epa.gov – US Environmental Protection Agency, National Center for Environmental Assessment.

Amobarbital *See* Barbiturates, Short-Acting.

Amphetamine

Michael Wahl

- Chemical Abstracts Service Registry Number: CAS 300-62-9
- Synonyms: 1-Phenyl-2-aminopropane; Phenylisopropylamine. In drug abuse, the word 'amphetamine' can also refer to a number of related compounds such as methamphetamine and other analogs with similar activity (e.g., meth, speed, wire, cross-tops, ice, dexies, black beauties, and hearts).
- Chemical/Pharmaceutical/Other Class: Central nervous system stimulant
- Chemical Formula: $C_9H_{13}N$
- Chemical Structure:



Uses

Amphetamine is used in the treatment of attention-deficit hyperactivity disorder and narcolepsy. It is also a drug of abuse.

Exposure Routes and Pathways

Oral and intravenous uses are probably the most common routes of exposure. Amphetamines can also be used via nasal insufflation and smoking. Peak concentrations after oral ingestion range from 1 to 4 h, depending on the specific amphetamine. Some pharmaceutical preparations are sustained or delayed release products, with lower absorption rates. More than 50% of a dose undergoes hepatic metabolism, and ~30% is excreted unchanged in urine. The amount of unmetabolized drug recovered in urine is greater with acidic urine pH. The apparent volume of distribution is 3–5 l kg^{-1} and also varies by specific drug. The half-life ranges from 8 to 15 h. Analytical methods used should distinguish the specific compound present since other compounds are structurally similar and may cross-react with antiamphetamine antibodies.

Toxicokinetics

Amphetamines are generally well absorbed from the gastrointestinal tract in therapeutic doses. Several commercially available amphetamines are formulated as sustained or delayed release products. Peak serum levels are expected within 30 min after intravenous injection and within 2–3 h after ingestion of immediate release products. In overdose and with exposure to sustained release products, delays in absorption are expected.

Amphetamines are widely distributed (generally several liters per kilogram) with low protein binding. These agents are generally extensively metabolized. Many metabolites have amphetamine activity. Elimination can vary greatly. Some amphetamines are primarily renally eliminated with the rate of elimination dependent upon the urine pH (e.g., amphetamine). Others have less than 1% of the parent compound renally excreted (e.g., methylphenidate). Half-lives vary as well with IV methylphenidate at 1–2 h and chlorphentermine at ~5 days.

Mechanism of Toxicity

The effects of amphetamines are due to the increase of neurotransmitters norepinephrine, serotonin, and dopamine in central synapses. This increase is from increased release and reuptake blockade of catecholamines. Amphetamines may also inhibit monoamine oxidase. These mechanisms combine to produce the sympathomimetic and central nervous system (CNS) effects seen with amphetamine abuse.

Acute and Short-Term Toxicity (or Exposure)

Animal

Effects in animals mimic those seen in humans. Expected signs and symptoms include hypertension, tachycardia, seizures, and hyperthermia.

Human

Toxicity primarily involves the CNS and cardiovascular system. CNS effects include increased alertness, restlessness, decreased appetite, irritability, stereotyped repetitive behavior, and insomnia with low doses. With larger exposures confusion, panic reactions, aggressive behavior, hallucinations, seizures, delirium, coma, and death can occur. Intracranial bleeding can result from untreated hypertension. Trauma is common secondary to the changes in behavior and decreased judgment. Frequent use results in fatigue, paranoia, and depression. Cardiovascular effects include increased heart rate and blood pressure, chest pain, myocardial ischemia or

infarction, dysrhythmias, cardiovascular collapse, and death. Other effects include increased temperature, rhabdomyolysis, increased respiratory rate, flushing, sweating, and dilated pupils.

Chronic Toxicity (or Exposure)

Animal

Animal models describe changes in behavior with toxicity and withdrawal. Chronic dosing of animals leads to stereotypic, compulsive behaviors of searching and examining in higher animals, sniffing and biting movements in lower animals. There has been no increased carcinogenic activity in rats and mice fed varying doses of amphetamine over studies as long as 2 years.

Human

Chronic use can result in psychosis and cardiomyopathy.

In Vitro Toxicity Data

Several amphetamines have been shown to have monaminergic neurotoxic properties. Recent studies of PC12 dopaminergic cells have shown increased activity of capsase-3 and mitochondrial cytochrome *c* release. These findings suggest that amphetamines (particularly substituted amphetamines) may induce apoptosis, possibly via a mitochondrial pathway.

Clinical Management

After assessment of airway, breathing, and circulation with necessary supportive care, decontamination of the gastrointestinal tract should be undertaken for substantial recent ingestions. Determination of specific toxic doses is difficult in chronic users of amphetamines due to the development of tolerance. Oxygen and benzodiazepines should be administered as needed for agitation, shortness of breath, or chest pain. Increased blood pressure can be managed with benzodiazepines or vasodilators. Benzodiazepines may be necessary for agitated or combative patients. Benzodiazepines, cooling, and rehydration are standard treatments for patients with increased temperature and rhabdomyolysis.

See also: Benzodiazepines; Catecholamines; Methylenedioxymethamphetamine.

Further Reading

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Kraemer T and Maurer HH (2002) Toxicokinetics of amphetamines: Metabolism and toxicokinetic data of designer drugs, amphetamine, methamphetamine and their *N*-alkyl derivatives. *Therapeutic Drug Monitoring* 24(2): 277–289.

McCann UD and Ricaurte GA (2004) Amphetamine neurotoxicity: Accomplishments and remaining challenges. *Neuroscience and Biobehavioral Review* 27(8): 821–826.

Amphibians

Prathibha S Rao

Background Information

The class of amphibia contains ~2600 species and is divided into anura, frogs.

Mechanism of Toxicity

The chemical compositions of amphibian toxins are highly diversified. Amphibians secrete substances to prevent desiccation, control the growth of microorganisms on skin, and discourage predators. These secretions have cytotoxic and/or hemolyzing effects.

Toads

The toxins from Bufo species of toads are venom complexes that have a distinct cardioactive digitalis-like action. Toxic signs include profuse salivation with pulmonary edema, cardiac arrhythmia, hypertension, and prostration. Convulsions and death due to cardiac arrest may occur as early as 15 min after exposure to the toxin. Susceptible populations include children and pet dogs or cats playing with toads.

Salamanders

Tetrodotoxin and additional toxic components are found associated with this group. Toxic effects are noted at 10 mg kg^{-1} body weight. Toxic signs include tingling of the oral cavity with salivation, muscle weakness, motor incoordination, skin numbness,

vomiting, diarrhea, and generalized paralysis with convulsions and death in severe cases.

Clinical Management

The victim's mouth should be washed out with copious amounts of water, atropine should be administered to control salivation. Barbiturates are used to control convulsions; calcium gluconate may be used to control some physiologic effects. Phenoxybenzamine and propranolol have been used experimentally to block α- and β-adrenergic receptors. Life-support therapy may be used to maintain respiration and other vital functions.

See also: Tetrodotoxin.

Further Reading

Daly JW (2004) Marine toxins and nonmarine toxins: Convergence or symbiotic organisms? *Journal of Natural Products* 67: 1211–1215.

Karalliedde L (1995) Animal toxins. *British Journal of Anaesthesia* 74: 319–327.

Relevant Websites

http://tolweb.org – The Tree of Life Web Project
http://www.livingunderworld.org.

Amaranth *See* Red Dye No. 2.

Amyl Nitrite

Michael Wahl

This article is a revision of the previous print edition article by Prathibha S Rao and Harihara M Mehendale, volume 1, pp. 65–66, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 110-46-3
- Chemical/Pharmaceutical/Other Class: Vasodilator
- Chemical Formula: $C_5H_{11}NO_2$
- Chemical Structure:

$$CH_3–CH–(CH)_3–CH_2–CH_2–O–NO$$

Uses

Amyl nitrite is a vasodilator that acts by relaxing vascular smooth muscle. It is also a component of the Taylor Cyanide Antidote Kit.

Exposure Routes and Pathways

Inhalation is the most common route of exposure.

Toxicokinetics

Amyl nitrite is a volatile liquid and is rapidly absorbed from the lungs. It is rapidly hydrolyzed to nitrite ion and the corresponding alcohol. About 60% of the ion is metabolized by the body. About 40% of the nitrites are excreted unchanged in the urine.

Mechanism of Toxicity

Nitrites bind to hemoglobin causing oxidation of hemoglobin to methemoglobin, which is unable to transport oxygen. When methemoglobinemia exceeds 10–15%, cyanosis may become apparent.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure of amyl nitrite in some animals may result in increases in intraocular pressure.

Human

Amyl nitrite causes methemoglobinemia, unconsciousness, dizziness, fatigue, shortness of breath, nausea, and vomiting. The skin is initially warm and sweaty and later becomes cold due to vasodilation. It causes coronary blood vessel contraction, bradycardia, atrial fibrillation, cardiac ischemia, headache, convulsions, and diarrhea.

Nitrite transferred through breast milk can cause methemoglobinemia in infants. Infants are at greater risk for the development of nitrite-related toxicity than adults due to their decreased ability to secrete gastric acid, higher levels of fatal hemoglobin, and diminished enzymatic capability to reduce methemoglobin to hemoglobin. Nitrites can combine with secondary amines to form dimethylnitrosamines, which are acutely toxic to the liver and cause

centrilobular necrosis, fibrous occlusion of central veins, and pleural and peritoneal hemorrhages in animals.

Chronic Toxicity (or Exposure)

Human

Chronic ingestion of $5\,mg\,kg^{-1}\,day^{-1}$ is considered unacceptable. Common findings associated with nitrite poisoning include unconsciousness, dizziness, fatigue, shortness of breath, nausea, vomiting, coma, cyanosis, dyspnea, and pallor.

Clinical Management

For substantial recent ingestions, activated charcoal may be considered in the emergency department. Methylene blue may be used to treat patients that develop significant methemoglobinemia. Benzodiazepines may be used to control seizures. Recurrent seizures may require use of phenobarbital. Dopamine or norepinephrine can be administered to control hypotension. Aggressive supportive therapy should be instituted for all symptomatic patients.

See also: Benzodiazepines; Cyanide; Liver.

Further Reading

Edwards RJ and Ujma J (1995) Extreme methemoglobinemia secondary to recreational use of amyl nitrite. *Journal of Accident and Emergency Medicine* 12: 138–142.

Haverkos HW and Dougherty J (1988) Health hazards of nitrite inhalants. *American Journal of Medicine* 84: 479–482.

Anabolic Steroids

Sharmilee P Sawant and Harihara M Mehendale

Introduction

The synthetic substances related to the male sex hormones (androgens) are called 'anabolic steroids'. The principal effects of these substances are to promote the growth of skeletal muscle (anabolic effects) and the development of male sexual characteristics (androgenic effects).

Developed in the late 1930s, anabolic steroids were primarily used to treat hypogonadism, a condition in which the testes do not produce sufficient testosterone for normal growth, development, and sexual functioning. These compounds are used medically to treat delayed puberty, some types of impotence, and wasting of the body caused by HIV infection or other diseases. Some of the commonly used/abused anabolic steroids are: Anadrol (oxymetholone), Oxandrin (oxandrolone), Dianabol (methandrostenolone), Deco-Durabolin (nandrolone decanoate), and Depa-Testosterone (testosterone cypionate).

During the 1930s, experiments in laboratory animals revealed that anabolic steroids facilitate the growth of skeletal muscles. This discovery resulted in anabolic steroids being used by athletes, particularly bodybuilders and weightlifters. Steroid abuse has become so widespread in athletics that it is almost assumed that leading contestants use either classic anabolic steroids or new designer drugs. The outcome of sports contests is therefore most often affected by the use/abuse of anabolic steroids.

Since their discovery, more than 100 different anabolic steroids have been developed. A prescription is required for the legal use of these compounds in the United States, though supplements such as dehydroepiandrosterone and androstenedione (street name 'Andro') can be purchased legally without a prescription through many commercial sources including health food stores. They are often referred to as dietary supplements, although they are not food products. They are often taken because the user believes they have anabolic effects. In 2003, the Food and Drug Administration (FDA) became aware of a substance called tetrahydrogestrinone (THG), which is illegally used by athletes to improve their performance. Based on its analysis of this product, FDA has determined that THG is an unapproved new drug. As such, it cannot be legally marketed without FDA approval. A number of so-called 'designer drugs' are emerging and are used for performance enhancement, with THG being one example. Even as regulating agencies such as FDA and sports authorities develop ways of detecting and monitoring drug use/abuse, new designer drugs appear before ways of detecting them and monitoring can be discovered.

It is believed that steroidal supplements get converted into testosterone (male sex hormone) or a similar compound in the body. Whether such conversion produces sufficient quantities of testosterone to promote muscle growth or whether the supplements

themselves promote muscle growth is unknown. Little is known about the side effects of steroidal supplements, but if large quantities of these compounds substantially increase testosterone levels in the body, they also are likely to produce the same side effects as anabolic steroids.

Anabolic Steroids Abuse

A driving motivation to abusing steroids is to improve their performance in sports. Steroid abuse is very high among competitive bodybuilders. The incidence of abuse probably varies among other athletes, depending on the specific sport. People suffering from behavioral syndrome (muscle dysmorphia), in which they have a distorted image of his or her body, use steroids to increase muscle size and/or reduce body fat. Men with this condition perceive themselves as small and weak, even if they are large and muscular. Similarly, women with the syndrome perceive themselves as fat and flabby, even though they are actually lean and muscular.

Some people who abuse steroids to boost muscle size have experienced physical or sexual abuse. They attempt to increase their muscle size to protect themselves. In one series of interviews with male weightlifters, 25% of steroid abusers reported memories of childhood physical or sexual abuse, as compared with none of the nonabusers. In a study of women weightlifters, twice as many of those who had been raped reported using anabolic steroids and/or another purported muscle-building drugs and markedly increased bodybuilding activities after the attack, compared to those who had not been raped. They believed that being bigger and stronger would discourage further attacks because men would find them either intimidating or unattractive.

Finally, some adolescents abuse steroids as part of a pattern of high-risk behaviors. These adolescents also take risks such as drinking and driving, carrying a gun, not wearing a helmet on a motorcycle, and abusing other illicit drugs.

While conditions such as muscle dysmorphia, a history of physical or sexual abuse, or engaging in high-risk behaviors may increase steroid abuse, researchers concur that most steroid abusers are psychologically normal when they start abusing the drugs.

Human Toxicity

Anabolic steroid abuse has been associated with a wide range of adverse side effects. Health consequences associated with anabolic steroid abuse include:

- *Disruption of hormonal system:* In boys and men, reduced sperm production, shrinking of the testicles, impotence, and irreversible breast enlargement are found to occur. In girls and women, decreased body fat and breast size, deepening of the voice, growth of excessive body hair, loss of scalp hair, changes in or cessation of the menstrual cycle, and clitoral enlargement are commonly found.
- *Musculoskeletal system effects:* Premature and permanent retardation of growth among adolescents of both sexes.
- *Cardiovascular effects:* Heart attacks and strokes.
- *Liver diseases:* Potentially fatal cysts and liver cancer.
- *Skin disorders:* Acne and cysts.
- *Infections:* In steroid abusers who use parentral routes for drug administrations, HIV/AIDS, hepatitis B and C, and infective endocarditis, a potentially fatal inflammation of the inner lining of the heart.
- *Behavioral effects:* Aggressive behavior, particularly when high doses are taken. Depression, mood swings, fatigue, restlessness, and loss of appetite when steroid abuse is stopped.

Addiction

A high percentage of steroid abusers become addicted to the drugs, as evidenced by their continuous use of steroids in spite of physical problems, negative effects on social relations, or nervousness and irritability. Also, they spend lot of time and money obtaining the drugs and experience withdrawal symptoms such as mood swings, fatigue, restlessness, loss of appetite, insomnia, reduced sex drive, and the desire to take more steroids. The most dangerous of the withdrawal symptoms is depression, because it sometimes leads to suicide attempts. If left untreated, some depressive symptoms associated with anabolic steroid withdrawal have been known to persist for a year or more after the abuser stops taking the drugs.

Early measures to prevent steroid abuse depend on the strategy of drug testing and educating students about the adverse effects of anabolic steroids. A few school districts test for abuse of illicit drugs, including steroids, and studies are currently under way to determine whether such testing reduces drug abuse.

Research on steroid educational programs has shown that simply teaching students about steroid's adverse effects does not convince them that they personally can be adversely affected. Nor does such instruction discourage young people from taking

steroids in the future. Presenting both the risks and benefits of anabolic steroid use is more effective in convincing adolescents about steroid's negative effects, apparently because the students find a balanced approach more credible and less biased, according to the researchers. However, the balanced approach still does not deter adolescents from abusing steroids.

A more sophisticated approach has shown promise for preventing steroid abuse among players on high school sports teams. In the Athletes Training and Learning to Avoid Steroids (ATLAS) program, developed for male football players, coaches and team leaders discuss the potential effects of anabolic steroids and other illicit drugs on immediate sports performance, and they teach how to refuse offers of drugs. They also discuss how strength training and proper nutrition can help adolescents build their bodies without the use of steroids. Later, special trainers teach the players proper weightlifting techniques. An ongoing series of studies has shown that this multicomponent, team-centered approach reduces new steroid abuse by 50%. A program designed for adolescent girls on sports teams, patterned after the program designed for boys, is currently being tested.

Clinical Management

Current knowledge is based largely on the experiences of a small number of physicians who have worked with patients undergoing steroid withdrawal. The physicians have found that supportive therapy is sufficient in some cases. Patients are educated about what they may experience during withdrawal and are evaluated for suicidal thoughts.

If symptoms are severe or prolonged, medications or hospitalization may be needed.

Some medications that have been used for treating steroid withdrawal restore the hormonal system after its disruption by steroid abuse. Other medications target specific withdrawal symptoms; for example, antidepressants to treat depression, and analgesics for head-aches and muscle and joint pains.

Some patients require assistance beyond simple treatment of withdrawal symptoms and are treated with behavioral therapies.

See also: Androgens; Drugs of Abuse.

Further Reading

Brower KJ (1997) Withdrawal from anabolic steroids. *Current Therapy in Endocrinology and Metabolism* 6: 338–343.

Fact sheets on anabolic steroids can be ordered free, by calling NIDA (National Institute of Drug Abuse) Infofax at 1-888-NIH-NIDA (1-888-644-6432).

Goldberg L, MacKinnon DP, Elliot D, *et al.* (2000) The adolescents training and learning to avoid steroids program: Preventing drug use and promoting health behaviors. *Archives of Pediatric and Adolescent Medicine* 154: 332–338.

Porcerelli JH and Sandler BA (1998) Anabolic–androgenic steroid abuse and psychopathology. *Psychiatric Clinics of North America* 21: 829–833.

Shahidi NT (2001) A review of the chemistry, biological action, and clinical applications of anabolic–androgenic steroids. *Clinical Therapeutics* 23(9): 1355–1390.

Relevant Website

http://www.steroidabuse.org – NIDA Steroid Abuse Website.

Analytical Toxicology

Shayne C Gad

Analytical toxicology is the use of qualitative and quantitative chemical and physical techniques used in sample preparation, separation, assay calibration, detection and identification, and quantification for the purposes of toxicological research and testing. Examples of the objectives of such analysis include:

- Determining the levels of exposure to potential toxicants via air, water, or food.
- Verifying exposure levels to doses for animals in experimental studies.
- Determining levels of xenobiotics and their metabolites in animal studies.
- Screening blood and urine for the presence of illicit drugs or their metabolites.
- Measuring levels of endogenous compounds and molecules to evaluate organ function and damage (clinical chemistry).
- Identifying metabolites and macromolecular adjuncts to identify mechanisms of action.

The diagnosis and treatment of health problems induced by chemical substances and the closely allied

field of therapeutic drug monitoring rely on analytic toxicology, and advances in the field have added both power and problems to toxicology, dual gifts of increases in sensitivity and specificity. Although the analytes are present in matrices similar to those seen in forensic toxicology, the results must be reported rapidly to be of use to clinicians in treating patients. This requirement of a rapid turnaround time limits the number of chemicals that can be measured because methods, equipment, and personnel must all be available for an instant response to toxicological emergencies. Investigations for an 'unknown' drug or poison are usually carried out on specimens of urine (30 ml for qualitative tests) and blood (10 ml for quantitative tests). No preservatives should be added to urine specimens and blood samples should be heparinized.

Occupational and regulatory toxicology requires analytic procedures for implementation or monitoring. In occupational toxicology, the analytical methods used to monitor threshold limit values and other means of estimating the exposure of workers to toxic hazards may utilize simple, nonspecific, but economical screening devices. However, to determine the actual exposure of a worker, it is necessary to analyze blood, urine, breath, or another specimen by employing methods similar to those used in clinical or forensic toxicology. For regulatory purposes, a variety of matrices (e.g., food, water, and air) must be examined for extremely small quantities of analytes. Frequently, this requires the use of sophisticated methodology with extreme sensitivity. Both of these applications of analytical toxicology impinge on forensic toxicology because an injury or occupational disease in a worker can result in a legal proceeding.

Other applications of analytical toxicology occur frequently during the course of experimental studies. Confirmation of the concentration of dosing solutions and monitoring of their stability often can be accomplished with the use of simple analytical techniques. The bioavailability of a dose may vary with the route of administration and the vehicle used. Blood concentrations can be monitored as a means of establishing this important parameter. In addition, an important feature in the study of any toxic substance is the characterization of its metabolites as well as the distribution of the parent drug, together with its metabolites, to various tissues. This requires sensitive, specific, and valid analytical procedures. Similar analytic studies can be conducted within a temporal framework to gain an understanding of the dynamics of the absorption, distribution, metabolism, and excretion of toxic chemicals.

Analysis of Common Toxic Substances

Analytical toxicology is intimately involved in many aspects of experimental and applied toxicology. Since toxic substances include all chemical types and because their measurement may require the examination of biological or nonbiological matrices, the scope of analytical toxicology is broad. Nevertheless, a systematic approach and a reliance on the practical experience of generations of forensic toxicologists can be used in conjunction with the sophisticated tools of analytical chemistry to provide the data needed to understand the hazards of toxic substances more completely. "All substances are poisons: There is none which is not a poison." Analytical toxicology potentially encompasses all chemical substances. Forensic toxicologists learned long ago that when the nature of a suspected poison is unknown, a systematic, standardized approach must be used to identify the presence of most common toxic substances. An approach that has stood the test of time was first suggested by Chapuis in 1873 in *Elements de Toxicologie*. It is based on the origin or nature of the toxic agent. Such a categorization can be characterized as follows:

1. gases,
2. volatile substances,
3. corrosives,
4. metals,
5. anions and nonmetals,
6. nonvolatile organic substances, and
7. miscellaneous.

In addition to considering the descriptive classification of the substance, one must determine the method for separating a toxic agent from the matrix in which it is embedded. The matrix is generally a biological specimen such as a body fluid or a solid tissue. The agent of interest may exist in the matrix in a simple solution or may be bound to protein and other cellular constituents. The challenge here is to separate the toxic agent in sufficient purity and quantity to permit it to be characterized and quantified. At times, the parent compound is no longer present in large enough amounts to be separated. In such cases, known metabolites may indirectly provide measure of the parent substance. With other substances, interaction of the poison with tissue components may require the isolation or characterization of a protein adduct. Methods for separation have long provided a great challenge to analytical toxicologists. Only recently have methods become available which permit direct measurement of some analytes without prior separation from the matrix.

The following sections provide a closer look at analytical toxicological issues related to substance class.

Gases

Gases are most simply measured by means of gas chromatography. Some gases are extremely labile, and the specimen must be collected and preserved at temperatures as low as that of liquid nitrogen. Generally, the gas is carefully liberated by incubating the specimen at a predetermined temperature in a closed container. The gas, freed from the matrix, collects over the specimen's 'headspace', where it can be sampled and injected into the gas chromatograph. Other gases, such as carbon monoxide, interact with protein, or the adduct can be measured independently, as in the case of carboxyhemoglobin.

Volatile Substances

Volatile substances are generally liquids of a variety of chemical types. Gas–liquid chromatography is the simplest approach for simultaneous separation and quantitation in many cases. The simple alcohols can be measured by injecting a diluted body fluid directly onto the column of the chromatograph. A more common approach is to use the headspace technique, as is done for gases, after incubating the specimen at an elevated temperature.

Corrosives

Corrosives include mineral acids and bases. Many corrosives consist of ions that are normal tissue constituents. Clinical chemical techniques can be applied to detect these ions when they are in great excess over normal concentrations. Because these ions are normal constituents, the corrosive effects at the site of contact of the chemical, together with other changes in blood chemistry values, can confirm the ingestion of a corrosive substance.

Metals

Metals are encountered frequently as occupational and environmental hazards. Elegant analytic methods are available for most metals even when they are present at extremely low concentrations. Classical separation procedures involve destruction of the organic matrix by chemical or thermal oxidation. This leaves the metal to be identified and quantified in the inorganic residue. Unfortunately, this prevents a determination of the metal in the oxidation state or in combination with other elements, as it existed when the metal compound was absorbed. For example, the toxic effects of metallic mercury, mercurous ion, mercuric ion, and dimethyl mercury are all different. Analytical methods must be selected which determine the relative amount of each form present to yield optimal analytical results.

Toxic Anions and Nonmetals

Toxic anions and nonmetals are a difficult group for analysis. Some anions can be trapped in combination with a stable cation, after which the organic matrix can be destroyed, as with metals. Others can be separated from the bulk of the matrix by dialysis, after they are detected by colorimetric or chromatopathic procedures. Still others are detected and measured by ion-specific electrodes. There are no standard approaches for this group, and other than phosphorus, they are rarely encountered in an uncombined form.

Nonvolatile Organic Substances

Nonvolatile organic substances constitute the largest group of substances which must be considered by analytical toxicologists. This group includes drugs, both prescribed and illegal, pesticides, natural products, pollutants, and industrial compounds. These substances are solids of liquids with high boiling points. Thus, separation procedures generally rely on differential extractions, either liquid–liquid or solid–solid in nature. These extractions often are not efficient, and recovery of the toxic substance from the sample matrix may be poor. When the nature of the toxic substance is known, immunoassay procedures are useful because they allow a toxicologist to avoid using separation procedures. Such compounds can be classified as organic strong acids, organic weak acids, organic bases, organic neutral compounds, or organic amphoteric compounds.

Miscellaneous

Finally, a miscellaneous category must be included to cover the large number of toxic agents that cannot be detected by the routine application of the methods described previously. Venoms and other toxic mixtures of proteins or uncharacterized constituents fall into this class. Frequently, if antibodies can be grown against the active constituent, immunoassay may be the most practical means of detecting and measuring these highly potent and difficult to isolate substances. Unfortunately, unless highly specific monoclonal antibodies are used, the analytic procedure may not be acceptable for forensic purposes. Frequently, specific analytic procedures must be developed for each

analyte of this type. At times, biological endpoints are utilized to semiquantify the concentration of the isolated product.

Analytical Techniques

Due to increased levels of sensitivity of analytical techniques and a range of legal requirements (including Good Laboratory Practices and issues in potential litigation), particular care must be taken in collecting and handling samples to both avoid contamination and maintain a chain of custody of samples and sample records. There are a vast variety of techniques now employed in analysis, as outlined below.

- Chromatography
  - Thin layer, gas, high-performance liquid (HPLC)
- Mass spectrophotomology
- Photometry/spectroscopy
- Spectrophotomology (ultraviolet, infrared, and visible light)
  - Flame photometry, atomic absorption, nuclear magnetic resonance (NMR) spectroscopy, electron spin resonance (ESR) spectrophotometry, Raman spectroscopy
- Immunoassays
  - Radioimmunoassay (RIA), enzyme immunoassay (EIZ), fluorescent immunoassay (FIA)
- Isotopic labeling
  - Positron emission tomography (PET)
- Magnetic resonance imaging (MRI)

Newer and more complex material analysis techniques are:

- Atomic absorption spectroscopy
- Auger electron spectroscopy
- Controlled potential coulometry
- Crystallographic texture measurement
- Electrogravimetry
- Electrometric titration
- Electron probe X-ray microanalysis
- Elemental and functional group analysis
- Extended X-ray absorption fine structure
- Ferromagnetic resonance
- Field ion microscopy
- High temperature combustion
- Image analysis
- Inert gas fusion
- Inductively coupled plasma
- Ion chromatography
- Low energy electron diffraction
- Low energy ion scattering spectroscopy
- Mass spectrometry
- Molecular fluorescence spectrometry
- Mossbauer spectroscopy
- Neutron activation analysis
- Neutron diffraction
- Optical emission spectroscopy
- Optical metallography membrane electrodes
- Particle induced X-ray emission potentiometric
- Radial distribution function analysis
- Radio analysis
- Rutherford backscattering spectroscopy
- Scanning electron microscopy
- Secondary ion mass spectroscopy
- Single crystal X-ray diffraction
- Small angle X-ray and neutron scattering
- Spark source mass spectrometry
- Transmission electron microscopy
- Voltametry
- Wet analytical chemistry
- X-ray diffraction residual stress techniques
- X-ray photoelectron spectroscopy
- X-ray powder diffraction
- X-ray spectrometry
- X-ray topography

See also: International Society of Exposure Analysis; Microarray Analysis.

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Ancient Warfare and Toxicology

Adrienne Mayor

In antiquity, natural toxins were exploited to make poison weapons to wage the earliest forms of biological and chemical warfare. A wide range of substances, from toxic plants and venomous insects and reptiles to infectious agents and noxious chemicals, were weaponized in ancient Europe, the Mediterranean, North Africa, the Middle East, Central Asia, India, China, and in the Americas. Evidence for the concept and practice of toxic warfare can be traced back thousands of years. For example, cuneiform tablets from about 1200 BC record that the Hittites of Asia Minor deliberately drove plague victims into enemy territory.

Such practices did not require a scientific understanding of toxicology, epidemiology, and chemistry, or depend on advanced technology, but were based on centuries of observation and experimentation with easily available toxic materials. Strategies based on insidiously attacking an opponent's biological vulnerabilities with poison agents could give an advantage when facing troops superior in numbers, courage, skill, or technology. Yet the use of toxic weapons also entailed practical and ethical dilemmas in antiquity.

The first poison projectiles were probably first devised for hunting and then turned toward war. The bow and arrow was a highly effective delivery system for toxins at an early date, since a mere scratch from a treated point could be fatal.

Toxic Weapons in Mythology

The concept of poisoned projectiles is embedded in the ancient Greek language, since the word for 'poison', *toxicon*, derived from *toxon*, the word for 'arrow'. Greek mythology offers further evidence of the antiquity of the concept. The great hero of myth, Hercules, for example, invented biological weaponry when he dipped his arrows in the venom of the Hydra monster, a many-headed serpent. Homer's *Iliad*, an oral epic first written down in the eighth century BC, contains indirect allusions to the use of toxic projectiles in the legendary Trojan War. Homer's descriptions of black (rather than red) blood oozing from wounds, battlefield doctors sucking out poisons, and never-healing wounds, are all hallmarks of snake venom poisoning. In his poem the *Odyssey*, Homer clearly describes the Greek hero Odysseus smearing lethal plant juices on arrows intended for enemies. According to myth, Odysseus himself died from a wound inflicted by a spear tipped with the toxic spine of a marbled stingray, a common species in the Mediterranean. Notably, spears tipped with stingray spines are used by natives in South America.

The epic poem recounting the legendary history of Rome, the *Aeneid* by Virgil, also refers to poisoned spears wielded by the early Romans, and poisoned weapons appear in the mythological epic of India, the *Rigveda*. Myth and legend likely reflect the original invention of biological arms in various cultures and they also offered models for the actual practice of biowar.

Plant Poisons in Warfare

About two-dozen toxic Eurasian plant species, often employed as medicines in minute dosages, were gathered to make arrow poisons or other biological weapons in the ancient world. One of the most popular plant drugs was hellebore, identified by the ancients as black hellebore (probably the Christmas rose of the buttercup Ranunculaceae family, *Helleborus niger*) and white hellebore (the lily family, *Liliaceae*). The unrelated plants are each laden with powerful chemicals that cause severe vomiting and diarrhea, muscle cramps, delirium, convulsions, asphyxia, and heart attack. Hellebore was one of the arrow drugs used by the Gauls, among other ancient groups, and it was also used to poison wells.

Another favorite biowar toxin was aconite or monkshood (also called wolfsbane). *Aconitum* (buttercup family) contains the alkaloid aconitine, a violent poison, which in high doses causes vomiting and paralyzes the nervous system, resulting in death. Aconite was employed by the archers of ancient Greece and India, and its use in warfare continued into modernity. For example, during the war between the Spanish and the Moors in 1483, Arab archers wrapped aconite-soaked cotton around their arrowheads. Nepalese Gurkhas poisoned wells with aconite in the nineteenth century, and during World War II, Nazi scientists created aconitine-treated bullets.

Henbane (*Hyoscyamine niger*), a sticky, bad-smelling weed containing the powerful narcotics hyoscyamine and scopolamine, was also collected as arrow poison in antiquity. Henbane causes violent seizures, psychosis, and death. Other plant juices used on projectiles included hemlock (*Conium maculatum*), yew (*Taxus*), rhododendron, and several species of deadly nightshade or belladonna, which causes vertigo, extreme agitation, coma, and death. The

fact that the Latin word for deadly nightshade was *dorycnion*, 'spear drug', suggests that it was smeared on weapons at a very early date, as noted by Pliny the Elder, a natural historian of the first century AD.

Snake Venom Arrow Poisons

Snake venom was another well-known arrow poison. Since snake venom is digestible, it could be safely used for hunting because the venom did not make game harmful to eat, but venom in the bloodstream of an enemy brought a painful death or a never-healing wound. Numerous poison snakes exist around the Mediterranean and in Africa and Asia. According to Greek and Roman writers, archers who steeped their arrows in serpents' venom included the Gauls, the Dacians and Dalmatians (of the Balkans), the Sarmatians of Persia (now Iran), the Getae of Thrace, Slavs, Armenians, Parthians between the Indus and Euphrates, Indians, North Africans, and the Scythian nomads of the Central Asian steppes. According to the ancient Greek geographer Strabo, the arrow poison concocted by the Soanes of the Caucasus was so noxious that its mere odor was injurious. Strabo also reported that people of what is now Kenya dipped their arrows "in the gall of serpents," while the Roman historian Silius Italicus described the snake venom arrows used by the archers of Libya, Morocco, Egypt, and Sudan. Ancient Chinese sources show that arrow poisons were also in use in China at early dates. In the Americas, Native Americans used snake, frog, and plant poisons on projectiles for hunting and warfare.

Complex recipes for envenomed arrows are recorded in Greek and Latin texts. One of the most dreaded arrow drugs was concocted by the Scythians, who combined snake venom and bacteriological agents from rotting dung, human blood, and putrefying viper carcasses bloated with feces. Even in the case of a superficial arrow wound, the toxins would begin taking effect within an hour. Envenomation accompanied by shock, necrosis, and suppuration of the wound would be followed by gangrene and tetanus and an agonizing death.

Several snake species contributed the venom used by the Scythians, including the steppe viper *Vipera ursinii renardi*, the Caucasus viper *Vipera kasnakovi*, the European adder *Vipera berus*, and the long-nosed or sand viper *Vipera ammodytes transcaucasiana*. In ancient India, one of the most feared poisons was derived from the rotting flesh and venom of the white-headed Purple Snake, described by the natural historian Aelian (third century AD). His detailed description suggests that the Purple Snake was the rare, white-headed viper discovered by modern herpetologists in the late 1880s, *Azemiops feae*.

A different snake venom tipped the arrows encountered by the army of Alexander the Great in his conquest of India in 327–325 BC. According to the historians Quintus Curtius, Diodorus of Sicily, and others, the Harmatelians (of what is now Mansura, Pakistan) had smeared their arrows and swords with an unknown snake poison. Most modern historians assume the Harmatelians used cobra poison, but the ancient historians' detailed description of the gruesome deaths suffered by Alexander's men points to the deadly Russell's viper. Even the slightly wounded went immediately numb and experienced stabbing pain and wracking convulsions. Their skin became cold and livid and they vomited bile. Black froth exuded from the wounds and then purple-green gangrene spread rapidly, followed by death. Death from cobra venom is relatively painless, from respiratory paralysis, but the Russell's viper causes numbness, vomiting, severe pain, black blood, gangrene, and death – as described by Alexander's historians.

Poisoning Water and Food

Tainting water and food was another ancient biological tactic. A legendary Greek account set in about 1000 BC tells how King Cnopus conquered Erythrae (in what is now Turkey) by drugging a bull and tricking the enemy into eating the poisoned meat. The earliest historically documented case of poisoning drinking water occurred in Greece in about 590 BC, during the First Sacred War. Athens and allied city-states made war on the strongly fortified city of Kirrha, which controlled the road to Delphi, the site of the famous Oracle of Apollo. According to several ancient Greek historians, Kirrha had offended the god and was therefore to be totally destroyed. During the siege, the league of allies gathered a great quantity of hellebore and placed it in the water pipes supplying Kirrha. The soldiers guarding Kirrha's walls – and the entire population – fell violently ill and the allies easily overran the city and wiped out combatants and civilians alike. After the war, Athens and her allies had second thoughts and agreed among themselves not to interfere with water supplies should they ever find themselves at war with each other.

Roman commanders also poisoned wells. Manius Aquillius, for example, ended a long-drawn-out war to quell insurrections in the Roman province of Asia Minor in 129 BC, by pouring poison into the springs supplying the rebelling cities. According to the Roman historian Florus, however, his victory was

dishonorable because of the resort to underhanded biological tactics.

Carthaginian generals, such as Himilco and Maharbal, overcame enemies in North Africa by tainting wine with mandrake, a heavily narcotic root of the deadly nightshade. In Europe, the Celts were known to drug their foes' food and wine with plant poisons. In North America, Native Americans poisoned enemy drinking water with rotting animal skins. In ancient India, numerous recipes for poisoning enemy food and water are given in the *Arthashastra*, a warfare manual dating to the fourth century BC, written by Kautilya, the advisor of King Chandragupta.

In 65 BC, naturally occurring toxic honey was used against the army of the Roman general Pompey during the war against King Mithridates VI of Pontus. In the Black Sea region, Mithridates' allies of set out tempting honeycombs along the Romans' route and hid. The honey was made by wild bees that gathered nectar from rhododendron blossoms, which contain devastating neurotoxins. As the legionnaires succumbed to the sweet treat, collapsing with vertigo, vomiting, and diarrhea, the enemy arrived to slaughter 1000 of Pompey's men.

Stinging Insects and Biting Snakes

Stinging insects such as wasps, deadly vipers, and scorpions could also be drafted for war. Perhaps as early as Neolithic times, hives filled with furious bees were thrown at enemies, who were driven into chaos by the painful stings; later, catapults were used to hurl beehives. The ancient Maya of Central Mexico created ingenious boobytraps to repel besiegers on their fortress walls, consisting of dummy warriors whose gourd heads were filled with hornets.

In the second century BC, the Carthaginian general Hannibal devised a plan of filling clay pots with live vipers during a naval battle in which he was outnumbered by ships commanded by Pergamum, a city on the coast of Turkey. The enemy sailors were routed when the catapulted pots smashed on their ships' decks, releasing masses of snakes.

At the fortified city of Hatra (Iraq), in AD 198–199, besieging Roman legions led by the emperor Septimius Severus were forced to retreat after the Hatreni defended their walls with insect bombs. The people of Hatra had packed terracotta pots with scorpions (arthropods), assassin bugs, and other poisonous insects from the surrounding desert. The historian Herodian wrote that as the insects rained down on the Romans scaling the walls, they "fell into the men's eyes and exposed parts of their bodies, digging in, biting, and stinging the soldiers, causing severe injuries." The terror effect would be impressive, no matter how many men were actually stung. Scorpion stings inject a complex combination of toxins, causing intense pain, great agitation and thirst, muscle spasms, convulsions, slow pulse, irregular breathing, and torturous death. Assassin bugs, predatory, bloodsucking insects with sharp beaks, inflict an extremely painful bite and inject a lethal nerve poison that liquefies tissues. It is possible that *Paederus* beetles were also collected by the Hatreni. Pederin, the virulent poison secreted by the predatory Staphylinidae (rove) beetles was well known in ancient India and China. One of the most powerful animal toxins in the world, pederin is a blistering agent on the skin and eyes, and in the bloodstream its toxicity is more potent than cobra venom.

Contagion as a Weapon

Many historians have considered the Mongols' ploy of catapulting of bubonic plague victims over the walls at Kaffa on the Black Sea in 1346 to mark the beginning of biological warfare. But an empirical understanding of contagion developed much earlier in history. In Mesopotamia in 1770 BC, for example, cuneiform tablets warned that disease could be spread by fomites, infectious pathogens on clothing, bedding, other items. Legends about King Solomon suggested that he hid plague in sealed jars in the Temple of Jerusalem to infect Babylonian and Roman invaders. During the Peloponnesian War (fourth century BC), the Athenians suspected that the Spartans had spread plague (apparently smallpox) by poisoning their wells. In the first century BC, King Mithridates was forced to withdraw from his siege of a city near the Black Sea after corpses thrown out in the area fatally infected his troops. In ancient India, Kautilya's *Arthashastra* suggested ways of infecting enemies with illnesses such as fevers, wasting lung disease, and rabies.

In Roman times, historians such as Seneca and Dio Cassius deplored "man-made pestilence," the malicious transmission of plagues by saboteurs who pricked victims with infected needles during the reigns of Domitian and Commodus in the first and second centuries AD. The Great Plague of AD 165–180, probably smallpox, was spread from Babylon (modern Iraq) to Syria, Italy, and Germany by Roman soldiers returning from the war to control Mesopotamia. According to historians of the era, the epidemic began when some Roman soldiers looted a treasure chest in an enemy temple in Babylonia. The implication of the historical accounts, that the chest was boobytrapped with plague-laden items, is plausible. The local population would have had some immunity to the epidemic while the invading Roman

army would have been vulnerable. At the very least, the reports demonstrate that the notion of deliberately spreading epidemics among the enemy was widely contemplated by that time.

Toxic Aerosols and Incendiaries

Asphyxiating clouds of smoke, dust, and gases were effective chemical weapons in antiquity. One of the earliest documented examples of toxic aerosols occurred during the Peloponnesian War in 429 BC, when Sparta besieged the city of Plataia, an ally of Athens. As reported by the historian Thucydides, the Spartans created a massive fire next to Plataia's city walls, and fueled the conflagration with liberal quantities of resinous pinetree sap and sulfur. The combination of pitch and sulfur created clouds of toxic sulfur dioxide gas, fumes that can be fatal when inhaled in large amounts. A few years later, in 424 BC, the Spartans' allies the Boiotians invented a 'flame-throwing' machine to propel noxious smoke from charcoal, resin, and sulfur used against the walled city of Delium.

The Greek strategist Aeneas the Tactician, writing in 360 BC, suggested the use of incendiaries made with pitch, hemp, and sulfur. Roman historians tell how burning chicken feathers created irritating, choking fumes propelled by bellows into siege tunnels.

In 80 BC, the Roman general Sertorius deployed choking clouds of dust to defeat the Characitani of Spain, who had taken refuge in inaccessible caves. The fine white soil in the area consisted of limestone and gypsum. Sertorius ordered his soldiers to pile great heaps of the powder in front of the caves. When the wind was right, the Romans stirred up the dust and raised great clouds of caustic lime powder, a severe irritant to the eyes and lungs. The Characitani surrendered.

A similar dust was used in China to quell an armed peasant revolt in AD 178, when 'lime chariots' equipped with bellows blew limestone powder into the crowds. The powdered lime interacts with the moist membranes of the eyes, nose, and throat with corrosive, burning effect, blinding and suffocating the victims.

In the Middle East, where petroleum is abundant, naphtha (the volatile and toxic light fraction of oil) was ignited and poured on attackers. The ancient Indians and Chinese added 'fire chemicals' to their incendiaries, explosive saltpeter or nitrite salts, a key ingredient of gunpowder, and they also mixed a great variety of plant, animal, and mineral poisons, such as arsenic and lead, in smoke and fire bombs. In the New World and in India, the seeds of toxic plants and hot peppers were burned to rout attackers.

Practical and Ethical Issues

The toxicity of plants, venoms, and other poisons used in armaments posed perils to those who wielded them, and the mythology and the history of poison weapons is rife with examples of accidental self-injury and unintended collateral damage. The use of windborne toxins also involved 'blowback' problems, as acknowledged by Kautilya in his *Arthashastra*. He cautioned that protective salves and other remedies must be applied before deploying poisonous smokes. Toxic weapons are notoriously difficult to control and often resulted in the destruction of noncombatants as well as soldiers, especially in siege situations.

The use of poisons in warfare led to a search for antidotes. Ancient sources list hundreds of substances believed to counteract specific weaponized poisons, from rust filings to poultices made from medicinal plants. It was also believed that one could become invulnerable to toxins by ingesting minute amounts of various poisons over time. King Mithridates VI of Pontus (d. 63 BC) was an early experimenter in creating a 'universal antidote', later known as *mithridatium* and ingested by Roman emperors such as Nero and Marcus Aurelius to gain immunity to poisoning.

The use of toxic weapons was surrounded by ambivalence in antiquity, although there were few rules of war governing their use. Weapons that delivered hidden poisons to make an enemy defenseless or experience excessive suffering aroused moral criticism in many cultures, even as their use was rationalized in numerous recorded instances. Ancient Greeks considered poisoned projectiles a cowardly weapon, for example, yet their most admired heroes, Hercules and Odysseus, resorted to such arms, and well poisoning and toxic aerosols were used in historical Greek conflicts. Poisoned arrows and tainting water and food supplies were deplored by many Romans, yet their generals occasionally turned to such strategies. The Hindu *Laws of Manu* (dating to ~500 BC) recommended spoiling the enemy's food and water but forbade the use of poisoned arrows. In the same era, Kautilya's *Arthashastra* extolled the advantages of poisoning projectiles, food, and water and asphyxiating foes with chemical and disease-laden clouds of smoke. Notably, Kautilya stressed the deterrent effect of publicizing the horrid ingredients of one's toxic arsenal, a strategy also embraced by the Scythians and others in broadcasting their recipes for poison arrows. Sun Tzu's *Art of War* (~500 BC) praised deceptive terror strategies based on fire and Chinese treatises give myriad recipes for toxic aerosols and incendiaries. On the other hand, humanitarian codes of war in China (~450–200 BC) forbade ruses of war and harming noncombatants.

Self-defense was often a rationale for the use of toxic weapons. Besieged cities and desperate populations overcome by overwhelming invaders turned to biological weapons as a last resort. Some commanders used poisons in frustration to break stalemates or long sieges. Other situations, such as holy wars, quelling rebellions, and fighting people considered 'barbarians', encouraged the indiscriminate use of bio-weapons against entire populations. The threat of horrifying toxic weapons could discourage would-be attackers or bring quick capitulation. Some commanders had no compunctions about using any weapons at hand, and in some cultures poison arrows were the customary weapons in both hunting and warfare.

The scope of human ingenuity in weaponizing natural forces in antiquity is impressive, and many of the ancient examples anticipated, in substance or principle, almost every basic form of biological and chemical weapon known today, from spreading plague to poisoning water. For example, asphyxiating smokes were precursors of mustard and other toxic gases first used in World War I. Red-hot sand catapulted onto Alexander the Great's men in the fourth century BC is analogous to modern thermite bombs of World War II. The burning, adhering effects of ancient petroleum incendiaries were reproduced in the modern invention of napalm so notorious during the Vietnam War. Even the advanced stench and noise weapons, the so-called calmatives in mists or water supplies, and top-secret insect and animal-based weapons being developed by Pentagon scientists all have antecedents in the ancient world. Nor are the dangers of self-injury and disposing of poison weapons anything new. The ancient myth of the Hydra with its ever-proliferating heads is a fitting symbol of the dilemmas of creating toxic arms. Faced with the problem of disposing of the immortal central head of the Hydra, Hercules buried it deep in the ground and placed a huge boulder as a marker over the spot. A similar geological solution is used today to dispose of toxic and nuclear weapons material, with burial deep underground in the deserts of New Mexico and Nevada, necessitating warnings to future generations about the perils of biochemical agents. A model for avoiding the proliferation of toxic weaponry is also found in Greek myth. The archer who inherited Hercules' Hydra-venom arrows had experienced grievous injury from the arrows himself, before he deployed them against the Trojans. After the Trojan War, he dedicated the poison arrows to a temple of Apollo, the god of healing, rather than passing them on to the next generation of warriors.

See also: Animals, Poisonous and Venomous; Chemical Warfare Delivery Systems; Chemical Warfare During WW1; Plants, Poisonous; Toxicology in the Arts, Culture, and Imagination.

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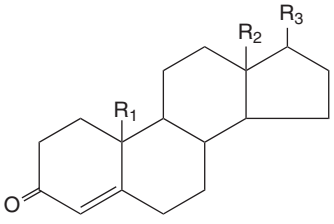
Androgens

Prathibha S Rao and Harihara M Mehendale

- SYNONYMS: Male sex hormones
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Androgens are natural and synthetic congeners of the steroid class of compounds
- CHEMICAL STRUCTURE:



	R_1	R_2	R_3
1. Testosterone	H	H	OH
2. Nandrolone decanoate	H	CH_3	$O-C(=O)-C_9H_{10}$
3. Testosterone propionate	CH_3	CH_3	$O-C(=O)-CH_2CH_3$
4. Ethylestrenol	H	CH_3	$CH(OH)-CH_2CH_3$

Uses

Therapeutic indications for androgens are deficient endocrine functions of the testes, such as hypogonadism,

treatment of refractory anemias in men and women, and hereditary angioneurotic edema. Testosterone has been known to have a palliative effect in some cases of breast cancer and in osteoporosis.

Toxicokinetics

Injected as an oil, androgens are so quickly absorbed, metabolized, and excreted that the effect is very small. Esters of testosterone are more slowly absorbed and are more effective. The majority of the androgens is inactivated primarily in the liver and involves oxidation of the hydroxy groups and reduction of the steroid ring. Alkylation at the 17-position retards hepatic metabolism and hence is effective orally.

About 90% of the androgens are excreted in the urine; 6% appear in the feces after undergoing enterohepatic circulation. Small amounts are also excreted as soluble glucuronide and sulfate conjugates. Many of the synthetic androgens have a longer half-life. Unaltered compounds are excreted in the urine and feces.

Acute and Short-Term Toxicity (or Exposure)

Animal

Six adult male baboons received weekly intramuscular injections of 200 mg testosterone enanthate (equivalent to $8\,mg\,kg^{-1}$ body weight) for up to 28 weeks, while two control animals received weekly injections of the vehicle only. Quantitative increases in the weight and volume of both prostatic lobes were seen after 15 weeks of treatment, and by week 28 there was an increase in stromal tissue with papillary ingrowth or invagination of glandular epithelium in the caudal lobe of the prostate. The serum concentrations of testosterone and dihydrotestosterone were significantly elevated, from 10 and 2–3 ng/ml to 30–40 and 5–6 ng/ml, respectively. The androstenedione concentrations were increased by three to four times and that of estradiol from 20 to 80–90 pg/ml. From this study, it was concluded that these steroids play a direct role in inducing early benign prostate hypertrophy in baboons and that their observations were similar to those in human benign prostate hypertrophy.

Human

Androgens may have a virilizing effect in women. The undesirable manifestations include acne, growth of facial hair, and coarsening of the voice. Profound virilization and serious disturbances in the growth and osseous development can occur when androgens are given to children. The capacity of androgens to enhance epiphyseal closure in children may persist for several months after discontinuation of the drug. All androgens should be used with great care in children. Androgens should not be used during pregnancy since they cross the placenta and cause masculinization of the female fetus. Feminizing effects, particularly gynecomastia, can occur in men who receive androgens. The feminizing effects are particularly severe in children and men with liver disease.

Water retention due to sodium chloride (salt) is a common manifestation that leads to weight gain. Edema is also found in patients with cardiac heart failure, renal insufficiency, liver cirrhosis, and hypoproteinemia. When large doses are used to treat neoplastic diseases, compounds with 17-alkyl substitutions can cause cholestatic hepatitis; at high doses, jaundice is the most common clinical feature with accumulation of bile in the bile capillaries. Jaundice usually develops after 2–5 months of therapy. It can be detected by increases in plasma aspartate aminotransferase and alkaline phosphatase.

Obstructive sleep apnea (OSA) causes a mild lowering of blood testosterone concentrations that is rectified by effective continuous positive airway pressure (CPAP) treatment. Although testosterone treatment has precipitated OSA and has potential adverse effects on sleep in older men, the prevalence of OSA precipitated by testosterone treatment remains unclear. It appears to be a rare idiosyncratic reaction among younger hypogonadal men but the risk may be higher among older men as the background prevalence of OSA rises steeply with age. Hence, screening for OSA by asking about daytime sleepiness and partner reports of loud and irregular snoring, especially among overweight men with large collar size, is wise for older men starting testosterone treatment although not routinely required for young men with classical hypogonadism.

Chronic Toxicity (or Exposure)

Animal

The effects of subcutaneously injected or implanted testosterone and its esters have been reviewed extensively. The working group convened by the International Agency for Research on Cancer (IARC) concluded that: "There is sufficient evidence for the carcinogenicity of testosterone in experimental animals. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard testosterone as if it presented a carcinogenic risk to

humans." The relevance of animal models to human prostate disorders has been reviewed. Besides humans, dogs are the only animals that develop prostatic cancer and benign prostatic hyperplasia at a high frequency. In this model, long-term treatment with androgens and estrogens is required to produce hyperplasia, although such synergism is not observed in other species. ACI rats spontaneously develop histologically evident prostatic cancer, which does not progress to clinically relevant disease when pharmacologically relevant amounts of exogenous androgen are administered. Prostate cancer has been induced only in the Noble and Lobund–Wistar strains of rat.

The role of hormones, including androgens, in the development of mammary neoplasia in rodents and their relevance to human risk assessment has been reviewed. Endogenous androgens are necessary for mammary development in rodents, and it was noted that rodent models mimic some but not all the complex external and endogenous factors involved in initiation, promotion, and progression of carcinogenesis. Tumor type and incidence are influenced by the age, reproductive history, and the endocrine milieu of the host at the time of exposure. The spontaneous incidence of tumors differs in different strains of rats and mice. In rats, most spontaneous neoplasias, with the exception of leukemia, occur in endocrine organs or organs under endocrine control. Russo and Russo concluded that mechanism-based toxicology is not yet sufficient for human risk assessment, and the approach should be coupled to and validated by traditional long-term bioassays.

Fischer 344 rats were given 3,2′-dimethyl-4-aminobiphenyl (a prostate carcinogen) at 50 mg kg^{-1} body weight 10 times at 2-week intervals, and then, from week 20, testosterone propionate and/or diethylstilbestrol by subcutaneous silastic implant for 40 weeks, as seven cycles of 30-day treatment and 10-day withdrawal. Intermittent administration of testosterone resulted in suppression of the development of ventral prostate adenocarcinomas and slight (nonsignificant) increases in the incidences of invasive carcinomas of the lateral prostate and seminal vesicles. Diethylstilbestrol completely suppressed tumorigenesis, and the combination with testosterone propionate inhibited prostate tumor development.

Hydroxyprogesterone caproate was given intramuscularly every other week at an average dose of 13 mg to 19 female rabbits, and testosterone ethanate was given intramuscularly every other week at an average dose of 15 mg to 21 animals; both treatments were given for up to 763 days. Rabbits treated with progesterone developed numerous endometrial cysts, sometimes associated with atypical hyperplasia; active mammary secretion was also seen. Treatment with testosterone induced two adenomatous polyps of the endometrium in one animal, but no other noteworthy endometrial changes were found and one control animal developed similar polyps. Neither significantly altered other tissues such as the ovary, adrenal, thyroid, or pituitary gland. No precancerous endometrial changes or cancers were found.

Human

With prolonged treatment, as in long-term use of androgens in mammary carcinoma, male pattern baldness, excessive body hair, prominent musculature, and hypertrophy of the clitoris may develop and may be irreversible. Patients receiving the 17α-alkyl substituted androgens may develop hepatic adenocarcinoma, the complications may be more common in people with Fanconi's anemia.

Clinical Management

Edema due to salt retention is generally treated with diuretics targeted at increased sodium excretion.

Environmental Fate

Hormones excreted in animal waste have been measured in surface and groundwater associated with manure that is applied to the land surface. Limited studies have been done on the fate and transport of androgenic hormones in soils. There were weak correlations of sorption with soil particle size, organic matter, and specific surface area. Testosterone was the dominant compound present in the soil column effluents, although it was found that testosterone degraded more readily than 17-β-estradiol, it appeared to have a greater potential to migrate in the soil because it was not as strongly sorbed.

Ecotoxicology

The EDMAR program investigated evidence of changes associated with endocrine disruption in marine life and, if so, the possible causes and potential impacts. It followed on from work that demonstrated that flounder in some UK estuaries had changes consistent with endocrine disruption. Male flounder from some industrialized estuaries showed strong vitellogenin induction. Caught sand gobies exhibited no vitellogenin induction or intersex, but feminization of secondary sexual characteristics was observed in male gobies in some estuaries. Viviparous blennies in some estuaries showed induction of vitellogenin, and incidence of intersex. Toxicity identification and

evaluation (TIE) procedures deployed on the Tyne and Tees estuaries identified three natural (steroidal) and two industrial (surfactant and phthalate) estrogenic compounds as possible causes of the observed effects.

A study utilizing fathead minnows was conducted to study the differences in the reproductive biology between groups of minnows from a stream directly below the effluent outfall from a feedlot, from a stream that receives runoff from an agricultural field with disbursed cattle, and from noncontaminated areas upstream from the two previous sample areas. The size, sex hormone levels and gonads of the sampled fish were tested for the effects of trenbolone-β, an active synthetic anabolic steroid. The female fish near the contaminated areas were found to have higher levels of androgens in their systems and smaller distances between internal organs than those from upstream. Similarly, male minnows had smaller testicles and closer internal organs than those from noncontaminated waters. No pathology was apparent in the ovaries or testicles of the fish collected in the contaminated water.

Other Hazards

For men and women, the use of male steroids (androgens) – either the hormone testosterone or the synthetic anabolic steroids – may also increase the risk of coronary artery disease. These drugs lower high-density lipoprotein (HDL) (the good) cholesterol levels, increase low-density lipoprotein (LDL) (the bad) cholesterol levels, and cause high blood pressure. All of these effects may contribute to having a heart attack at an early age or to having a stroke. What effects the use of anabolic steroids early in life has later in life are unclear.

Although mind-altering drugs typically are those that have potential for abuse, several other drugs that do not alter the mind (or do so only occasionally) are often taken without medical need, even when doing so endangers the quality of life or health and safety of the user. Using a drug this way is considered drug abuse. People who stop abusing any of these drugs do not experience withdrawal symptoms, but they may experience medical problems when the drug is discontinued abruptly (problems that are usually preventable if discontinuation is supervised by a doctor). Anabolic steroids are very similar to the hormone testosterone. They have many physical effects on the body, including muscle growth and increased strength as well as increased energy level. Thus, anabolic steroids are often abused to gain a competitive edge in sports. Users are often athletes, typically football players, wrestlers, or weight lifters, and almost all users are male. Many side effects are associated with the abuse of anabolic steroids. Very high doses of anabolic steroids may cause erratic mood swings, irrational behavior, and increased aggressiveness (often called steroid rage). Anabolic steroids can damage the liver and cause jaundice. Regular use of any amount also tends to increase body hair. Acne commonly gets worse with anabolic steroid use and is one of the few side effects for which an adolescent may visit a doctor. Laboratory tests can measure anabolic steroid breakdown products in the urine.

Up to 6% of boys in high school, including a number of nonathletes, have used anabolic steroids at least once. A particular problem with anabolic steroid use in adolescents is early closure of the growth plates at the ends of bones, resulting in permanent short stature. Other side effects are common to both adolescents and adults.

See also: Endocrine System; Reproductive System, Female; Reproductive System, Male; Toxicity Testing, Reproductive.

Further Reading

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Anesthetic Agents

Jeffrey W Allen

Anesthetic agents are a diverse class of chemicals which are extremely important in modern medicine. They are generally used to produce a loss of sensation to all stimuli, either in a specific anatomical area, or a total loss of consciousness. Anesthetics differ from analgesics in that analgesics such as aspirin, acetaminophen, ibuprofen, or morphine act to decrease pain, but not other sensations. Anesthetics can be broadly categorized into two general classes, local anesthetics and general anesthetics. These classes are independent as far as indication, chemical class, routes of administration, and toxicity, and thus will be considered separately. It will be noted when one compound within a class differs from the others.

Local Anesthetics

- Prototypical Compounds: Lidocaine; Mepivacaine; Bupivacaine; Procaine; Tetracaine; Prilocaine; Benzocaine; Cocaine
- Chemical Abstracts Service Registry Numbers:
  Amides
  Lidocaine: HCl (CAS 6108-05-0); Mepivacaine HCl (CAS 1722-62-9); Bupivacaine HCl (CAS 14252-80-3); Procaine HCl (CAS 51-05-8).
  Esters
  Tetracaine: HCl (CAS 136-47-0); Prilocaine HCl (CAS 1786-81-8); Benzocaine (CAS 94-09-7)
- Synonyms:
  Amides
  Lidocaine: 2-(Diethylamino)-2′,6′-Acetoxylidide; Lida-Mantle; Xilina; Xllina; 2-(diethylamino)-*N*-(2,6-dimethylphenyl)-Acetamide; Xyloneural; Cappicaine; α-(Diethylamino)-2,6-acetoxylidide; Duncaine; Gravocain; Isicaina; Isicaine; Leostesin; Lignocaine; Maricaine; Xycaine; Xylestesin; Xylocain; Xylocaine; Xylocitin; Xylotox; 2-(Diethylamino)-2′,6′-acetoxylidide; Diethylaminoaceto-2,6-xylidide; α-Diethylamino-2,6-dimethylacetanilide; α-Diethylaminoaceto-2,6-xylidide
  Mepivacaine: Carbocaine hydrochloride; *N*-(2,6-dimethylphenyl)-1-methyl-2-piperidinecarboxamide monohydrochloride
  Bupivacaine: Marcaine; 1-Butyl-*n*-(2,6-dimethylphenyl)-2-piperidine carboxamide
  Procaine: Novocain hydrochloride; Ethocaine; Paracain; Allocaine; 2-(Diethylamino)ethyl-4-aminobenzoate hydrochloride; 4-Aminobenzoic acid 2-(diethylamino)-ethyl ester hydrochloride; *p*-Aminobenzoyldiethylaminoethanol hydrochloride; Alocaine; Aminocaine; Anesthol; Anestil; Atoxicocaine; 4-Aminobenzoic acid, 2-(diethylamino)ethyl ester, monohydrochloride; Bernacaine; Cetain; Eugerase; Irocaine; Isocaine-Asid; Isocaine-Heisler; Jenacaine; Kerocaine; Medaject; Naucaine; Neocaine; Novocaine; Planocaine; Scurocaine; Sevicaine; Sycaine; Syncaine; Topocaine
  Esters
  Tetracaine: Amethocaine hydrochloride; 2-dimethylaminoethyl 4-*n*-butylaminobenzoate hydrochloride; 4-(Butylamino)benzoic acid 2-(dimethylamino)ethyl ester hydrochloride; Anethaine; Butethanol; *p*-butylaminobenzoyl-2-dimethylaminoethanol hydrochloride; Pontocaine hydrochloride; Tonexol; 4-(butylamino) Benzoic acid, 2-(dimethylamino)ethyl ester, monohydrochloride; Tetracainhydrochlorid; Tetracaina, clorhidrato; Tétracaine, chlorhydrate
  Prilocaine: Propitocaine HCl; Xylonest; *n*-(2-Methylphenyl)-2-(propylamino)-propanamide hydrochloride; 2-(Propylamino)-*o*-propionotoluidide hydrochloride; *n*-(α-Propylaminopropionyl)-*o*-toluidine hydrochloride; α-Propylamino-2′-methylpropionanilide hydrochloride
  Benzocaine: Anesthesin; Parathesin; Auralgan Otic; Ethyl aminobenzoate
- Chemical Formula
  Amides
  Lidocaine: $C_{14}H_{22}N_2O \cdot HCl$; Mepivacaine: $C_{15}H_{22}N_2O \cdot HCl$; Bupivacaine: $C_{18}H_{28}N_2O \cdot HCl$; Procaine: $C_{13}H_{20}N_2O_2 \cdot HCl$

Esters
Tetracaine: $C_{15}H_{24}N_2O_2 \cdot HCl$; Prilocaine: $C_{13}H_{20}N_2O \cdot HCl$; Benzocaine: $C_9H_{11}NO_2$

Uses

Local anesthetics are generally a hydrophobic aromatic ring separated from a hydrophilic tertiary or secondary amine linked by an ester or amide as seen in **Figure 1**. The more hydrophobic (nonpolar or lipid-soluble) the molecule, the more potent, and more toxic, are the compounds. They all act by binding to a hydrophobic site in the intracellular region of voltage-gated Na^+ channels on nerve fibers, blocking Na^+ entry and preventing local membrane depolarization. This action prevents the spread and generation of action potential in these previously excitable membranes. As this blockade is frequency- and voltage-dependent, an active nerve is more susceptible to the effects of local anesthetics than resting nerves. In addition, small unmyelinated nerves, the C and Aδ fibers which carry pain, and pain and temperature messages, respectively, are more sensitive to local anesthetics than the larger myelinated Aβ, Aγ, Aα, and B nerves that carry touch, pressure, proprioceptive, and motor information. Thus a highly active nociceptive (pain-transmitting) neuron's activity would be blocked before neighboring motor and pressure-sensitive nerves. It is this ability to obtain a relative differential sensory and motor block that has given these compounds their widespread clinical usefulness.

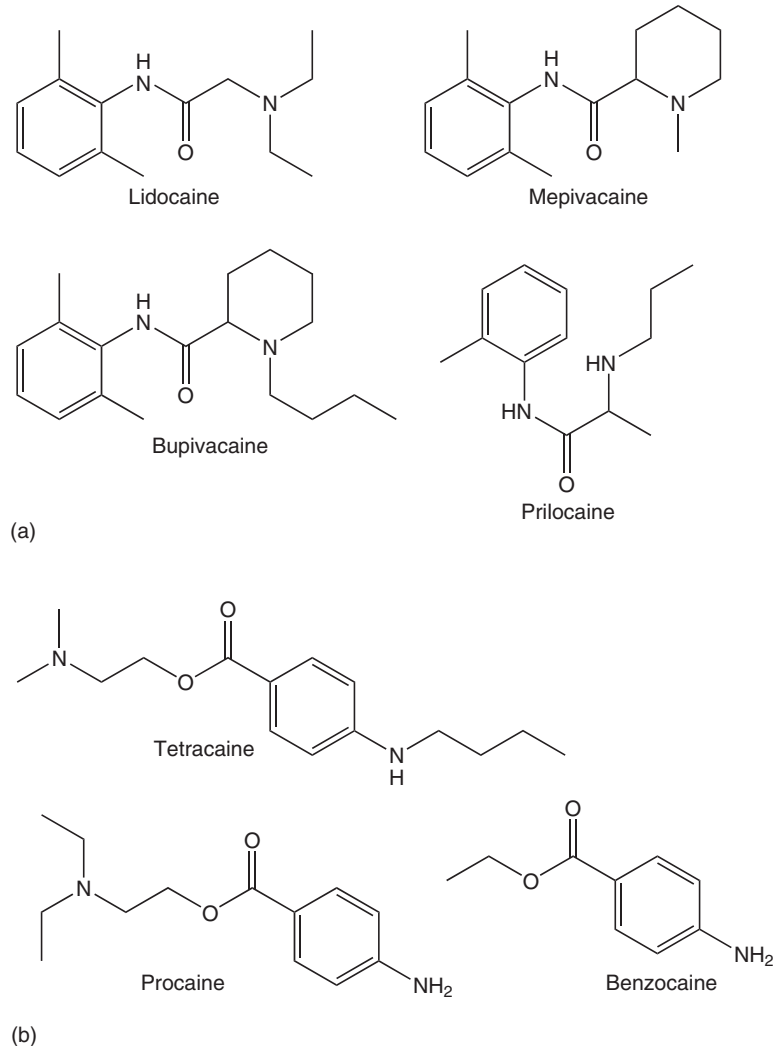

Figure 1 Chemical structures for (a) the amide-linked local anesthetics and (b) the ester-linked local anesthetics.

Exposure Routes and Pathways

Local anesthetics, particularly benzocaine and lidocaine, are found in a number of topical mixtures for treatment of minor superficial pain. These may be found in both prescription and over-the-counter formulations. An adhesive patch that contains 5% lidocaine is available by prescription. This has been successfully used for treatment of focal tactile hyperalgesia, such as that seen in post-herpetic neuralgia. A eutectic mixture of 2.5% lidocaine and 2.5% prilocaine (EMLA) provides anesthesia for superficial procedures including venipuncture and some skin graft harvesting, but requires placement of an occlusive dressing and $\sim$60 min to reach full efficacy. Local anesthetics are commonly administered by focal infiltration to provide temporary loss of sensation for minor invasive procedures such as closure of a laceration with sutures. Mepivacaine is not effective for topical or infiltration anesthesia. If anesthesia is desired in a larger region, local anesthetics can be injected directly adjacent to major nerves or nerve roots such as is often performed for dental procedures and minor surgical procedures, especially on extremities, producing a nerve block. In addition, regional anesthesia in the lower leg and foot or lower arm and hand can be performed by administering intravenous lidocaine or prilocaine in a region that has been isolated from the general circulation by compressive exsanguination and application of a tourniquet. This is commonly known as a Bier's block. Injections into the spinal space, either epidurally or intrathecally are often used for peri- and postoperative pain, in addition to neuropathic pain states. With chronic pain states, delivery of local anesthetics with or without the addition of opiates via an implanted permanent intrathecal catheter and subcutaneous pump can provide a high degree of pain relief in selected patients. Low dose, intravenous lidocaine infusions have also been effective in treating some neuropathic pain states. Lidocaine can be used in the treatment of ventricular tachycardia or fibrillation. As will be discussed below, cardiovascular effects are an important toxicological consideration of lidocaine.

Toxicokinetics

Local anesthetics are readily absorbed and distributed. They cross the intact placenta and blood–brain barrier. Recommended maximum dosages, distribution, and elimination kinetics for three of the most commonly prescribed local anesthetics are presented in **Table 1**.

Approximately 50–70% of an injected dose of amide local anesthetic (lidocaine, mepivacaine, bupivacaine) is taken up by the hepatic system and undergoes *N*-dealkylation and hydrolysis. An important caveat is that prilocaine has a hydrolytic first step which produces *o*-toluidine metabolites that can produce methemoglobinemia. Significant methemoglobinemia has been seen in patients using ELMA. Amide local anesthetics are highly protein-bound in plasma (55–95%) with α_1-acid glycoprotein being the primary protein.

Ester-containing anesthetics such as cocaine, benzocaine, and tetracaine are extensively hydrolyzed by plasma esterases in addition to a contribution from hepatic esterases.

The majority of local anesthetics and their metabolites are excreted renally. The ester anesthetics display very little ($<2\%$) excretion of unmetabolized drug while amide anesthetics can range for 10–16%. Up to 80% of lidocaine and 40% of mepivacaine and their metabolites are found in the urine of normal patients.

Local anesthetics are weak bases and are usually made as HCl salts which are soluble and stable in water. The pK_a of the compound determines when the ionized and unionized forms are equal (see **Table 1** for values of pK_a and percentage ionization at pH 7.4). The time of onset of the block is related to diffusion of the anesthetic into the nerve fiber, which occurs only in the unionized, or non-protonated form. Sodium bicarbonate is often added ($1\,\text{mEq}\,(10\,\text{ml})^{-1}$ lidocaine or $0.1\,\text{mEq}\,(10\,\text{ml})^{-1}$

Table 1 Pharmacokinetic, physiochemical characteristics, and maximal recommended doses of some commonly used amide local anesthetics

	Distribution half-life (min)		*Elimination half-life (h)*	*Time of onset (min)*	*pK_a*	*Percent non-protonated at pH 7.4*	*Maximum dose (mg kg^{-1})*
	α	β	γ				
Lidocaine	1	9.6	1.6	2–4	7.7	25	4
Mepivacaine	0.7	7.2	1.9	2–4	7.6	40	4
Bupivacaine	2.7	28	3.5	5–8	8.1	18	2

Note: The local anesthetics with a pK_a closer to physiological pH of 7.4 have a higher percentage of molecules in the unionized form and have a faster time of onset.

bupivacaine) to raise the pH of the solution. This pushes the ionization state of the local anesthetic from the protonated to the non-protonated state, thus increasing the diffusion of the local anesthetic into the cell and decreasing the time of onset of the block. The addition of bicarbonate does not have an effect on the quality or duration of the block, only the time of onset. It may also make the injection more comfortable for the patient. Once inside the fiber the anesthetic is ionized and trapped in the intracellular space.

Acute and Short-Term Toxicity (or Exposure)

The toxicity of local anesthetics is related to their potency which is directly related to their hydrophobicity. The more hydrophobic drugs such as bupivacaine produce toxicities at concentrations lower than the less potent anesthetics such as lidocaine and mepivacaine.

The two toxicological consequences of greatest concern following acute exposure of local anesthetics are the central nervous and cardiovascular systems. Local anesthetics are able to cross the blood–brain barrier and enter the central nervous system (CNS). Local anesthetic toxicity can be characterized by three phases. In the initial phase signs and symptoms such as lightheadedness, tinnitus, confusion and euphoria or dysphoria, or circumferential numbness are reported by the patient. In the second or excitation phase, clonic–tonic seizures are seen, and in the final or depressive phase unconsciousness, generalized CNS depression followed by respiratory depression and arrest can be present. This seemingly paradoxical initial increase in CNS activity including convulsions can be explained in that small inhibitory interneurons appear to have the greatest to blockade.

Following the appearance of CNS effects cardiovascular effects are often noted. Just as local anesthetics block conduction in peripheral nerves, they can also block Na^+ channels in the myocardium producing decreased action potential duration, rate of depolarization, and refractory period. At very high levels, they may block the sinoatrial and atrioventricular nodes. These agents also have a direct negative ionotropic action and produce a bi-phasic dose-related increase then decrease in vascular resistance. In addition, epidural and intrathecal administration can produce a sympathetic block, removing the sympathetic tone and producing profound hypotension. At equianalgesic doses, bupivacaine is more cardiotoxic than lidocaine.

Because of bupivacaine's longer half-life and greater tendency toward sensory than motor block it is commonly used epidurally during labor. This is in contrast to mepivacaine, which is not used in obstetrics due to increased toxicity in neonates. This toxicity is due to ion trapping because of the lower pH of the neonatal blood compared with that of the mother and the higher pK_a of mepivacaine. The decreased plasma protein in neonates also makes them more susceptible to amide local anesthetic toxicity.

Allergic reactions, including anaphylaxis, have been reported to local anesthetics. This is much more common for amide than ester-containing anesthetics. Local tissue, especially nerve fiber, toxicity can occur. This was most dramatically noted with the advent of microbore intrathecal catheters used to inject high-concentration (5%) lidocaine. It is thought this resulted in high local tissue concentrations and produced a number of cases of cauda equine syndrome and radiculopathy. As a result of these incidents, microbore catheters were removed from the market in 1993.

When local anesthetics are to be administered spinally or for regional blocks they should be preservative-free saline preparations as the neurotoxicity of preservatives and excipiants generally have not been systematically studied.

The invasive procedures used to deliver local anesthetics carry their own risk that must be evaluated in addition to any toxicity due to direct or indirect actions of the drugs.

Chronic Toxicity (or Exposure)

Animal No mutagenic or carcinogenic potential was found in preclinical testing of lidocaine or prilocaine. The 2,6-xylidine metabolite of lidocaine did display carcinogenicity in a 2 year oral toxicity study in rats 60, but not 30 times the single administration dose. The procaine metabolite *o*-toluidine has been carcinogenic in mice at 60–960 and 60–320 times standard dosing levels.

No data have been published concerning pre-clinical carcinogenic or reproductive studies for mepivacaine and no carcinogenic studies have been published for bupivacaine. Decreased pup survival in rats and embryocidal effect in rabbits have been observed when bupivacaine was administered to these species in doses comparable to nine and five times, respectively, the maximum recommended daily human dose.

Human As noted above in acute and subchronic effects, local anesthetics can produce an allergic syndrome that can manifest as urticaria in some patients. This nearly always occurs with amide anesthetics so changing to amine anesthetics greatly reduces this side effect.

There are no reports suggesting mutagenic, carcinogenic, or teratogenic potential of local anesthetics in humans.

In Vitro Toxicity Data

Mutagenesis tests of 2,6-xylidine have proved equivocal. *o*-Toluidine has displayed positive results in DNA repair assays and phage induction assays. The parent compounds displayed no mutagenicity. The mutagenesis potential has not been evaluated in the majority of local anesthetics.

Clinical Management

Reports of signs and symptoms by patients such as noted in the Acute Toxicity section should be regarded as the onset of toxicity. Administration of anesthetics should be halted and proper supportive care initiated. Benzodiazepines can be given prophylactically to prevent or decrease the expected seizure activity. If seizures have begun, then benzodiazepines or fast-acting barbiturates such as pentothal can be given intravenously.

General Anesthetics

General anesthetics are either liquids, which are delivered in their gaseous forms via inhalation using carrier gases of oxygen and air or oxygen and nitrous oxide, or they are relatively lipid-soluble compounds which are given intravenously. They all share a common property of producing a loss of awareness and recall. In addition, they have a rapid onset and also a rapid recovery following discontinuation. They also share the feature of relatively small therapeutic indexes of approximately two to four between effective dosing and cardiopulmonary arrest, making them among the most potentially dangerous drugs that are commonly used in patients.

Inhalational anesthetics are volatile organic hydrocarbons or ethers which are liquid at room temperature (**Figure 2**). Due to their high vapor pressure it is possible to volatilize these agents in a stream of gas, and using highly accurate calibrated vaporizers, to consistently deliver concentrations generally between 0.25% and 6% over a range of temperatures and gas flow rates. Their potency is directly correlated to their gas–oil partition index (Meyer–Overton correlation) with greater oil partitioning providing greater potency. It should be noted that there is a cut-off phenomenon in which lipid-soluble molecules with carbon chains longer than 10 molecules no longer have the increased anesthetic potency as would be predicted. The potency of inhalational anesthetics is measured in terms of minimal alveolar concentration (MAC). A dose of 1 MAC is defined as the concentration at which 50% of patients do not respond with movement to surgical stimulation. By using additional anesthetic or analgesic agents, the commonly used doses range from 0.5 to 2 MAC, which would produce sufficient anesthesia in a majority of patients. Unlike prior generations of volatile anesthetics such as diethyl ether and cyclopropane, the current agents are nonflammable, and unlike chloroform, are relatively nontoxic. Halothane has been the gold standard for evaluating new inhalation agents since its introduction in 1956, but it is now largely being replaced with newer less toxic agents that undergo less biotransformation.

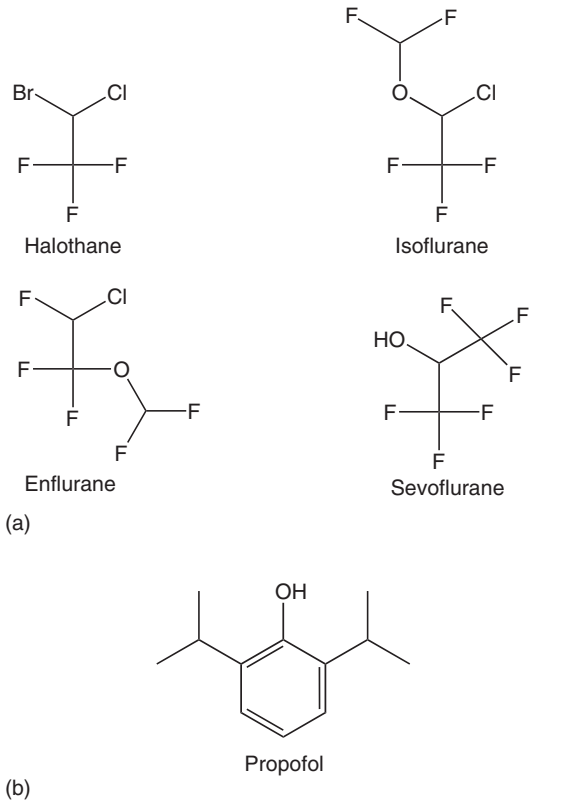

Figure 2 Chemical structures for (a) inhalational anesthetics and (b) the intravenous anesthetic propofol.

Intravenous anesthetics are also relatively lipid-soluble, which helps account for their rapid onset. This high degree of lipid solubility allows them to rapidly cross the blood–brain barrier and partition into the brain. Barbiturates such as thiopental and methohexitol and the nonbarbiturates etomidate and propofol are often used to induce anesthesia, but only propofol is commonly used today as a general anesthetic by continuous infusion, thus only propofol will be discussed.

- Prototypical Compounds: *Inhalational agents*: Halothane; Isoflurane; Enflurane, Sevoflurane. *Intravenous agents*: Propofol
- Chemical Abstracts Service Registry Numbers:
  Inhalational
  Halothane: (CAS 151-67-7); Isoflurane (CAS 26675-46-7); Enflurane (CAS 13838-16-9); Sevoflurane (CAS 28523-86-6).
  Intravenous
  Propofol: (CAS 218-206-6)
- Synonyms
  Inhalational
  Halothane: Fluothane; 2-Bromo-2-chloro-1,1,1-trifluoroethane; Bromochlorotrifluoroethane; 1,1,1-Trifluoro-2,2-chlorobromoethane
  Isoflurane: Forane; 2-Chloro-2-(difluoromethoxy)-1,1,1-trifluoro ethane; 1-Chloro-2,2,2-trifluoroethyl difluoromethyl ether
  Enflurane: Ethrane; 2-Chloro-1,1,2-trifluoroethyldifluoromethyl ether; Efrane; Alyrane
  Sevoflurane: Ultane; Propane-1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy); Fluoromethyl-2,2,2-trifluoro-1-(trifluoromethyl)ethyl ether
  Intravenous
  Propofol: Diprivan; 2,6-Diisopropylphenol; Diisopropylphenol; 2,6-Bis(1-methylethyl)Phenol; Disoprofol
- Chemical Formula: *Inhalational* – Halothane: $C_2HBrClF_3$; Isoflurane: $C_3H_2ClF_5O$; Enflurane: $C_3H_2OClF_5$; Sevoflurane: $C_4H_3F_7O$. *Intravenous* –Propofol: $C_{12}\ H_{18}O$

Uses

General anesthetics are used to produce loss of awareness and recall during invasive medical procedures such as surgery and certain diagnostic or therapeutic procedures. They can also provide varying degree of muscular relaxation which is advantageous for most procedures. In addition, agents such as propofol can be used to provide either deep or light sedation during the time spent in intensive care units (ICUs) when unwanted activity would be detrimental to the recovery process. The rapid immergence from sedation with the termination of propofol administration, even after days of sedation, is especially useful in that it can allow periodic assessments of mental and neurological functions of patients during extended recovery periods.

Intravenous anesthesia does not require the elaborate equipment needed to deliver inhalational anesthetics, but it should be recognized that intravenous anesthetics cause respiratory and cardiovascular collapse as seen with inhalation agents. Thus a general anesthetic should never be administered in a setting without appropriate monitoring, resuscitation equipment, and personnel trained in their use.

Exposure Routes and Pathways

Volatile anesthetics are administered exclusively via inhalation. Propofol is administered only intravenously. Exposure by other routes would not be anticipated.

Toxicokinetics

Inhalation anesthetics: The uptake of inhalational anesthetics by the lung, diffusion into the bloodstream and partitioning into the brain are determined by a number of factors including their solubility in blood and tissue and the adequacy of respiratory and cardiovascular function. Due to centrally mediated respiratory depression, patients are often mechanically ventilated while being administered general anesthetics. Cardiovascular function is both directly and indirectly decreased by inhalational anesthetics. This direct effect is thought to be due to decreased Ca^{2+} entry into myocytes producing deceased contractile force. Heart rate is also slowed due to the loss of sympathetic tone; however, the sensitivity of the myocardium to sympathomimetics can be increased resulting in a sensitized or irritable heart that is prone to tachyarrhythmias if large amounts of epinephrine are used in the presence of volatile anesthetics. Anesthetics may possibly also decrease cardiac function by blunting baroreceptor reflexes. While they vary somewhat in the degree of cardiac depression produced, it is a trait common to all inhalational anesthetics.

Inhalational anesthetics are largely removed in the same route as they were administered, by the lungs. For halothane, up to 80% of the gas can be exhaled unchanged by the lungs. This generally occurs over the first 24 h, but some amounts may be exhaled for many days. The remaining nonexhaled are either excreted or undergo biotransformation, largely by hepatic mixed function oxidases. The oxidative and reductive metabolism of halothane is shown in detail in **Figure 3**. Trifluoroacetic acid (TFA) is the major metabolite of halothane and is found in the urine with a half-life of 16 h. The consequence of this biotransformation following halothane exposure has been a driving factor for the development of newer inhalational agents and will be discussed below.

Propofol is widely used for the induction of anesthesia and often for maintenance during procedures when rapid recovery is beneficial. Pain is sometimes

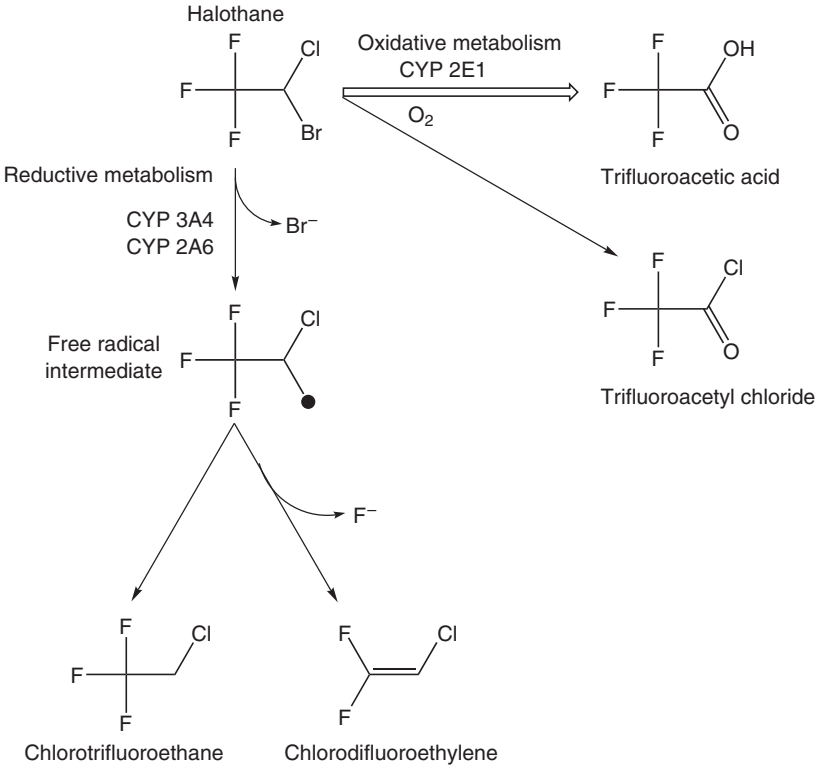

Figure 3 Halothane biotransformation. Approximately 80% of inhaled halothane is exhaled unchanged. The majority of the remaining 20% undergoes oxidative metabolism. This produces trifluoroacetic acid (TFA) as the major metabolite which is excreted in the urine, and smaller amounts of trifluoroacetyl chloride. The trifluoroacetyl chloride covalently binds to a variety hepatic proteins. Antibodies to these trifluoroacetylated proteins are thought to be a causative factor in the development of type II hepatotoxicity. Some reductive metabolism also occurs which involves the production of a free radical intermediate. It is believed that this intermediate is the hepatotoxic compound in type I hepatotoxicity. The metabolites chlorotrifluoroethane (CTF) and chlorodifluoroethylene (CDF) are exhaled and can serve as markers for reductive metabolism.

reported with propofol infusion and often a local anesthetic is added to the solution during its use for induction. Unlike inhalational anesthetics, propofol has little direct cardiovascular depressant effects. Decreases in blood pressure of up to 30% can be seen, but this is likely due to a decreased peripheral vascular resistance and is often reversed by stimulation such as endotracheal intubation. Because propofol is poorly soluble in water ($\sim 0.01\,mg\,ml^{-1}$), it is formulated for injection as an emulsion of $10\,mg\,ml^{-1}$ propofol, $100\,mg\,ml^{-1}$ soybean oil, $22.5\,mg\,ml^{-1}$ glycerol, $12\,mg\,ml^{-1}$ egg lethicin, and 0.005% EDTA. It has a molecular weight of 178.24 and pK_a is 11. The octanol–water partition coefficient for propofol is 6761:1 at a pH of 6–8.5. Propofol is chiefly eliminated by hepatic conjugation to inactive metabolites which are excreted by the kidney. A glucuronide conjugate accounts for $\sim$50% of the administered dose.

Acute and Short-Term Toxicity (or Exposure)

Inhalation anesthetics: Hepatic injury is a concern following halothane exposure due to its large amount of biotransformation which is illustrated in **Figure 3**. Two major types of hepatotoxicity are associated with halothane administration. They appear to be unrelated and are termed type I (mild) and type II (fulminant). Type I hepatotoxicity is benign, self-limiting, and relatively common with incidence rates as high as 25–30%. Type I is marked by mild, transient increases in serum transaminase and glutathione S-transferase concentrations and by altered postoperative drug metabolism. Type I probably results from reductive (anaerobic) biotransformation of halothane rather than the normal oxidative pathway. It does not occur following administration of other volatile anesthetics because they are metabolized to a lesser degree and do not undergo this reductive metabolism.

In contrast, type II hepatotoxicity is associated with massive centrilobular liver cell necrosis that can lead to fulminant liver failure. Type II hepatotoxicity is characterized by fever, jaundice, and very high serum transaminase levels. It may be immune-mediated and is thought to occur in genetically predisposed individuals. The incidence of type II hepatotoxicity is $\sim$1:35 000 with one exposure to halothane and

increases to 1:3700 on second exposure. Type II hepatotoxicity is initiated by oxidative halothane metabolism (see **Figure 3**) to an intermediate acyl halide compound, trifluoroacetyl chloride, which produces covalent triflouroacetylation of proteins in the hepatic endoplasmic reticulum. Antibodies against these trifluoroacetylated proteins are thought to be responsible for the autoimmune destruction of the lever. Approximately 20% of halothane is oxidatively metabolized, compared with only 2% of enflurane and 0.2% of isoflurane as described in **Table 2**. The occurrence of type II hepatotoxicity after enflurane or isoflurane administration is extremely rare.

A second rare, but potentially fatal, condition associated with acute inhalational anesthetic exposure is malignant hypothermia. This is an autosomal dominant disease in which there is excessive sarcoplasmic release of intracellular Ca^{2+} in skeletal muscles during exposure to inhalational anesthetics. This produces a hypermetabolic state that is manifested as increased muscle rigidity and contracture, tachycardia and metabolic acidosis. Extreme hyperthermia is also present. There are currently a number of worldwide registries for tracking this disease and an *ex vivo* testing paradigm exists to determine a potentially susceptible persons phenotype.

Propofol: Propofol has been relatively free of acute side effects other than those associated with its mechanism of action. Continuous infusions lasting greater than 10 days in ICUs have demonstrated no significant apparent toxicities. Propofol is not recommended for obstetrics, including cesarean section deliveries. It crosses the placenta, and as with other general anesthetic agents, may be associated with neonatal depression. Propofol is not recommended for use in nursing mothers because it is excreted in human milk, and the effects of oral absorption of small amounts of propofol in newborn and infants are not known.

Chronic Toxicity (or Exposure)

Animal *Inhalation anesthetics*: An 18 months inhalational carcinogenicity study of halothane at 0.05% in the mouse revealed no evidence of anesthetic-related carcinogenicity. This concentration is equivalent to 24 h of 1% halothane. Some studies have shown halothane to be teratogenic, embryotoxic, and fetotoxic in the mouse, rat, hamster, and rabbit at subanesthetic and/or anesthetic concentrations. Reproduction studies of halothane (10 ppm) and nitrous oxide in the rat caused decreased fertility. This trace concentration corresponds to 1/1000 the human maintenance dose. Studies of isoflurane have not demonstrated these effects.

Propofol: Animal carcinogenicity studies have not been performed with propofol. *In vivo* animal tests failed to show any potential for mutagenicity by propofol. Studies in rats revealed no impairment of fertility. Reproduction studies have been performed in rats and rabbits at intravenous doses of $15\,mg\,kg^{-1}\,day^{-1}$ (approximately equivalent to the recommended human induction dose on a $mg\,m^{-2}$ basis) and have revealed no evidence of impaired fertility or harm to the fetus due to propofol. Propofol, however, causes maternal death in rats and rabbits and decreases pup survival during the lactating period in dams treated with $15\,mg\,kg^{-1}\,day^{-1}$ (approximately equivalent to the recommended human induction dose on a $mg\,m^{-2}$ basis). The pharmacological activity of the drug (anesthesia) on the mother is thought to be responsible for the adverse effects seen in the offspring.

Human *Inhalation anesthetics*: Occupational exposure to inhalational anesthetics, especially halothane, produces an increase in miscarriage rate in individuals. There are no reports of inhalational anesthetic-related carcinogenesis, teratology, or mutagenesis. A recent analysis suggests anesthesiologists, who presumably have the highest exposure levels, have mortality rates equivalent to that seen in nonexposed physicians, such as internists. To date, no definitive long-term detrimental effects have been noted with chronic low-level inhalational anesthetic exposure in humans.

Propofol: There are no adequate and well-controlled studies in pregnant women. Although animal reproduction studies are not always predictive of

Table 2 Physical constants and anesthetic values for the four most commonly used inhalational anesthetics

	MAC (%)	*MAC awake (%)*[a]	*Boiling point (°C)*	*SVP (mmHg)*[b]	*Partition coefficients (37°C)*			*Biotransformation (%)*
					Oil:gas	*Blood:gas*	*Brain:blood*	
Halothane	0.75	0.41	50.2	243	225	2.2	2.9	20
Isoflurane	1.2	0.4	48.5	238	98	1.4	2.6	0.2
Enflurane	1.6	0.4	56.5	175	98	1.9	1.4	2.4
Sevoflurane	2.0	0.6	58.5	160	53	0.45	1.7	3–5

[a] MAC awake is the concentration where responses to verbal commands are lost, this is also usually the point of amnesia and loss of awareness.

[b] Vapor pressure at standard conditions of 20°C.

human responses, this drug should be used during pregnancy only if clearly needed.

In Vitro Toxicity Data

Mutagenesis testing of halothane revealed both positive and negative results. In the rat, 1 year exposure to trace concentrations of halothane (1 and 10 ppm) and nitrous oxide produced chromosomal damage to spermatogonia cells and bone marrow cells. Negative mutagenesis tests included Ames bacterial assay, Chinese hamster lung fibroblast assay, sister chromatid exchange in Chinese hamster ovary cells, and human leukocyte culture assay.

Propofol: Tests for mutagenicity included the Ames mutation test, gene mutation/gene conversion using *Saccharomyces cerevisiae*, *in vitro* cytogenetic studies in Chinese hamsters, and a mouse micronucleus test. None of these have shown mutagenic potential.

Clinical Management

Decreased respiratory and cardiovascular function due to general anesthetics must be carefully monitored and appropriate actions taken when necessary. Given the short time of induction and recovery for general anesthetics, discontinuation of drug administration may quickly resolve the depressant effect.

Malignant hyperthermia is a rapidly progressing life-threatening condition. On suspected diagnosis, all anesthetic drugs are stopped and 100% oxygen is given. Intravenous dantrolene, an inhibitor of sarcoplasmic Ca^{2+} release, should be administered and the patient should be cooled as rapidly as possible with ice.

Environmental Fate

Like the conventional hydrochlorofluorocarbon refrigerants, inhalation anesthetics are known to oxidize in the atmosphere. As in human oxidative metabolism, atmospheric transformation produces TFA and trifluoroacetyl chloride. TFA appears to be very resistant to degradation by nonbiological physicochemical and photochemical processes due to its light-absorption properties. TFA salts are highly soluble and will not precipitate from solution at concentrations expected in the environment. Thus, the stability and solubility of TFA suggest that it will tend to remain dissolved in water. The bulk of existing data suggests that TFA is resistant to biodegradation in natural environments. However, certain bacterial strains maintained in the laboratory have been shown to degrade TFA with release of carbon dioxide. The fate of the fluorine atoms in this process is unknown.

Tests have generally shown that mammals, fish, and crustaceans are resistant to TFA at concentrations many thousands of times higher than expected in the environment. Because TFA has very low affinity for lipids there is no potential for passive accumulation in fatty tissues, even after long exposure at low levels. The amount of TFA contributed to the environment by use of inhalational anesthetics is thought to be minor compared to those that are released by industrial and manufacturing processes.

Exposure Standards and Guidelines

In the past, concerns have been raised about potential health risks of exposure to airborne anesthetic agents in clinical settings where scavenging devices have either been inadequate, malfunctioned, or not been installed. Although there is no clear evidence of significant effects among occupational groups studied thus far, as was noted above, there is biological plausibility for concerns about adverse neurological, reproductive, and developmental risks. Therefore, the Occupational Safety and Health Administration provides guidelines for minimizing workplace exposures to fugitive and waste anesthetic gases. In addition, both the National Fire Protection Association (NFPA) and the American Society for Testing and Materials (ASTM) specify consensus standards, codes, and performance requirements for equipment (e.g., ventilators, medical gas systems, and gas-scavenging equipment).

The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned halothane a threshold limit value of 50 ppm ($404\,mg\,m^{-3}$) as a time-weighted average for a normal 8 h workday and a 40 h workweek. This is $\sim$1.5 times the odor threshold of 33 ppm for halothane.

The National Institute for Occupational Safety and Health (NIOSH) has established a recommended exposure limit for halothane as a waste anesthetic gas of 2 ppm ($16.2\,mg\,m^{-3}$) as a 60 min ceiling limit that should not be exceeded during any part of the workday.

See also: Benzodiazepines; Cocaine; Lidocaine; Procainamide.

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Relevant Websites

http://www.osha.gov – Occupational Safety & Health Administration (OSHA). This United States government agency sets and enforces standards for workplace safety and health, including exposure levels of inhalational anesthetics.

http://www.astm.org – American Society for Testing and Materials. The agency sets and provides standardization for equipment such as inhalational anesthetic vaporizers.

http://www.niehs.nih.gov – National Institute of Environmental Health Sciences that examines the human effects of environmental contaminates such as metabolites of inhalational anesthetics.

Aneuploidy

David A Eastmond

Aneuploidy is a condition in which the chromosome number of a cell or individual differs from a multiple of the haploid complement for that species. In common terms, an aneuploid cell will have one or more chromosomes in addition to or less than, what is normal for that cell type, such as a human somatic cell having 45 or 47 chromosomes rather than 46, the normal diploid number. Similarly, an aneuploid human germ cell would possess $\leqslant 22$ or $\geqslant 24$ chromosomes rather than the haploid chromosome number of 23. In cytogenetics, aneuploidy is considered one type of numerical chromosome aberration. The other type of aberration is polyploidy, where the chromosome number of a cell is increased by a multiple of the haploid complement for the species. (For example, a human cell having 69 or 92 chromosomes rather than the diploid 46 would be considered polyploid.) Aneuploid cells may be further described as hyperploid – having additional chromosomes, hypoploid – possessing fewer chromosomes, or as having trisomy – possessing three copies of one chromosome, or monosomy – with a single copy of a chromosome. In some cases, researchers have expanded the definition of aneuploidy to include partial or segmental aneuploidies, conditions resulting from structural rearrangements where portions of chromosomes have been added to or lost from a cell. In this article, aneuploidy will be used based on its original and more widely accepted definition, which involves the loss or gain of entire chromosomes.

Aneuploidy occurring in germ cells and early embryos is a major cause of morbidity and mortality in humans. It is associated with infertility, pregnancy loss, congenital malformations, and mental retardation. Congenital aneuploidy involving autosomal chromosomes affects $\sim 0.15\%$ of all live births, and aneuploidy involving the sex chromosomes affects another 0.175%. In addition, the frequency of chromosomal abnormalities is much higher among pregnancies that terminate at birth ($\sim 5\%$) or during gestation ($\sim 50\%$ in fetuses dying between weeks 8 and 11 of gestation). In most cases, these abnormalities are believed to have contributed to the embryonic and fetal deaths. Overall, chromosome abnormalities are estimated to be responsible for $\sim 30\%$ of lost pregnancies, with aneuploidy accounting for $\sim 75\%$ of the total. As a consequence, it has been estimated that $\sim 13\,000$ aneuploid babies will be born each year in the United States and that another 150 000–200 000 chromosomally abnormal embryos will be spontaneously aborted. Among the surviving offspring, the most common type of congenital aneuploidy is Down's syndrome, which results from trisomy of chromosome 21 and occurs in ~ 1 in 800 newborns (0.13%). Similarly, Klinefelter's syndrome (XXY), YY males (XYY), triple X females (XXX), and Turner's syndrome (XO) are congenital aneuploidies of the sex chromosomes that individually affect ~ 0.05–0.005% of live births. Because most of these individuals are infertile and exhibit developmental abnormalities, aneuploidy is responsible for a significant portion of the recognized cases of infertility, congenital malformations, and mental retardation. Indeed, aneuploidy has been reported to be the leading genetic cause of mental retardation in the United States.

Nonrandom patterns of numerical aberrations are frequently observed in cancer cells, implicating aneuploidy in carcinogenesis. Associations between

aneuploidy and neoplastic development have also been observed in patients with congenital and familial predispositions for cancer, as well as in patients with cancers resulting from chemical exposures. Similar results have been observed in animals and cellular systems in which the nonrandom gain or loss of specific chromosomes has been associated with tumorigenesis or neoplastic transformation. These patterns have been seen in tumors occurring spontaneously as well as those induced by chemical, radiation, or viral agents.

While in some tumors, chromosomal changes appear to be a secondary effect related to cell proliferation or genomic instability, a growing body of molecular and cytogenetic evidence indicates that the induction of aneuploidy plays an important role in neoplastic transformation. This has perhaps been best characterized in the case of retinoblastoma where it has been shown that the loss of the allele containing the functional Rb tumor suppressor gene frequently occurred through a mechanism involving nondisjunction of chromosome 13. Similarly, alterations in gene dosage resulting from aneuploidy are believed to contribute to the development of many other cancers.

Mechanistically there are many ways by which aneuploidy can occur. Almost any process that interferes with mitosis or meiosis during cell division can affect chromosome segregation and result in aneuploidy. In germ cells, aneuploidy appears to originate, in part, from aberrant meiotic recombination, premature separation of sister chromatids, and possibly altered DNA methylation. During carcinogenesis, aneuploidy has been reported to result from mechanisms including spontaneous errors of mitosis, chemical interference with the mitotic spindle, viral integration resulting in chromosomal instability, as well as mutations affecting the kinetochore, the centrosome or other cellular structures and organelles.

The ability of chemicals to interfere with proper chromosome segregation has been an area of considerable concern within genetic toxicology. Many chemical and physical agents including those used as pesticides, pharmaceuticals, consumer products, and industrial chemicals have been shown to induce aneuploidy *in vitro* and/or *in vivo*. Indeed, drugs such as vincristine sulfate and griseofulvin are used specifically because of their ability to induce aneuploidy, which gives them cytostatic or cytotoxic properties. In spite of the common use of aneugenic chemicals, the extent to which aneuploidy induced by these agents contributes to cancer and reproductive dysfunction in the general population remains uncertain. However, due to the clear involvement of aneuploidy in carcinogenesis and in adverse reproductive outcomes, there continues to be concern about the safety of aneuploidy-inducing agents.

Many different assays have been developed to detect aneuploidy, all of which have significant limitations. The conventional approach has been to count the number of chromosomes in metaphase preparations of dividing cells. Unfortunately, this restricts the detection to actively dividing cells, which may not be present in the tissue of interest. In addition, this technique is laborious and prone to technical artifacts, such as chromosome loss during metaphase preparation. The micronucleus assay, particularly as modified with antibodies or probes to detect centromere-containing micronuclei, has emerged as a simple way to detect aneugenic agents. While valuable, this assay is only able to detect chromosome loss and breakage, and may not detect agents that specifically induce nondysjunction or chromosome gain. Other techniques involving fluorescence *in situ* hybridization with DNA probes allow chromosome gains to be detected in many tissues but are relatively insensitive unless multiple probes and other modifications are used. As a result, efforts continue to develop assays or combinations of assays that will allow the efficient detection of aneugenic agents.

See also: Carcinogenesis; Chromosome Aberrations; Dominant Lethal Tests; Genetic Toxicology; *In Vitro* Test; *In Vivo* Test; Molecular Toxicology–Recombinant DNA Technology; Sister Chromatid Exchanges; Toxicity Testing, Mutagenicity.

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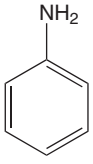
Angiotensin-Converting Enzyme Inhibitors

See ACE Inhibitors.

Aniline

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 62-53-3
- Synonyms: Phenylamine; Aminobenzene; Blue oil
- Chemical/Pharmaceutical/Other Class: Aromatic amine
- Chemical Formula: C_6H_7N
- Chemical Structure:



Uses

Intermediate in dyestuff production and in the manufacture of pharmaceuticals, photographic developers, shoe polish, resins, varnish, perfumes, and organic chemicals.

Exposure Routes and Pathways

Exposure is primarily by dermal and inhalation routes.

Toxicokinetics

Aniline is rapidly absorbed by the skin, lungs, and the gastrointestinal tract of experimental animals. After intravenous injection of radiolabeled aniline to rats, radioactivity is distributed throughout the body; highest concentrations were found in blood, liver, kidney, urinary bladder, and the gastrointestinal tract. The major urinary metabolites in various animal species tested are *o*-, *p*-amino-phenol, and their conjugates. *p*-Aminophenyl- and *p*-acetylaminophenylmercapturic acids are also excreted in rats and rabbits. *N*-Hydroxylation of aniline by liver microsomes from several species has been observed *in vitro*. The formation of phenylhydroxylamine from aniline appears to be the reactive metabolite responsible for its toxic activity.

Mechanism of Toxicity

The formation of phenylhydroxylamine from aniline appears to be the reactive metabolite responsible for its toxic activity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Moderate skin and severe eye irritant in rabbits; reproductive toxin in mice. Rat LC_{Lo} 250 ppm h^{-1}; dermal LD_{50} 1400 mg kg^{-1}; mouse oral LD_{50} 464 mg kg^{-1}. Aniline is a mutagen, that is, it is positive in the *in vivo* mouse micronucleus and sister chromatid exchange assays. DNA strand breakage was induced in the livers and kidneys of rats exposed *in vivo*.

Human

Human acute LD_{Lo} 350 mg kg^{-1}. Systemic exposure leads to methemoglobin formation, and metabolic formation of aniline from a number of drugs leads to methemoglobinemia associated with their use. Normal systemic levels should be less than 1 mg l^{-1}. Toxic oil syndrome (TOS) is a multisystemic disease

that occurred in epidemic proportions in Spain in 1981 caused by the ingestion of rapeseed oil denatured with aniline. It was one of the largest intoxication epidemics ever recorded. This oil had been illegally sold as olive oil, and many aniline-derived oil components have been identified in the oil. The pathologic findings in TOS showed primary endothelial injury, with cell proliferation and perivascular inflammatory infiltrates, and an immunological mechanism has been directly implicated in this illness.

Chronic Toxicity (or Exposure)

Animal

Can cause methemoglobin formation, and liver and endocrine effects. Causes kidney, urethra, bladder, and hematologic neoplasia. For example, aniline administered to rats for 5, 10, or 20 days resulted in splenic congestion, increased hematopoiesis and hemosiderosis, and bone marrow hyperplasia, and the dietary intake of aniline hydrochloride by rats for 104 weeks at levels of 10, 30, or 100 mg kg^{-1} diet is associated with an increased incidence of primary splenic sarcomas. Several species of animals exposed to 5 ppm of aniline vapour daily for 6 months resulted in no effects other than a slight increase in methemoglobin in the blood of rats. Repeated subcutaneous injections of 1.25 mg aniline in lard produced no tumours in mice that survived 2 years, and no tumours were observed after 15 months in mice given eight subcutaneous injections of aniline (5 mg in olive oil), or after 12 months in mice given 13 subcutaneous injections of aniline hydrochloride.

Human

The World Health Organization, International Agency for Research on Cancer, has evaluated the data for aniline and has placed aniline in its group 3 'classification of carcinogenicity', that is, aniline is not classifiable as to its carcinogenicity to humans.

In Vitro Toxicity Data

Although aniline per se is not mutagenic in the Ames *Salmonella typhimurium* assay system, the urine of rats that received aniline orally was mutagenic for *S. typhimurium* when the assay was performed in the presence of liver microsomes from PCB-induced rats. Aniline is positive in the *in vitro* sister chromatid exchange and mouse lymphoma assays. Further, results for cytogenetic effects in Chinese hamster ovary cells were positive for both chromosome aberrations and sister chromatid exchanges.

Clinical Management

Methemoglobin levels should be managed and/or reduced with suitable agents such as methylene blue.

Ecotoxicology

Toxic to aquatic organisms; for example, there was an inhibiting effect of 20–40 ppm aniline on the pigmentation of *Xenopus laevis* embryos, and of a concentration as low as 1 ppm on the body size of the young toads. Investigation of the death of pine trees in the United States found air pollution from aniline as the most likely causal agent for the needle necrosis and needle abscission.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists has established an 8 h time-weighted average of 2 ppm. The US Occupational Safety and Health Administration (OSHA) permissible exposure limit is 5 ppm.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Genetic Toxicology.

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Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Aniline.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Aniline.

Animal Models

Shayne C Gad

Introduction

The use of animals in experimental medicine, pharmacological study, and toxicological assessment is a well-established and essential practice. Whether serving as a source of isolated cells or tissues, a disease model, or as a prediction for drug or other xenobiotic action in humans, experiments in animals have provided the necessary building blocks that permitted the explosive growth of medical and biological knowledge in the latter half of the twentieth century. Animal experiments also have served rather successfully as identifiers of potential hazards to and toxicity in humans for synthetic chemicals with many intended uses.

Animals have been used as models for centuries to predict what chemicals and environmental factors would do to humans. The earliest uses of experimental animals are lost in prehistory, and much of what is recorded in early history about toxicology testing indicates that humans were the test subjects. The earliest clear description of the use of animals in the scientific study of the effects of environmental agents appears to be by Priestley (1792) in his study of gases. The first systematic use of animals for the screening of a wide variety of agents was published by Orfila (1814) and was described by Dubois and Geiling (1959) in their historical review. This work consisted of dosing test animals with known quantities of agents (poisons or drugs) and included the careful recording of the resulting clinical signs and gross necropsy observations. The use of animals as predictors of potential ill effects has grown since that time.

Current Animal Studies

The current regulatory required use of animal models in acute testing began by using them as a form of instrument to detect undesired contaminants. For example, canaries were used by miners to detect the presence of carbon monoxide – a case in which an animal model is more sensitive than humans. By 1907, the US Food and Drug Administration started to protect the public by the use of a voluntary testing program for new coal tar colors in foods. This was replaced by a mandatory program of testing in 1938, and such animal testing programs mandated by regulations have continued to expand until recently.

The knowledge gained by experimentation on animals has undoubtedly increased the quality of our lives, an observation that most reasonable people would find difficult to dispute, and has also benefited animals. As is the case with many tools, animals have sometimes been used inappropriately. These unfortunate instances have helped fuel an increasingly vituperative animal rights movement. This movement has encouraged a measure of critical self-appraisal on the part of scientists concerning the issues of the care and usage of animals. The Society of Toxicology, for example, has established an Animals in Research Committee, and has published guidelines for the use of animals in research and testing. In general, the purpose of this committee is to foster thinking on the four 'Rs' of animal-based research: reduction, refinement, research into replacements, and responsible use.

The media commonly carry reports that state that most (if not all) animal testing and research is not predictive of what will happen in humans and, therefore, such testing is unwarranted. Many of the animal rights groups also present this argument at every opportunity and reinforce it with examples that entail seemingly great suffering in animals but which add nothing to the health, safety, and welfare of society. This is held to be especially the case for safety testing and research in toxicology. Animal rights activists try to 'prove' this point by presenting examples of failure; for example, thalidomide may be presented as an example without pointing out that, in the case of thalidomide, there was lack of adequate testing (or of interpretation of existing test results) prior to marketing. In light of the essential nature of animal research and testing in toxicology, this is equivalent to seeking to functionally disarm us as scientists. Our primary responsibility (the fourth 'R') is to provide the information to protect people and the environment, and without animal models we cannot discharge this responsibility.

When confronted with this argument, all too many toxicologists cannot respond with examples to the contrary. Indeed, many may not even fully understand the argument at all. Also, very few are familiar enough with some of the history of toxicity testing to be able to counter with examples where it has not only accurately predicted a potential hazard to humans but also where research has directly benefited both humans and animals. There are, however, many such examples. Demonstrating the actual benefit of toxicology testing and research with examples that directly relate to the everyday lives of most people and not esoteric, basic research findings (which are the most exciting and interesting products to most scientists) is not an easy task. Examples that can be

seen to affect neighbors, relatives, and selves on a daily basis would be the most effective. The problem is that toxicology is, in a sense, a negative science. The things we find and discover are usually adverse. Also, if the applied end of our science works correctly, then the results are things that do not happen (and therefore are not seen).

If we correctly identify toxic agents (using animals and other predictive model systems) in advance of a product or agent being introduced into the marketplace or environment, then generally it will not be introduced (or it will be removed) and society will not see death, rashes, renal and hepatic diseases, cancer, or birth defects (for example). Also, as these things already occur at some level in the population, it would seem that seeing less of them would be hard to firmly tie to the results of toxicity testing that rely on animals. In addition, the fact that animals are predictive models for humans is controversial.

Origins of Predictive Animal Testing

The actual record of evidence for the predictive value of animal studies and how they have benefited man and domestic animals will be reviewed in the following. However, the negative image needs to be rebutted. First, it must be remembered that predictive animal testing in toxicology, as we now know it, arose largely out of three historical events.

The 'Lash Lure' Case

Early in the 1930s, an untested eyelash dye containing i-pheylenediamine (Lash Lure) was brought onto the market in the United States. This product (as well as a number of similar products) rapidly demonstrated that it could sensitize the external ocular structures, leading to corneal ulceration with loss of vision and at least one fatality.

The Elixir of Sulfanilamide Case

In 1937, an elixir of sulfanilamide dissolved in ethylene glycol was introduced into the marketplace. One hundred and seven people died as a result of ethylene glycol toxicity. The public response to these two tragedies helped prompt US Congress to pass the Federal Food, Drug, and Cosmetic Act of 1938 (FD&C Act). This law mandated the premarket testing of drugs for safety in experimental animals. It is a fact that since the imposition of animal testing as a result of these two cases, no similar occurrence has happened even though society uses many more consumer products and pharmaceuticals today than during the 1930s.

Thalidomide

The use of thalidomide, a sedative-hypnotic agent, led to some 10 000 deformed children being born in Europe. This in turn led directly to the 1962 revision of the FD&C Act, requiring more stringent testing. Current testing procedures (or even those at the time in the United States, where the drug was never approved for human use) would have identified the hazard and prevented this tragedy. In fact, tragedies like this have not occurred in Europe or the United States except when the results of animal tests have been ignored. **Table 1** presents an overview of cases in which animal data predicted adverse effects in humans.

Birth defects, for example, have occurred with isotretinoin (Accutane) where developmental toxicity had been clearly established in animals and presented on labeling, but the drug has continued to be used by potentially pregnant women.

Choosing an Animal Model

Choosing the appropriate animal model for a given problem is sometimes guesswork and often a matter of convenience. One often uses a species with which

Table 1 Animal models that predicted adverse effects of xenobiotics in humans

Agent	*Effect*	*Animal species*	*In human*
Thalidomide	Phocomelia	Rat	No/yes
Accutane	Developmental toxicity of CNS (neural tube defects)	Rat, rabbit, dog, primate	Yes
AZT	Bone marrow depression	Dog, rat, monkey	Yes
Valproic acid	Cleft palate	Rat, mouse, rabbit	Yes
Cyclosporine	Nephropathy, reversible immune response suppression (essential aid to organ transplantation)	Rat, monkey	Yes
Benoxaprofen (Oraflex)	Hepatotoxicity,	No	Yes
	photosensitivity	Guinea pig	Yes
Zomepirac (Zomax)	Anaphylactic shock	No	Yes
MPTP	Parkinsonism	Monkey	Yes
Cyclophosphamide	Hemorrhagic cystitis	Rat, dog	Yes
Mercury	Encephalopathy	Rat, monkey	Yes
Diethylene glycol	Nephropathy	Rat, dog	Yes
Razoxin	Myelomonocytic leukemia	Mouse	Yes

one is most familiar, with little consideration as to whether the chosen species is actually the most appropriate for the problem at hand. For example, the rat is probably a poor model for studying the chronic toxicity of any new nonsteroidal antiinflammatory drug (NSAID) because the acute gastrointestinal toxicity will probably mask any other toxic effects. The guinea pig is less sensitive to most NSAIDs than the rat and closer in sensitivity to humans and thus would be a more appropriate species for investigating the chronic (nongastrointestinal) toxicity of an NSAID. This practice of not rationally choosing an appropriate species for an experiment undoubtedly results in questionable science. This alone should be considered a waste of animals and resources. It results also in additional, and sometimes duplicative, experiments.

Research into replacements for test animals, such as cellular cultures, organs harvested from slaughterhouses, *in silico* (computer) modeling, and physical/chemical systems, has been extensive. While each of these has their utility, they will not replace animals for the foreseeable future. Some degree of animal use will continue, and the future is bright for the ongoing development, refinement, and usage of animal models, for example, building on the quite recent development of transgenic and knockout models for research on various diseases to go along with xenograft and other types of animal models.

See also: Analytical Toxicology; *In Vitro* Test; *In Vivo* Test; Society of Toxicology; Thalidomide; Toxicity Testing, Alternatives; Toxicity Testing, Aquatic; Toxicity Testing, Behavioral; Toxicity Testing, Carcinogenesis; Toxicity Testing, Dermal; Toxicity Testing, Developmental; Toxicity Testing, Inhalation; Toxicity Testing, Irritation; Toxicity Testing, Modeling.

Further Reading

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Relevant Websites

http://dels.nas.edu – US National Academy of Sciences, Institute of Laboratory Animal Research (ILAR). Animal Models & Strains Search Engine.

http://www.toxicology.org – Society of Toxicology, Animals in Research.

Animal Testing Alternatives *See* Toxicity Testing, Alternatives.

Animals, Poisonous and Venomous

Teresa Dodd-Butera and Molly Broderick

This article provides an overview of some of the more commonly encountered terrestrial animals that produce toxins, often referred to as venoms, with a focus on spiders, snakes, and other reptiles.

Arthropods

Phylum Arthropoda is the largest phylum in the animal kingdom. Most of the species are nontoxic. However, Class Arachnida contains spiders. Arachnidism means envenomation from a spider. Most spiders are venomous; however, the black widow, brown recluse, and hobo spiders are responsible for a significant number of toxicity events in humans, so these will be discussed in more detail.

Black Widow Spider

Lactrodectus mactans (lactrodectism is produced by a bite from the female spider). The female is larger than the male. It is noted for a black color that is shiny, with a rounded abdomen and a red hourglass mark on the ventral surface. The black widow spider produces neurotoxic venom. Alpha latrotoxin is the protein of the neurotoxin.

Exposure is usually through a painful bite, although the bite may occasionally go unnoticed until symptoms develop. The mechanism of action of black widow spider venom involves binding of the gangliosides and glycoproteins of the motor end plate in the neuromuscular junction, which affects the opening of sodium channels and the release of acetylcholine (Ach) and norepinephrine. This results in excessive stimulation and allows for penetration and circulation of the venom into the lymphatic system.

Red spots, a slight local reaction, and a 'target lesion' may appear at the site. Symptoms may occur 15 min to several hours after envenomation. Depending on the severity, symptoms may last for several days. Milder symptoms may last for 1–2 weeks. Skeletal muscle cramps are noteworthy for envenomations and may produce a tightness and pain in the chest and abdomen. 'Facies lactrodectismica' is characterized by unique facial sweating and grimacing. Other symptoms may include nausea, tremor, weakness, joint pain, seizures, hypertension, hyperreflexia, extreme restlessness, and priapism (rare). Death has occurred due to seizures and respiratory difficulty. Sensitive populations include the very old and the very young, pregnant women, and those with chronic illnesses. Medical attention should be sought if a bite occurs, particularly in members of these high-risk groups.

Treatment is primarily symptomatic and supportive for a black widow spider bite. Hypertension and severe pain may be present. Pain control may require over-the-counter antiinflammatory agents, or may be severe enough to warrant parenteral opioids. Diazepam may be used for sedation and as an anxiolytic. Effective results have been achieved using lactrodectus antivenin; however, anaphylaxis and serum sickness have been reported. The antivenin may be needed if severe pain and hypertension are refractory to treatment. However, caution must be exercised in individuals allergic to horse serum products. Monitoring the site of the wound for proper healing and tetanus prophylaxis are also important in treatment of these envenomations. Patients should be instructed on using precaution and prevention measures to avoid further envenomations. These may include avoidance of a particular area where the black widow spiders are, or in some instances, careful spraying of areas with creosote may be necessary. Wearing protective clothing may also prevent further bites, if high-risk areas cannot be avoided.

Brown Recluse Spider

Loxosceles reclusa; Violin or Fiddleback Spider (loxoscelism is a systemic syndrome due to the bite of a female brown recluse spider). This is a small reddish brown spider with a violin-shaped mark on the dorsum surface of the cephalothorax. It is generally found in the southern United States. Exposure is through a bite, which may go unnoticed. Spiders may be found in woodpiles and basements.

Loxosceles venom contains hyaluronidase, alkaline phosphatase, 5-ribonucleotide phosphohydrolase, and sphingomyelinase D. Sphingomyelinase D is a component of the cytotoxic venom, with a MW of 32 000 Da. Sphingomyelinase causes release of choline and *N*-acylsphingosine phosphate from the red blood cell membrane, which stimulates platelet aggregation and dermonecrosis.

Initially, a local lesion and swelling may appear at the site of the bite. A blister may appear with worsening pain. Ulceration with delayed healing may occur. Systemic symptoms manifest as high fever, weakness, nausea, vomiting, arthralgias, jaundice, abnormal bleeding, and rashes. Left untreated, life-threatening reactions may progress from hemolytic anemia, thrombocytopenia, and disseminated intravascular coagulation (DIC). Hemolysis may lead to shock, renal failure, and death (rare). Multiple organs may be affected including lungs, heart, pancreas, and liver.

Most of these spider bites do not result in serious toxicity and are managed without medical intervention. However, when symptoms do occur, treatment of both the wound and systemic toxicity are required. Keeping the bite area clean is necessary to avoid secondary infection and further tissue damage. There is no proven preventive measure to avoid dermonecrosis; and surgical excision may delay wound healing. However, surgery may be indicated with abscess formation. Corrective surgery, in severe cases, may be needed for skin grafts or debridement. Systemic reactions require hospitalization in order to provide aggressive supportive care for multiple organ involvement. Patients who present with mild symptoms immediately after a bite with a suspicion of *Loxosceles*, may be monitored as outpatients with adequate medical follow-up. Young children, the elderly, and chronically ill patients need to be monitored carefully, after envenomation from these spiders. Prevention is difficult, but shaking out items carefully and avoiding areas where *Loxosceles* reside may be helpful.

Hobo Spider

Tegenaria agrestis (hobo spiders) are found in Europe and the Pacific Northwestern United States. *T. agrestis* is brown with gray markings and $\sim$10 mm in length. Hobo spiders are found in woodpiles, basements, and moist areas.

Envenomation from a painless bite by *T. agrestis* may result in necrosis, similar to *L. reclusa*. Unlike the previous spiders discussed, male Hobo spiders are more venomous than females. Local symptoms of blistering may occur after envenomation. The blister may rupture and create a necrotic ulcer. Scarring and healing range from 1 month to 3 years. Systemic symptoms may include headache, nausea, intractable

vomiting, profuse diarrhea, weakness, impaired vision, memory loss, pancytopenia, and death.

Treatment is symptomatic and supportive for wound care (similar to loxoscelism) and hematologic complications. Surgical graft repair for severe ulcerative lesions may be warranted.

Scorpions

Scorpion stings occur most commonly in the southwestern United States. There is a range of symptoms which may occur, but children under 6 are at higher risk for mortality. Poisonous scorpions in the United States are *Centruroides sculpturatus* (exilicauda) and *Centruroides gertschii*. Toxin from a sting consists of phospholipase, acetylcholinesterase, hyaluronidase, serotonin, and neurotoxins. Venom of the *Centruroides* genus may cause neurotoxicity. Sodium channels are affected with prolonged action potentials. There are also scorpions outside of the United States that may cause hemorrhaging to a victim. Symptoms may include pain, numbness, restlessness, shaking movements, blurred vision, slurred speech, and respiratory collapse. Local wound care is the most common type of treatment required, but severe toxicity requires hospitalization. Therapy is symptomatic and supportive, dependent on the effects from the specific scorpion toxin. Prevention strategies include shaking out shoes, sleeping bags, and tents, and careful attention when in an area of scorpions, particularly at night.

Hymenoptera: Bees, Wasps, Hornets, and Yellow Jackets

Bites and stings from this subclass may cause toxic and allergic reactions, in numbers greater than those from poisonous snakes. Hymenoptera venom is a combination of biogenic amines, phospholipase, hyaluronidase, and contains other various substances depending on the particular species. Symptoms usually involve local swelling and pain without a systemic reaction. Swelling of the upper airway is a hazard, but is a rare occurrence with one sting. The danger arises when multiple stings occur to the victim, and large numbers have been fatal. An anaphylactic reaction may occur shortly after a sting, in sensitive individuals. Fatalities can occur within minutes. Treatment is symptomatic and supportive for life support and care of the local area of the bite, depending on the severity of the symptoms. Avoidance, if possible, and emergency epinephrine kits for sensitive individuals can be helpful prevention measures.

Snakes

Snakes belong to the phylum Chordata, Class Reptilia. Two major families of venomous snakes are Crotalidae and Elapidae.

Crotalids

Three genera of crotalids are *Crotalus* (Rattlesnake), *Sistrurus* (Massasauguas or pigmy rattlesnakes), and *Agkistrodon* (Copperhead and Cottonmouth). Crotalids ('Pit Vipers') have triangular heads, elliptical pupils, a single row of subcaudal scales behind the anal plate, and facial pits which serve as heat sensors. Crotalids have hinged front fangs ~ 2 cm in length, which are curved and hollowed. Rattlesnakes usually have a rattle – keratin scales at the end of the tail that produce a rattling sound when rubbed together. Venom glands are located posterior to the eyes and connected to fangs by venom ducts. Identifiable characteristics of copperheads are the rust-colored heads, and a white buccal cavity is noteworthy of cottonmouths or 'water moccasins'.

Envenomation from a crotalid bite leaves one or more puncture wounds with a potential for progressive edema and ecchymosis. Crotalid venom contains a mixture of proteins, lipids, and metals. The venom forms fibrin polymers, which are susceptible to normal fibrinolysis and phagocytosis. It is represented by falling fibrinogen levels. Copperhead venom has a weak effect on this series of events in coagulation, resulting in lower morbidity after envenomation.

Initial pain at the site of the bite may be followed with a 'metallic sensation' in the mouth. Victims may become weak, and experience nausea, diarrhea, diaphoresis, and chills. Edema may begin around the bite area or may be delayed. Observation of the site for edema is a clue as to whether or not a 'dry bite' has occurred; that is, that no venom was injected into the site. Envenomation is most serious if venom is injected directly into joints, muscles, or veins. Hemorrhagic blisters and tissue destruction are possible. Neurotoxicity from rattlesnakes (but generally not from cottonmouths or copperheads) may be manifested as fasciculations, which are fine continuous contractions. In some cases, systemic neurotoxicity may involve respiratory failure. In the most serious cases, massive envenomation may lead to serious bleeding, hypotension, shock, multiple organ failure, and a high incidence of mortality.

Despite popular belief, crotalid envenomation does not generally result in life-threatening symptoms. Maintaining a patent airway, intravenous access, clinical observation of edema and the bite area, adequate laboratory work, and the use of antivenin

when necessary are the essentials of treatment in snakebite envenomation. Antivenin should only be used in moderate to severe envenomations, usually within 8 h postenvenomation. This is an equine-derived product and thus skin testing for sensitivity is usually performed after the decision that antivenin is necessary has been made. Serum sickness may occur from the antivenin. Hospital monitoring, wound care, and patient follow-up are important for the recovery of these patients.

Elapidae

This family includes coral snakes, cobras, mambas, and kraits. In the United States, Elapidae are responsible for 1–2% of poisonous snakebites. The incidence of envenomations is greater in some other parts of the world. Examples of coral snakes commonly found in the United States are the eastern coral snake, the Sonoran coral snake, and the Texas coral snake. Coral snakes are smaller than pit vipers. They do not have facial pits, and the head is rounded, as are the pupils. Fangs are $\sim$2 mm and fixed to the jaw. Coral snakes are also more brightly colored, with bands of black and red, separated by yellow and white bands. Coral snakes are timid, nocturnal creatures.

Envenomation from a coral snake exerts minimal local pain, and appears as rows of teeth marks. Victims may report that the snake was 'chewing' on the bite site and had to be forcibly removed. Coral snake venom is composed of peptides and enzymes that have not all been identified, but which exert neurotoxicity rather than cytotoxicity.

Minimal local pain may be present initially; however, systemic toxicity may be delayed for several hours. Nausea, vomiting, weakness, dizziness, and numbness have been reported. Drowsiness or euphoria may occur. Central and peripheral nervous system effects and paralysis may be quite serious, but do not always occur. Life support measures should be instituted, as necessary, with stabilization of vital signs, and evaluation of pulmonary and neurological symptoms. Antivenin may be given to a patient, based on history and circumstances of the bite. Skin testing for sensitivity to horse serum is performed when that decision is made. Patients who are treated with the antivenin need to be monitored for serum sickness. In addition, monitoring the patient for respiratory symptoms, wound, and skin infections should continue. Victims may take weeks to recover, if paralysis has occurred. Long-term prognosis is generally good for these patients.

Other Reptiles

Venomous Lizards

The Gila monster (*Heloderma suspectum*) and the beaded lizard (*Heloderma horridum*), Order Squamata, are found in desert areas; for example, in the southwestern United States and Mexico. They are large, timid, nocturnal creatures. Beaded lizard bites are rarely encountered. In fact, bites of both types of lizards are uncommon; however, Gila monsters are known for their tenacity when they do bite. They may need to be forcibly disengaged from their victims. Gila monster venom contains enzymes, hyaluronidase, phospholipase A, and serotonin, in addition to other toxins. Envenomation does not always occur with bites. Symptoms following envenomation include pain, swelling, and possible anaphylactic reactions. Symptomatic and supportive care for the victim is the treatment plan.

See also: Diazepam; Marine Organisms; Scorpions; Snakes.

Further Reading

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Relevant Website

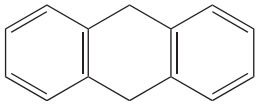
http://www.calpoison.org

Antagonism *See* Chemical Interactions.

Anthracene

Prathibha S Rao

- Chemical Abstracts Service Registry Number: CAS 120-12-7
- Synonyms: Anthracin; Paranaphthalene; Green oil; Tetra oil N2G
- Chemical/Pharmaceutical/Other Class: Polycyclic aromatic hydrocarbon
- Chemical Structure:



Background Information

Anthracene is a solid white to yellow crystal, has a weak aromatic odor, and sinks in water. Its characteristics are boiling point, 342°C; melting point, 218°C; molecular weight, 178.22; density/specific gravity, 1.25 at 27 and 4°C; octanol–water coefficient, 4.45. It is soluble in absolute alcohol and organic solvents. Maximum absorption occurs at 218 nm.

Exposure Routes and Pathways

Inhalation is the primary exposure pathway. Natural occurring sources include a high boiling fraction of coal tar, consisting of anthracene, phenanthrene, and other solid hydrocarbons as well as acridine. Other sources include volcanoes and forest fires. Artificial sources include exhaust from motor vehicles and other gasoline and diesel engines; cigarette, marijuana, and cigar smoke; emissions from coal-, oil-, and wood-burning stoves, furnaces and power plants; smoke and soot. Air pollution sources include coke oven emissions, space heating installation burning, emissions from typical European gasoline engines, dielectric in the manufacture of battery electrodes, and electric arc furnace electrodes; felt, roof, and paper manufacturing; and alumina reduction.

Toxicokinetics

Polycyclic aromatic hydrocarbons were detected in human fat and liver and their average concentrations were 1100 and 380 ppt, respectively. Anthracene was found at high levels in the liver and fat. When administered orally to animals 70–80% of the dose is excreted unchanged in the feces but metabolites present in rat urine include *N*-acetyl-*S*-(1,2-dihydro-2-hydroxy-1-anthryl)-cysteine and conjugates of *trans*-1,2-dihydroanthracene-1,2-diol and 1,2-dihydroxyanthracene. The cysteine conjugate is decomposed by mineral acids to yield 1-anthrylmercapturic acid, 1- and 2-anthrols, and anthracene. Rats metabolize anthracene into *trans*-9,10-dihydroanthracene-9,10-diol, which gives rise to anthrone and several hydroxylated metabolites.

Acute and Short-Term Toxicity (or Exposure)

Human

Anthracene is photosensitizing. It can cause acute dermatitis with symptoms of burning, itching, and edema, which are more pronounced in the exposed bare skin regions. Other symptoms are lacrimation, photophobia, edema of the eyelids, and conjunctival hyperemia. The acute symptoms disappear within several days after cessation of contact. Systemic effects of industrial anthracene manifest themselves by headache, nausea, loss of appetite, slow reactions, and adynamia.

Chronic Toxicity (or Exposure)

Animal

Anthracene showed no mutagenic activity in *Salmonella thyphimurium* TA100 and TA98 with and without addition of rat liver microsomes (S9) and no carcinogenic activity in Swiss albino mice. A significant increase in the formation of nonneoplastic melanotic tumors was observed among first- and second-generation progeny of *Drosophila melanogaster* that had been exposed chronically as larvae to low concentrations of anthracene.

Human

Chronic exposure may lead to inflammation of the gastrointestinal tract, patchy areas of increased yellow–brown pigment changes, loss of skin pigment, thinning or patchy thickening of skin, skin warts, skin cancer, and pimples. Repeated breathing of 'fumes', especially from heated anthracene, may cause a chronic bronchitis with cough and phlegm. Repeated exposure of male scrotum can cause skin thinning and increased skin pigmentation.

No occupational exposure limits have been established for anthracene. However, safe work practices should always be followed.

Clinical Management

No specific treatments have been prescribed. The patient should be moved to fresh air in case of respiratory distress.

See also: Coke Oven Emissions; Polycyclic Aromatic Hydrocarbons (PAHs).

Relevant Websites

http://www.speclab.com – Spectrum Laboratories Inc.
http://www.state.nj.us – State of New Jersey.
http://www.1.nature.nps.gov – National Park Service Nature & Science.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Anthracene.
http://rais.ornl.gov – The Risk Assessment Information System.

Anthrax

Kartik Shankar and Harihara M Mehendale

Epidemiology

Anthrax is a zoonotic disease with worldwide distribution. Anthrax is caused by *Bacillus anthracis*, a gram-positive, spore-forming, rod-shaped bacterium that primarily infects herbivores such as cattle and deer. The earliest known description of anthrax is found in the Book of *Genesis*, in which the fifth plague is said to have killed Egyptian cattle. Further, there are numerous descriptions of anthrax in animals and humans in Hindu, Greek, and Roman literature. Between 20 000 and 100 000 cases of anthrax have been estimated to occur worldwide. Human anthrax is most common in enzootic areas in developing countries, among people who work with livestock, eat undercooked meat from infected animals, or work in establishments where wool, goatskins, and pelts are stored and processed. West Africa is the most affected part in the world. Anthrax is also a significant problem in other parts of Africa, Central America, Spain, Greece, Turkey, and the Middle East. In economically advanced countries, where animal anthrax is controlled, incidence in humans is rare. Further infections have been dramatically reduced by the vaccination of high-risk individuals and improvements in industrial hygiene. Incidence in the United States declined to less than one case per year till the recent biological terrorism attacks in the fall of 2001.

Microbiology

B. anthracis is nonmotile, catalase positive, nonhemolytic on blood agar, and frequently occurs in long chains. Chains of virulent forms are surrounded by a capsule. Sporulation occurs in soil and on culture media but not in living tissue. Spores are highly resistant to UV light, high-temperature extremes, high pH, drying, high salinity levels, and routine methods of disinfection.

Mechanism of Toxicity

Anthrax toxin is composed of three proteins: protective antigen (PA; 83 kDa), lethal factor (LF; 90 kDa), and edema factor (EF; 89 kDa). Individually, none of the three proteins are toxic but interact synergistically with at least one of the others. PA and LF (called LeTx) can cause lethal shock in experimental animals, and a mixture of PA and EF (edema toxin, EdTx) induces edema at the site of injection. Since two discrete units of the toxin are required for its action, the term binary toxin has been used to this and other bacterial toxins. Anthrax is unique from other binary toxins in that the binary moieties (EF and LF) interact only after being secreted from the bacteria. Further, EF and LF enter the cell via a single PA protein. Assembly of the three toxin proteins is initiated when PA binds to a proteinaceous cellular receptor and is activated by a member of the furin family of cellular proteases. The exact mechanisms of internalization of the toxin moieties are subject of scientific enquiry. Inside the cellular cytoplasm, EF (a calcium and calmodulin-dependent adenylate cyclase) causes a dramatic increase in intracellular cAMP concentrations and LF acts proteolytically to cleave certain MAPK kinases.

Clinical Forms of Anthrax

Anthrax mainly occurs in three forms: cutaneous, inhalation, and gastrointestinal. Exposure to *B. anthracis* most likely in an occupational setting is the cause of cutaneous anthrax. The incubation period varies from 1 to 12 days. In most cases, the disease remains localized to the skin lesion. Major diagnostic characteristic is the development of edema around the

lesion. The fatality rate is 20% without and less than 1% with antibiotic treatment. Inhalation anthrax is the most lethal form of anthrax resulting from inhalation of pathogenic endospores. The US Department of Defense estimates that the lethal dose in humans is ~8000–10 000 spores. The illness is biphasic after exposure to large numbers of spores. The first phase is characterized by a 'flu-like' illness with nonproductive cough. After several days of apparent improvement there is a sudden onset of rapidly progressive respiratory failure, acute dyspnea, circulatory collapse, and pleural effusion. The mortality rate is very high, is spite of supportive care and antibiotics, generally within 24 h of the onset of the second stage due to toxemia and suffocation. Gastrointestinal anthrax, although rare, occurs after an incubation period of 1–7 days following ingestions of *B. anthracis* via contaminated food or drink. Mortality rates are estimated to be between 25% and 60% unless treatment is begun early enough. Severe abdominal pain, fever, nausea, vomiting, and bloody diarrhea are manifested during the disease. Death occurs due to toxemia and shock.

Clinical Management

Prompt clinical diagnosis and treatment with effective antimicrobial drugs is necessary for successful treatment of anthrax. Although *B. anthracis* is susceptible to penicillin *in vitro* it is not used as monotherapy. Several historical strains produce an inducible β-lactamase and are resistant to penicillin. Ciprofloxacin (400 mg intravenously twice daily) and possibly other quinolones or doxycycline (200 mg intravenously twice daily) should be used as initial therapy. The duration of treatment for inhalation anthrax should be 60 days. Corticosteroid therapy should be considered for patients with inhalation anthrax associated with meningitis or severe edema. Supportive therapy should be initiated to prevent shock, fluid and electrolyte imbalance, and loss of airway patency.

Potential for Use as a Biological Weapon

Anthrax is classified as a category A biological weapon (most dangerous) along with smallpox, plague, *Clostridium botulinum* toxins, filovoviruses, etc. *B. anthracis* has several biological, technical, and virulence characteristics that make it an attractive bioweapon. These include easy procurement from a variety of sources and relative ease to grow, process, and store. The World Health Organization estimates that 50 kg of weapon-grade anthrax spores released by an aircraft over an urban population of 5 million would result in 250 000 cases of mainly inhalation anthrax.

See also: Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents; Botulinum Toxin; Chemical Warfare Agents.

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Anticholinergics

Swarupa G Kulkarni and Harihara M Mehendale

- SYNONYMS: Parasympatholytics; Cholinergic blockers; Sympatholytics; Antispasmodics

Uses

Anticholinergics have a wide range of therapeutic uses: prior to anesthesia, as a prophylactic for preventing motion sickness, in symptomatic control of Parkinson's disease, in abnormal slowing of the heart in poisoning with organophosphates and other cholinergic drugs, and in the treatment of peptic ulcer and irritable bowel syndrome.

Since these agents act as smooth muscle relaxants they are used as antispasmodics and may be used to reduce spasms of the stomach. Antimuscarinics appear to be useful in the treatment of gastrointestinal hypersecretory states (e.g., Zollinger Ellison syndrome) and may be used in conjunction with an H_2 receptor antagonist. In addition to atropine, belladonna, and other semisynthetic derivatives, a number of other synthetic compounds are also used in gastrointestinal disorders. These compounds consist of a large blocking group linked by a short chain to a strongly basic tertiary or quaternary group. Synthetic drugs used as antispasmodics or antisecretory agents in gastrointestinal disorders include oxyphenonium bromide, isopropamide iodide, mepenzolate bromide, and dicyclomine.

Antimuscarinics are potent bronchodilators and are used in the treatment of chronic bronchitis and asthma.

Toxicokinetics

Antimuscarinics having a quaternary ammonium group are incompletely absorbed from the gut since these are completely ionized. The tertiary amine antimuscarinics are readily absorbed from the gut. The presence of food may reduce absorption. Quaternary ammonium antimuscarinics exhibit poor lipid solubility, do not cross the blood–brain barrier, and thus exhibit minimal central nervous system (CNS) effects. Also due to their poor lipid solubility they do not penetrate the eye and are unlikely to appear in the milk. Atropine and other tertiary amines are capable of crossing the CNS. Atropine is capable of crossing the placenta and has been stated to distribute into milk in small quantities. It is oxidized primarily in the liver. Atropine is apparently metabolized in the liver to tropic acid, tropine, and possibly esters of tropic acid and glucuronide conjugate.

Antimuscarinics are mainly eliminated in urine as unchanged drug and its metabolites. Following oral administration substantial amounts of antimuscarinics may be eliminated in feces as unabsorbed drug.

Mechanism of Toxicity

Antimuscarinics competitively inhibit the action of acetylcholine or other cholinergic stimuli at the muscarinic receptor. At usual doses these have little or no effect on the cholinergic stimuli at nicotinic receptors. Autonomic ganglia, where cholinergic transmission involves nicotinic receptors, produce a partial cholinergic block at relatively high doses. Receptors at various sites are not equally sensitive to inhibitory effects of antimuscarinics. Atropine acts by competitive antagonism at the receptor sites of the effector organs. It may also inhibit responses to histamine, serotonin, and norepinephrine and may block transmission at the autonomic ganglia and the skeletal neuroeffector junction.

Acute and Short-Term Toxicity (or Exposure)

Human

Single, 10 mg oral doses of atropine have produced signs of acute toxicity in adults. Children are more susceptible than adults to the toxic effects of atropine. Deaths have been reported in children following ingestion of 10 mg of atropine.

Acute overdosage with antimuscarinics produces both peripheral and CNS symptomatology. The quaternary ammonium compounds do not readily penetrate the CNS and thus exhibit minimal central effects even at toxic doses. Patients with anticholinergic toxicity will typically show peripheral symptoms including dry mouth, thirst, fixed dilated pupils, flushed face, fever, hot, dry, red skin, urinary retention, hyperthermia, hypotension, tachycardia, and increased respiratory rate. In addition to tachycardia, cardiac manifestations may include EKG abnormalities similar to those produced by quinidine. Speech and swallowing may be impaired in association with blurred vision. Other peripheral signs and symptoms may include nausea and vomiting.

In large doses, atropine induces stimulation of the CNS, which in humans is characterized by overactive coordinated movements, hallucinations, and delirium. After the stimulation has lasted for some time, depression sets in and may proceed to complete paralysis of the CNS, which is fatal through cessation of respiration. In infants, particularly those ingesting antihistamines, paradoxical excitement may occur subsequently followed by a more characteristic CNS depression. CNS manifestations may resemble acute psychosis characterized by incoherence, confusion, hallucinations, delusions, paranoia, and abnormal motor behavior.

In severe overdosage, CNS depression, circulatory collapse, and hypotension may occur. Coma and skeletal muscle paralysis may also occur followed by death due to respiratory failure. Acute overdosage with quaternary ammonium antimuscarinics may produce a curariform neuromuscular block and ganglionic blockade manifested as respiratory paralysis.

Clinical Management

Immediate treatment should include instituting emesis, with syrup of ipecac or gastric lavage, followed by administration of activated charcoal and saline cathartics if the patient is not comatose. Induced emesis may be ineffective in ingestion of antihistaminics related to phenothiazines or in massive ingestion. The use of physostigmine should generally be reserved for treatment of patients with extreme delirium or agitation. Physostigmine in a dose of 0.5–2 mg administered intravenously, which can be repeated every 30 min as needed, may be used to alleviate symptoms like confusion, agitation, or coma. Other cholinergic antagonists have not been useful since they do not cross the blood–brain barrier. Measures such as forced diuresis and dialysis have not yet been shown to be effective.

Fluid therapy and other standard treatments of shock should be administered as needed.

Miscellaneous

As a class, anticholinergics include the antihistamines, atropine and homatropine; anti-Parkinsonian agents like benzotropine, procyclidine, and trihexyphenidyl; the antimuscarinics of which atropine is the prototype; and antispasmodics like dicyclomine and oxybutymin. Most antimuscarinics are aminoalcohols or their derivatives (usually esters or ethers), aminoamides, or other amines. Antimuscarinics can be divided into two groups. These are the naturally occurring alkaloids and their semisynthetic derivatives like atropine, homatropine, scopolamine, and hyoscyamine and the synthetic amine compounds such as anisotropine, dicyclomine, and ipratropium.

See also: Cholinesterase Inhibition; Gastrointestinal System; Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates.

Further Reading

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Scarpero HM and Dmochowski RR (2003) Muscarinic receptors: What we know. *Current Urology Reports* 4: 421–428.

Relevant Website

http://bnf.org – British National Formulary: Antimuscarinic.

Antimony

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst, Shirley B Radding, and Kathryn A Wurzel, volume 1, pp. 76–77, © 1998, Elsevier Inc.

- Representative Compounds: SbH_3 (Stibine); Antimony trioxide
- Chemical Abstracts Service Registry Number: CAS 7440-36-0
- Synonym: Stiblum
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Sb^{3+}; Sb^{5+}

Uses

Antimony is used in white metal, which is any of a group of alloys having relatively low melting points. White metal usually contains tin, lead, or antimony as the chief component (e.g., the alloys Britannia and Babbitt). Antimony is used as a hardening alloy for lead, especially in storage batteries and cables, bearing metal, type metal, solder, collapsible tubes and foil, sheet and pipe, semiconductor technology, and pyrotechnics. It is also used in thermoelectric piles, and for blackening iron or coatings. Antimony-containing compounds are used in materials for refrigerators, air conditioners, aerosol sprays, paints, and flameproofing agents. Approximately half of the antimony used in the United States is recovered from lead-based battery scrap. Antimony is also used medicinally (e.g., antimony potassium tartrate as an emetic and antimony as an antiparasitic agent).

Exposure Routes and Pathways

The emission of antimony into the human environment appears to be the result of human activity, with the emission of antimony trioxide being the most significant source. Antimony trioxide is emitted as a result of coal burning, or with fly ash when antimony-containing ores are smelted. In addition, medicines containing antimony are administered orally. Antimony is present in food and drinking water is present mostly in the low μg/kg-wet weight range or less, including vegetables grown on Sb-contaminated soils. Daily oral uptake of Sb ranges from 10 to $70\,\mu g\,day^{-1}$ and appears to be significantly higher than exposure by inhalation.

Toxicokinetics

Normally, antimony is absorbed slowly when ingested or administered orally. Many antimony compounds are gastrointestinal irritants. The emetic antimony potassium tartrate is easily absorbed and, within 24 h, 50% is excreted in the urine (hamsters). Antimony can concentrate in lung tissue, the thyroid gland, the adrenal glands, the kidneys, and the liver. The trivalent compounds of antimony concentrate in the red blood cells and liver and the pentavalent compounds concentrate in the blood plasma. Both forms are excreted in feces and urine, but generally,

more trivalent compounds are excreted in urine and more pentavalent compounds in feces.

Presumably by reacting with the sulfhydryl groups, antimony can inhibit oxidative and phosphorylating enzymes like monoamine oxidase, succinoxidase, pyruvate brain oxidase, and phosphoftuctokinase. Inhibition of these enzymes can alter activities such as glucose metabolism and nerve transmission. Ten percent of the trivalent form is excreted by the kidney in 24 h; 50–60% of the pentavelent form is found in the urine within 24 h.

Mechanism of Toxicity

The toxicity of Sb is a function of the water solubility and the oxidation state of the Sb species under consideration. Antimony toxicity often parallels that of arsenic, although antimony salts are less readily absorbed than arsenic. It is presumed that antimony, like arsenic, complexes with sulfhydryl groups of essential enzymes and other proteins. By analogy, antimony can uncouple oxidative phosphorylation, which would inhibit the production of energy necessary for cellular functions. Antimony's trivalent compounds are more toxic than its pentavalent compounds.

Acute and Short-Term Toxicity (or Exposure)

Animal

The rat oral LD_{50} is $100\,mg\,kg^{-1}$. Antimony administered intravenously to experimental animals resulted in abnormal electrocardiograms.

Human

Accidental poisonings can result in acute toxicity, which produces vomiting and diarrhea. Most information regarding antimony toxicity has been obtained from industrial exposures. Occupational exposures usually occur through inhalation of dusts containing antimony compounds. Workers exposed to antimony trisulfide (used as a pigment and in match production) at concentrations greater than $3.0\,mg\,m^{-3}$ experienced heart complications and died. In addition, a temporary skin rash, called 'antimony spots', can occur in persons chronically exposed to antimony in the workplace. Inhalation of antimony hydride (stibine gas) can lead to hemolytic anemia, renal failure, and hematuria. Stibine gas is produced when antimony alloys are treated with acids.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to a dose level of $4.2\,mg\,m^{-3}$ airborne antimony trioxide dust for 1 year were reported to develop lung tumors; at a dose level of $1.6\,mg\,m^{-3}$, lung tumors were not found. Guinea pigs exposed to airborne antimony trioxide developed interstitial pneumonia. Oral feeding of antimony to rats does not induce an excess of tumors or teratogenesis. In rats it is a tumorigen, and high levels cause decreased red blood cell counts, hematocrit, hemoglobin levels, and plasma protein concentrations.

Human

Inhalation of antimony compounds produces different effects at different concentrations. Chronic inhalation of low concentrations causes rhinitis and irritation of the trachea. At high concentrations, acute pulmonary edema occurs, and bronchitis may occur (the bronchitis may lead to emphysema). Inhaled antimony concentrates in lung tissue; as a result, pneumoconiosis with obstructive lung disease has been recorded. Antimony is a suspected human carcinogen.

In Vitro Toxicity Data

The compounds $SbCl_3$ and $SbCl_5$ were reported to be genotoxic in the rec-assay with *B. subtilis*. Sb(III)acetate enhanced the Simian-Adenovirus-7-mediated transformation of SHE-cells, and enhanced rates of chromosomal breaks in human leukocytes were reported after treatment with potassium antimony tartarate (APT). $SbCl_3$ did not induce DNA/protein-crosslinks in V79-cells and peripheral human lymphocytes.

Clinical Management

The oil-soluble BAL (British anti-Lewisite; 2,3-dimercaptopropanol) administered intramuscularly appears to be the antidote of choice for antimony poisoning. The antidotal action of BAL depends on its ability to prevent or break the union between antimony and vital enzymes.

Ecotoxicology

Antimony is highly toxic to amphibians and zooplankton.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA) in the United States have the following airborne exposure limits:

- OSHA Standard: Permissible exposure limit – 8 h time-weighted average (TWA) = $0.5\,mg\,m^{-3}$.
- ACGIH threshold limit value: 8 h TWA = $0.5\,mg\,m^{-3}$ (antimony and compounds, as Sb). ACGIH classifies antimony as a suspected human carcinogen.

In the United States, antimony is listed as a Clean Air Act hazardous air pollutant generally known or suspected to cause serious health problems. Antimony and its compounds are listed as Clean Water Act toxic pollutants, subject to effluent limitations. The Federal Drinking Water Standards is $6\,\mu g\,l^{-1}$.

See also: Antimony Trioxide; Metals.

Further Reading

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.

Winship KA (1987) Toxicity of antimony and its compounds. *Adverse Drug Reactions and Acute Poisoning Reviews* 2: 67–90.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Antimony.

Antimony Trioxide

Shayne C Gad

Txcl.00081

- Chemical Abstracts Service Registry Number: CAS 1309-64-6
- Synonyms: Antimony white; Antimony oxide; Antox; Thermogrand B; ATO
- Chemical/Pharmaceutical/Other Class: Metal oxide
- Chemical Formula: Sb_2O_3

Uses

Antimony trioxide is used in flameproofing of textiles, paper, and plastics; as paint pigments, ceramic opacifier, catalyst, staining iron and copper; and as a mordant.

Exposure Routes and Pathways

Inhalation and oral routes from pottery glaze. The emission of antimony into the human environment appears to be the result of human activity, with the emission of antimony trioxide being the most significant source. Antimony trioxide is emitted as a result of coal burning or with fly ash when antimony-containing ores are smelted.

Toxicokinetics

Antimony trioxide is poorly absorbed orally and through the lungs. Trivalent antimony readily leaves the plasma but remains in the circulation bound to erythrocytes excreted in the bile after conjugation with glutathione.

Mechanism of Toxicity

The toxicity of antimony is a function of the water solubility and the oxidation state of the antimony species under consideration. It can react with red cell membrane and interfere with hemoglobin function. It has high affinity for sulfhydryl groups.

Acute and Short-Term Toxicity (or Exposure)

Animal

Antimony trioxide is a mild primary eye irritant in rabbits. Rat oral $LD_{50} > 34\,g\,kg^{-1}$. Mouse intraperitoneal $LD_{50} = 172\,mg\,kg^{-1}$. The *in vivo* genotoxicity of antimony trioxide was studied using single and repeat dose mouse bone marrow micronucleus tests, and the rat liver unscheduled DNA synthesis assay. All three studies were negative. In contrast, chromosomal damage by antimony trioxide was reported in mouse bone marrow cells after repeat dosing but not after single dosing. This discrepancy with respect to repeat dosing may be explained by the 'not specified

purity' and much higher systemic toxicity of the antimony trioxide sample used in one study; for this reason and because of the poor water solubility of antimony trioxide, it has been concluded that antimony trioxide was not genotoxic *in vivo*.

Human

Eye and respiratory irritation occur due to antimony exposure. Normal human serum levels of antimony should be from 0.05 to 0.50 mg dl^{-1}.

Chronic Toxicity (or Exposure)

Animal

Antimony trioxide is a mutagen in bacteria and human lymphocytes. Chronic exposure leads to reproductive and developmental effects. There was one experimental lifetime study of antimony trioxide with rats; however, this study was reported to have had many methodological shortcomings.

Human

The International Agency for Research on Cancer evaluated antimony trioxide and concluded that antimony trioxide was possibly carcinogenic to humans (group 2b) on the basis of the inhalation study in rats.

In Vitro Toxicity Data

Antimony trioxide was genotoxic in a number of older bacterial mutation assays but not in more recent studies. Positive results were observed with antimony trioxide in the *in vitro* cytogenetic assay with human lymphocytes and the sister chromatid exchange assay with V79-cells, but not in the L5178Y mutation assay.

Clinical Management

The oil-soluble BAL (British antilewisite; 2,3-dimercaptopropanol) administered intramuscularly appears to be the antidote of choice for antimony poisoning. The antidotal action of BAL depends on its ability to prevent or break the union between antimony and vital enzymes.

Environmental Fate

Antimony does not bioaccumulate so exposure to naturally occurring antimony through food is very small.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA) in the United States have the following airborne exposure limits:

- OSHA standard: permissible exposure limit (PEL): 8 h time-weighted average (TWA): 0.5 mg m^{-3}.
- ACGIH threshold limit value: 8 h TWA: 0.5 mg m^{-3} (antimony and compounds, as Sb). ACGIH classifies antimony as a suspected human carcinogen.

In the United States, antimony is listed as a Clean Air Act hazardous air pollutant generally known or suspected to cause serious health problems. Antimony and its compounds are listed as Clean Water Act toxic pollutants, subject to effluent limitations. The Federal Drinking Water Standard is 6 μg l^{-1}.

See also: Antimony; Metals.

Further Reading

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Anxiolytics

Swarupa G Kulkarni and Harihara M Mehendale

- SYNONYMS: Minor tranquilizers; Antianxiety drugs; Sedative-hypnotics; Benzodiazepines (BZDs)

Uses

Anxiolytics are used for preoperative relief of anxiety, for conscious sedation, as hypnotics in the treatment of insomnia, for short-term relief of symptoms of anxiety, or for the management of anxiety disorders. Benzodiazepines (BZDs) are also used for the

management of agitation associated with alcohol withdrawal, for their anticonvulsant properties, and as skeletal muscle relaxants. BZDs are preferred over barbiturates since these are less likely to produce tolerance and physical dependence and are remarkably safe in large suicidal doses.

Toxicokinetics

The BZDs meprobamate and buspirone are well absorbed from the gut. Plasma concentration of the BZDs and their metabolites exhibits considerable interpatient variation. Onset and duration of action varies depending on the BZD and the route of administration. BZDs are widely distributed into body tissues and cross the blood–brain barrier. Generally, BZDs and their metabolites cross the placenta. The concentration of diazepam in fetal circulation has been reported to be equal to or greater than the maternal plasma concentration. The drugs and their metabolites are distributed into milk. BZDs and their metabolites are highly bound to plasma proteins. Meprobamate is uniformly distributed throughout the body and is 20% bound to plasma proteins. It is capable of crossing the placenta and is distributed in milk. Buspirone is extensively distributed and is 95% bound to plasma proteins, mainly albumin. These are metabolized in the liver and may undergo conjugation. Meprobamate is metabolized to form the 2β-hydroxymeprobamate and glucosyluronide and glucuronide conjugate of meprobamate. Buspirone is metabolized in the liver, mainly via oxidation, to form the hydroxylated metabolites, which may further undergo conjugation. BZD metabolites are excreted principally in urine. Meprobamate is excreted mainly via the urine. Buspirone is excreted principally in the urine and to a lesser extent in feces.

Mechanism of Toxicity

The advantage of using BZDs is that they have a larger therapeutic index. The exact sites and mode of action of the BZDs have not been elucidated. However, their effects seem to be mediated through the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Allosteric interaction of central BZD receptors with $GABA_A$ receptors and subsequent opening of chloride channels are involved in eliciting the central nervous system (CNS) effects of the drugs. These drugs appear to act at the limbic, thalamic, and hypothalamic levels of the CNS. In usual doses, BZDs appear to have very little effect on the autonomic nervous system, respiration, or the cardiovascular system. These do not produce extrapyramidal side effects or interfere with the autonomic nervous system function. The mechanism of action of meprobamate is unknown. The mechanism of action of buspirone probably involves several neurotransmitter systems.

Acute and Short-Term Toxicity (or Exposure)

Human

The BZDs have a low order of toxicity unless ingested with other CNS depressants. Deep coma is rare. The BZDs have been known to cause dose-dependent adverse CNS effects. BZD overdosage may result in somnolence, impaired coordination, slurred speech, confusion, coma, and diminished reflexes. Hypotension, seizures, respiratory depression, and apnea may also occur. Although cardiac arrest has been reported, death from overdosage of BZDs in the absence of concurrent ingestion of alcohol and other CNS depressants is rare.

BZDs should be avoided during the first trimester and at delivery. Malformation and CNS dysfunction have been described in infants born of mothers using BZDs during pregnancy. Both animal data and human epidemiological studies suggest that BZDs are teratogens.

Severe anaphylactic reactions following intravenous administration of diazepam have been reported. Meprobamate causes toxicity similar to that of a barbiturate overdosage. Death may result from respiratory failure or hypotension. Limited information is available about the acute toxicity of buspirone. Effects are merely extensions of pharmacological effects. Nausea, vomiting, dizziness, drowsiness, miosis, and gastric distention may be seen.

Chronic Toxicity (or Exposure)

Human

Tolerance and psychologic and physical dependence may occur following prolonged use of BZDs. Such effects may occur following short-term use of BZDs particularly at high doses. Drowsiness, ataxia, slurred speech, and vertigo may be seen on dependence. Withdrawal symptoms, including anxiety, agitation, tension, dysphoria, anorexia, insomnia, sweating, blurred vision, irritability, tremors, and hallucinations, may be seen. Milder withdrawal symptoms such as insomnia have also been reported. Since some BZDs and their metabolites have long elimination half-lives, withdrawal symptoms may not occur until several days after the drug has been discontinued.

Meprobamate causes physical dependence similar to that seen with barbiturate dependence. No physical dependence on buspirone administration has been seen.

Clinical Management

Emesis is not recommended following an overdosage of BZD because of the potential of CNS depression. Gastric lavage soon after ingestion and activated charcoal/cathartic may be administered. Pulse, respiration, and blood pressure should be monitored and the patient should be closely observed. Intravenous fluids should be administered and adequate airway maintained. Hypotension may be controlled, if necessary, by intravenous administration of norepinephrine or metaraminol. Although some manufacturers recommend use of caffeine and sodium benzoate to combat CNS depression, most authorities believe that caffeine and other analeptic agents should not be used. Flumazenil (BZD antagonist) may be used in treatment. Flumazenil is an adjunct to and not a substitute for appropriate supportive and symptomatic therapy. Flumazenil (0.2–3 mg) intravenously in 0.2 to 0.3 mg increments for BZD overdose in adults and 0.2–1 mg intravenously in 0.2 to 0.3 mg increments for reversal of BZD sedation in adults may be used. Gradual dosage tapering is required. Occasionally temporary reinstitution of BZD therapy to suppress withdrawal symptoms may be necessary. Initial withdrawal symptoms may be managed with phenobarbitone or diazepam, followed by decreasing the dose by $\sim$10% per day of the initial dose required to control symptoms. Treatment of BZD physical dependence consists of cautious and gradual withdrawal of the drug using a dosage tapering schedule. In the case of meprobamate toxicity general supportive therapy should be maintained. Forced diuresis may be beneficial. In the case of withdrawal symptoms, the patient may be stabilized on phenobarbitone, which is then withdrawn over 10–14 days. No specific antidote is available for the treatment of an overdosage of buspirone and treatment involves symptomatic and supportive care.

Miscellaneous

This class of compounds includes the BZDs like diazepam (Valium) and oxazepam (Serax), chlordiazepoxide (Librium), meprobamate (carbamate derivative), and related compounds, and buspirone (aryl piperazine derivative), which is an anxioselective drug. A miscellaneous group of drugs includes certain antihistaminic and anticholinergic drugs that are difficult to classify (e.g., hydroxyzine and buclizine).

See also: Barbiturates, Long-Acting; Barbiturates, Short-Acting; Neurotoxicity.

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Relevant Website

http://bnf.org – British National Formulary: 4.1 Hypnotics and anxiolytics.

Apoptosis

Sidhartha D Ray and Harihara M Mehendale

Definition

Apoptosis, a form of cell death (Ap oh' tosis or A 'pop tosis: Greek 'apo', meaning leaf; 'ptosis', meaning falling off), is a genetically self-orchestrated naturally occurring cell death process that is associated with the course of development and induced during pathological situations for the overall benefit of the organism. In contrast, necrosis (necroh' sis), another form of cell death, typically affects groups of contiguous cells, and an inflammatory reaction usually develops in the adjacent viable tissue in response to the released cellular debris.

Introduction

Cell death is a necessary event in the life of a multicellular organism. Cells predominantly die via

apoptosis or necrosis. Since apoptosis is a form of tightly regulated genetically controlled self-orchestrated cell death, it is often referred to as programmed cell death (PCD). In contrast, necrosis is termed unprogrammed cell death since it occurs accidentally in an unplanned manner. However, occasionally, incomplete execution of biochemical cascade leads to the expression of morphological features of both apoptosis and necrosis yielding to a third type of death, also called apocrosis or aponecrosis. Apocrotic cells do not deliberately bypass the common biochemical machinery shared by both apoptosis and necrosis and are morphologically distinguishable from both apoptosis and necrosis (display signs of both apoptosis and necrosis; molecular mechanism discussed below in a separate section). The term 'apoptosis' appeared in the 1970s, but the phenomenon had been known long before. Apparently PCD was discovered by C. Vogt in the middle of the nineteenth century through observations on the morphology of dying cells during metamorphosis of amphibians. By 1885, there were publications unequivocally diagramming apoptosis, and simultaneously several researchers had noted the death of metamorphosing tissues in insects.

When apoptosis was first described over three decades ago, the terminologies describing these changes (councilman or acidophillic bodies in liver diseases; civatte bodies in lichen planus; tingible bodies in lymphoid germinal centers; basophilic or Benirschke granules characteristic of premenstrual endometrial glands) were already in the perusal of scientists who coined, defined and differentiated 'apoptosis' from 'necrosis' based on changes in cellular ultrastructures. Naming of the process evolved from 'shrinkage necrosis' or 'coagulative necrosis' (early) exclusively on morphologic grounds to 'apoptosis' (later) when it was found to play a role in regulating tissue morphogenesis and tissue size. In contrast, historically, the only form of cell death known to human kind for centuries used to be 'necrosis'. From mechanistic standpoint, necrotic cells pass through a reversible phase, often followed with explosive rapidity, by irreversible changes resulting from the insulting stimuli.

What is Cell Death?

Generally cell death or loss of cell viability can be defined as irreversible failure of vital cellular functions coupled with irreparable structural damage. Therefore, cell death is considered a near equilibrium terminal end-stage, which can be induced by a variety of physiological or nonphysiological perturbations (e.g., ischemia, hypoxia, drugs and chemicals, immune reactions, infectious agents, and high temperature or radiation), including a variety of disease states or disorders such as infectious, immunological, iatrogenic, idiopathic, or neoplastic. Paradoxically, it is now well established that exposure to any such perturbation(s) to a cell population either *in vivo* or *in vitro* could be either apoptogenic (apoptosis-inducing) or necrogenic (necrosis-inducing). For several reasons the idea that physiological cell death was medically important and biologically interesting did not capture the attention of researchers until fairly recently. This is partly because majority of life scientists focused researching on programmed cell life, mechanisms of cell injury and mechanisms of protection of cell life, rather than cell death. Surprisingly, everyone has a new sense of the importance of apoptosis or PCD, and necrosis or unprogrammed cell death. Interestingly, cell death literature also include several other terminologies, such as secondary necrosis and oncotic necrosis, which are yet to be substantiated with biochemical and morphological criteria to distinguish from necrosis.

Importance of Apoptosis or Programmed Cell Death

Positive implications of cell death are currently the subject of intense debate and considerable research activity. This interest stems, in part, from the potential for understanding oncogenesis and the possibility of exploiting the cell death program for therapeutic purposes. For example, inhibition of cell death might contribute to oncogenesis by promoting cell survival instead of death. Likewise, triggering cell death might provide the means for eliminating unwanted cells such as tumor cells. Apoptosis has been affirmatively identified as an important mechanism in both development and homeostasis. Removal of superfluous, infected, transformed or damaged cells by activation of an intrinsic suicide program is achieved via apoptosis. One form of apoptosis involves death and subsequent withdrawal (shrinkage) from the surrounding tissues so as to allow phagocytosis by neighboring cells. The other form is characterized by maintenance of intact cell membranes during the suicide process in order to allow practically any type of cell to engulf the apoptotic bodies or fragments of dying cell. These two suicidal modes circumvent release of degraded dead cell debris and bypass the emergence of a local inflammatory reaction. Beneficial roles of this process begin early on during prenatal life and continue until death.

Biological Significance of Apoptosis

Cell death by apoptosis has been reported in plants, nematodes, insects, fish, birds, amphibians, and

mammals. Examples of elimination of transitory organs and tissues via apoptosis include phylogenetic vestiges (pronephros and mesonephros in higher vertebrates), anuran tails and gills and larval organs of holometabolous insects. Regression of the tadpole tail during amphibian metamorphosis serves as one of the prime examples of PCD during early development, and perhaps is only rivaled by the cataclysms of insect metamorphosis. Tadpole tail fin collapse is followed by degradation of tail muscle, a spectacular event recorded by classical pathobiologists. Another classic example is vertebrate limb bud development. If PCD fails, in formation of the digits, digits remain joined by soft tissue. Formation of heart loops during vertebrate development is another biological architecture by apoptosis. The sloughing off of the inner lining of the uterus (the endometrium) at the start of menstruation occurs by apoptosis. Depletion of cells in spinal ganglia occurs during development of the chick embryo, and there is precise chronological and spatial control over this process. Enormous numbers of cells are deleted especially in the nervous system by apoptosis to give rise to the final configuration of the brain. In mammals, the secondary palate separating oral and nasal cavities develops by growth, rotation and fusion of left and right palatal shelves. Decreased proliferation, increased adhesiveness coupled with PCD of medial edge epithelial cells engineer this fusion of the shelves. The reproductive organs of vertebrates show stunning changes during sexual differentiation and maturation which involve massive PCD. Despite the fact that both male and female embryos have the same reproductive rudiments, the Wolffian duct differentiates into the epididymis and vas deferens, whilst the Mullerian duct regresses in the male and the opposite occurs in the female. The Mullerian duct differentiates into the uterus in mammals or the oviduct and shell gland in avian species. These events are hormone-mediated but cell removal occurs by apoptosis. All these observations indicate that 'apoptosis' or PCD is an equal and opposite force to mitosis. In certain tissues the cells survive until the organism dies while other cells are continually produced in self-renewing tissues, and then differentiate to perform specific functions, and eventually die. Noted examples of these events are the life cycles of skin cells (keratinocytes) and hemopoietic cells (cells contained in the blood such as lymphocytes, leukocytes, monocytes, and erythrocytes).

True importance of this unique cell death process was unknown until a recent upsurge in interest to unravel the mechanisms underlying this process. After three decades of research, it is now well understood that apoptosis is a very tightly regulated, energy-dependent, genetically programmed, and evolutionarily conserved self-destruction process through which cells undergo organized suicide for beneficial purposes. The idea that life requires death seems paradoxical, but cell suicide is essential for an animal to survive. It is very interesting that PCD can be affirmatively predicted in certain cells of the famous nematode *Caenorhabditis elegans*. *Caenorhabditis elegans* matures as an adult hermaphrodite with 1090 cells of which 131 undergo PCD. These PCD-designated cells at various locations automatically trigger suicidal death program at different but precisely defined times. The wide range of cells that can undergo apoptosis in micro (well controlled *in vitro* models) and macro (invertebrates, vertebrates, and plants: *in vivo* models) environments suggests that practically each and every type of cell is potentially capable of executing terms and conditions of apoptosis. For example, without selective destruction of 'nonself' T cells, an animal would lack immunity. Similarly, meaningful neural connections in the brain are whittled from a mass of cells. Apoptosis research, with roots in developmental and cell biology, genetics, and immunology, embraces this long-ignored natural law. Apoptosis as part of normal development is a strategy to select certain cells for survival, sculpting a tissue's specificity. Interestingly, the fetal thymus allows only specific population of T cells (with 'self-antigen' surface markers) to complete development. A cell whose DNA is damaged by ultraviolet radiation in sunlight is either repaired or jettisoned via apoptosis-assisted peeling. Apoptosis is the mechanism by which natural killer (NK) cells nonspecifically kill virally infected cells, stimulating the infected cell to undergo unscrupulous PCD. In multicellular organisms, such beneficial functions include maintenance of optimal tissue growth and development, and physiologic activity (by a balance between proliferation, growth arrest, and PCD). Apoptotic cells, upon completion of their mission, execute a suicidal program and form apoptotic bodies which are rapidly removed by a variety of resident and nonresident scavenger cells. Phagocytic scavengers, such as macrophages, have specialized receptors that recognize phosphatidylserine (PTS) moieties on the surface of apoptotic bodies and carry out their disposal job in an organized manner without eliciting an inflammatory response. While PTS is actively transported from the outer to the inner leaflet of the plasma membrane by ATP-dependent aminophospholipid translocase, the implication of a scramblase that moves phospholipids bidirectionally across the membrane is still debated. Cyt *c* released from mitochondria selectively peroxidizes PTS. Peroxidized PTS inhibits the ATP-dependent aminophospholipid translocase, allowing

oxidized-PTS and phosphatidylethanolamine (PTE) to be exposed also on the extracellular layer of cell membrane. Unlike necrosis, apoptotic cells do not release any enzyme; however, activation of a tissue enzyme transglutaminase has been reported in several systems. Massive cellular demise by apoptosis generally does not lead to organ dysfunction as opposed to necrotic cell death. In contrast, necrosis typically affects groups of contiguous cells, and an inflammatory reaction usually develops in the adjacent viable tissue in response to the released cellar debris.

Stages of Apoptosis

The cellular machinery of apoptotic death turns out to be as intrinsic to the cell as mitosis. Apoptotic process is executed in an organized fashion and usually solicits participation of all the intracellular organelles. The time to onset of apoptosis after a lethal stimulus is variable (minutes to hours) but the changes are extremely rapid. In apoptosis, coordinated changes occur in the nucleus, the cytoplasm, and at the cell surface. *In vivo*, apoptosis usually affects single cells or small groups of cells in an asynchronous fashion and the entire process can be primarily divided into four distinct stages: (1) cell shrinkage, (2) nuclear condensation and fragmentation, (3) cellular fragmentation and formation of apoptotic bodies, and (4) apoptotic body or debris clearance by phagocytosis. The major sequence of events that are associated with apoptosis and necrosis are presented in **Table 1**.

Step I: Cellular Shrinkage

The earliest changes observed include the loss of cell junctions and other specialized plasma membrane

Table 1 Sequence of cellular events associated with apoptosis contrasted with those associated with necrosis

Characteristic	*Apoptosis*	*Necrosis*
Distribution	Affects individual cells scattered throughout the tissue	Affects massive and contiguous cells
Adhesion between cells and to basement membrane	Lost early	Lost but late
Cellular morphology	(a) Chromatin condensation (karyorrhexis) followed by margination as large crescents to the periphery of the nuclear membrane; fragmentation in large masses (convolution). See **Figure 1b**	(a) Irregular clumping of chromatin, pyknosis or karyolysis, nucleolysis occasionally precedes collapse of nuclear membrane; cells occasionally maintain their boundaries with some or no organelles. See **Figure 1c**
	(b) Loss of cell volume (cytoplasmic compaction)	(b) Very early swelling of cell, ballooning occurs frequently
Damage to organelles (e.g., mitochondrion)	Late (organelles mostly retain integrity), occasionally organellar swelling and bleb formation on cell surface appear very late (organelles found in blebs)	Very early swelling of organelles; cells disintegrate and lyse, appear chaotic, form blebs early (organelles are not found in blebs)
DNA breakdown pattern	Internucleosomal cleavage (ladder-like pattern on agarose gel)	Random or irregular damage (appears as a smear on gel)
Release of lysosomal enzymes	Absent	Present
Duration of biochemical and morphological changes	Minutes to hours	Hours to days
Ultimate outcome	Forms apoptotic bodies, occasionally containing intact organelles	Swelling, disintegration, dissolution
Cell removal	Usually phagocytosis by all types of resident and nonresident cells	Usually cells are not removed
Inflammation	Absent	Present
Energy requirement and overall regulation	Strictly energy-dependent, very tightly regulated, signaling-dependent, can easily be delayed but can be inhibited with difficulty	Energy and signaling-independent, occasionally energy-dependent, can be blocked prior to irreversible changes (e.g., plasma membrane leakage)
Genomic control	Strictly dependent	Usually independent
Scar formation	Absent	Present
Cellular osmotic regulation	Intact	Lost leading to cell swelling

structures such as microvilli. In intact organs or tissues, a withdrawal mode from the surrounding sets in. Typically, the cytoplasm begins to shrink following the cleavage of lamins and actin filaments, and in some instances cytoplasm becomes hypertrophied.

Step II: Nuclear or Chromatin Condensation

This is the most noticeable distinguishing feature of apoptosis, which shows classic stereotypical changes. This stage goes through very complex biochemical and molecular changes. At this stage, chromatin condenses (coalesces; generation of pyknotic nucleus), fragments in an orderly fashion into one or more large (or small) masses, and migrates toward the periphery of the nuclear membrane (in many cases the nuclei of apoptotic cells take on a 'horse-shoe' like appearance). As the process continues the nucleus breaks into several fragments. Under an electron microscope, these fragments appear very dense and dark in the near-total absence or loss of volume regulation of other organelles such as the mitochondria. The contraction of cytoplasmic volume is apparently associated with loss of intracellular fluid and ions. Photomicrographs of normal (panel a), apoptotic (panel b), and necrotic (panel c) liver cells are presented in **Figure 1a–c**.

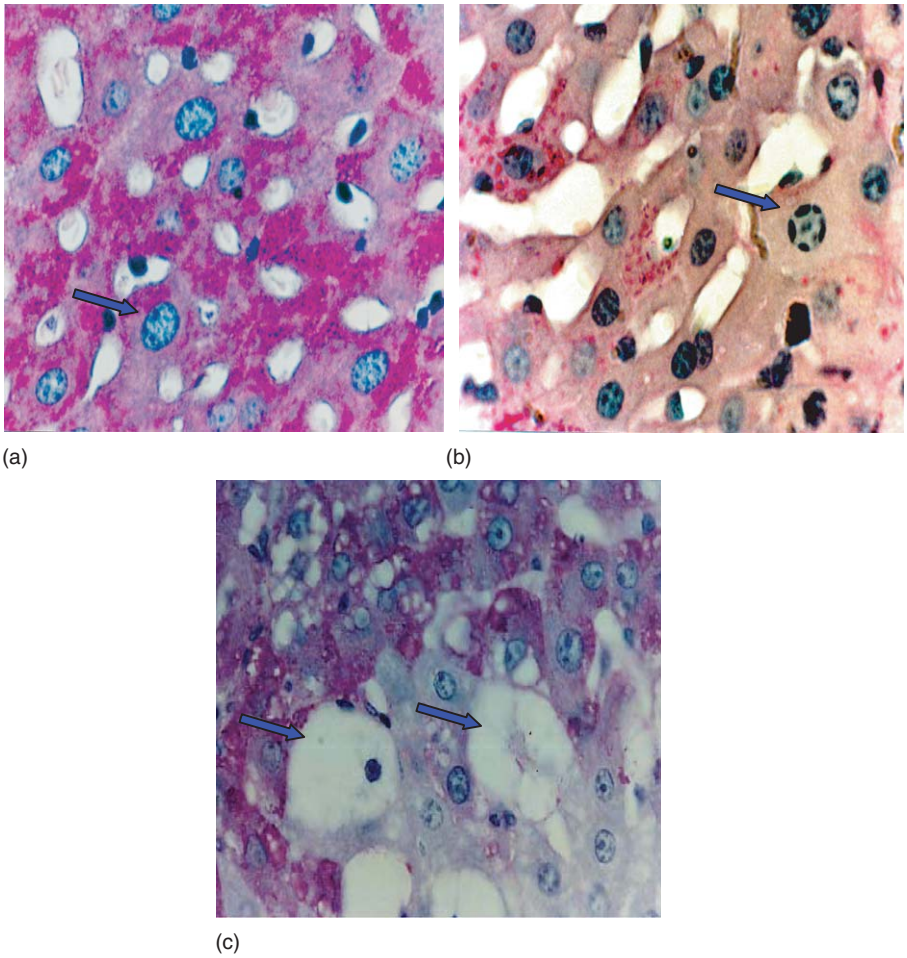


Figure 1 Light photomicrographs (PAS-stained; × 1000) of liver sections showing architecture of a normal hepatocyte with a normal nucleus (panel a: see arrow), apoptotic hepatocyte with an apoptotic nucleus (panel b: see arrow; in the vicinity of normal, damaged- and glycogen-depleted hepatocytes), and abnormally ballooned necrotic hepatocytes with necrotic changes (see panel c: a liver cell with a nucleus with disintegrated cytoplasm, and another liver cell without a nucleus with disintegrated cytoplasm; both arrows indicate necrotic cells). Liver injury and apoptosis was induced by a single hepatotoxic dose of acetaminophen (500 mg kg^{-1}, i.p.). (Reproduced with permission from Ray SD and Jena N (2000) A hepatotoxic dose of acetaminophen modulates expression of BCL-2, BCL-X(L), and BCL-X(S) during apoptotic and necrotic death of mouse liver cells *in vivo*, *Archives of Toxicology* **73**: 594–606; © Springer-Verlag. Ray, *Proceedings of the Society of Free Radical Research*, 2004.)

Step III: Cellular Fragmentation or Formation of Apoptotic Bodies

Cells committed to apoptosis continue to shrink, packaging themselves into a form that allows for easy engulfment by all types of cells. The cell transiently adopts a deeply convoluted outline and shows extensive surface blebbing. In order to promote their phagocytosis by macrophages, apoptotic cells often elicit biochemical changes on the plasma membrane surface that appeal to the macrophage response. One such change is the translocation of PTS from the inner leaflet of the cell to the outer surface. Lysophosphatidylcholine generated as a result of caspase-3-mediated activation of the Ca^{2+}-independent phospholipase-A_2 renders cells vulnerable to phagocytosis. Membrane changes can often be observed morphologically through the appearance of membrane blebs or blisters which often appear toward the end of the apoptotic process. Subsequently, the cell breaks up into several membrane-bound smooth-surfaced 'apoptotic bodies' that contain a variety of tightly compacted organelles and some nuclear fragments. Under the microscope, appearance of apoptotic bodies is a common feature used by trained pathologists to identify apoptosis in any tissue.

Step IV: Phagocytosis of Apoptotic Cells or Bodies

Apoptotic bodies show a great diversity in size, and shape, and there is no limit to the number of apoptotic bodies formed from one cell. Apoptotic bodies are typically phagocytosed by 'professional phagocytes' (macrophages) or neighboring cells serving as 'semiprofessional' phagocytes (glomerular mesangial cells, Kupffer cells, or liver cells). These phagocytic cells are responsible for removing apoptotic cells from tissues in a clean and tidy fashion that avoids many of the problems associated with necrotic cell death. Although professionally trained engulfing cells are typically members of the reticuloendothelial system (mononuclear phagocytes such as macrophages, phagocytes, etc.), any other normal or abnormal cell capable of phagocytosis may participate in cell clearance process. The endocytosed apoptotic debris is rapidly degraded by a series of enzymes within lysosomes, and the adjacent cells move around or if necessary, proliferate to replace the gap created by the just-deleted apoptotic cell.

Mechanisms of Apoptosis

Now, it has become clear that the regulatory mechanisms controlling apoptosis are as fundamental, and as complex, as those regulating cell proliferations. Based on the observations of the past three decades, apoptotic response of cells may depend upon a multitude of intrinsic and extrinsic factors, for example, (1) the stimulus (e.g., physical, chemical, or biological); (2) cellular defense mechanisms (intracellular redox status; oxidant/antioxidant balance); (3) signal transduction pathways (receptors involved); (4) level of expression of relevant pro and anti-apoptotic genes, and (5) intrinsic cellular susceptibility to apoptosis (half-life of a cell). However, the overall process appears to go through three distinct phases at the molecular level: an induction phase, an effecter phase, and a degradation phase. The induction phase depends on death-inducing signals to stimulate pro-apoptotic signal transduction cascades. These death-inducing signals include oxidative stress typically produced during drug metabolism by reactive oxygen or nitrogen species (ROS, RNS), or their hybrids (peroxynitrite), overactivation of Ca^{2+}-dependent pathways, and modulation of gene expression (Bcl-2 family proteins such as Bcl-XL, Bax, and Bad). In phase 2, the effector phase, the cell becomes committed to death by the action of a key regulator, which arguably is the mitochondrion. At this phase mitochondrial permeability transition (MPT) pores are formed by several mechanisms resulting in excessive leakage and flooding of the cytosol with Cyt *c* from the mitochondrion. The last phase, a degradation phase, involves both cytoplasmic and nuclear events. In the cytoplasm, a complex cascade of protein-cleaving enzymes called caspases is activated. In the nucleus, the chromatin condenses, the nuclear envelope breaks down, and the DNA undergoes orderly fragmentation. Finally, the cell is fragmented into apoptotic bodies, phophatidylserine on the membranes is recognized, and apoptotic bodies are cleared as described above.

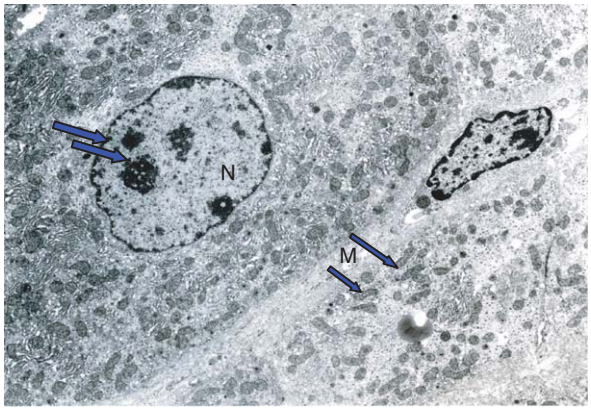
Biochemical and Molecular Events That Trigger Apoptosis

Prolonged cellular stresses, such as oxidative stress and DNA damage, if not defended against, can trigger the apoptotic pathway. Biochemical markers such as DNA fragmentation have been used to identify apoptotic cells. Unfortunately, cells dying by necrosis, a traumatic form of cell death that is accompanied by cell swelling and lysis also exhibit DNA fragmentation. In addition, cells with the characteristic morphologic appearance of apoptosis, occasionally, may not show evidence of DNA fragmentation. In this context, it is worth mentioning that induction of apoptosis in enucleated cells has also been reported. Cells exhibiting evidence of DNA damage and oxidative stress may, however, recover

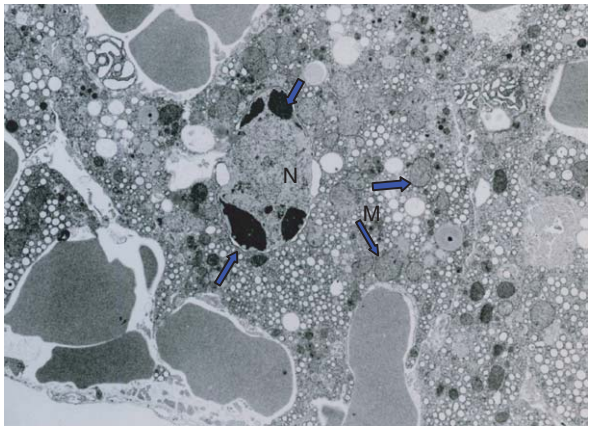
from those stresses, provided they are equipped with an adequate DNA repair system and an adequate level of stress-response proteins and/or antioxidant defenses.

One of the hallmarks of apoptosis is the cleavage of chromosomal DNA into nucleosomal units. The degradation of DNA in the nuclei of apoptotic cells is accomplished in a number of ways following activation of caspases (a family of cystein proteases). The fragmentation of DNA into nucleosomal units – as seen in DNA laddering assays – is caused by an enzyme known as caspase-activated DNase (CAD). Normally CAD exists as an inactive complex with ICAD (inhibitor of CAD, also known as DNA fragmentation factor 45 or DFF45). During apoptosis, ICAD is cleaved by caspases, including caspase 3, to release CAD. Active CAD migrates into the nucleus and cleaves DNA. Since CAD is a DNase with a high specific activity (comparable to or higher than DNase I and DNase II) rapid fragmentation of the nuclear DNA follows. Formerly, CAD was thought to be a Ca^{2+}/Mg^{2+}-dependent nuclear enzyme (endonuclease), a master operator of this beneficial form of death. Therefore, xenobiotics that are known to induce intracellular Ca^{2+}-dysregulation were classified as possible apoptosis inducers. The other viable pathway through which genomic fragmentation is achieved is via lamins, a family of intra-nuclear proteins. Lamins (there are two forms, Lamin-A and Lamin-B) maintain the shape of the nucleus and mediate interactions between chromatin and the nuclear membrane. Degradation of lamins by caspase 6 may result in the chromatin condensation and nuclear fragmentation commonly observed in apoptotic cells (see **Figure 2**).

Since mitochondrial morphology remains intact throughout apoptosis, until recently, mitochondria were not assumed to be critical players in the effecter phase of apoptosis. But accumulating evidence indicates that mitochondria exhibit major functional roles and structural changes that serve to regulate apoptosis. Data from diverse models systems conclude that: (1) collapse of mitochondrial inner transmembrane potential ($\Delta\Psi_m$), (2) mitochondrial proteins that serve as rate-limiting factors for the activation of endonucleases and caspases, (3) modulation of expression of anti-apoptotic protein, Bcl-2, is localized to the mitochondrial inner membrane, and (4) release of cytochrome *c* (cyt *c*) into the cytosol, may be of paramount importance to apoptosis regulation. Some recent reports claim the ability of this organelle to release procaspase-3 (inactive form of caspase-3) and zymogens of caspase-2 and caspase-9. Intriguingly, mitochondrial oxidative stress can also release Cyt *c* and apoptosis-inducing factor (AIF).



(a)



(b)

Figure 2 Electron photomicrographs ($\times$ 8000) of mouse liver sections showing ultrastructural details of a normal hepatocyte (panel a) and an apoptotic hepatocyte (panel b). Normal hepatocyte shows classic features of an intact nucleus with intact chromatin (see arrow) and other organelles (N, nucleus; M, mitochondria). Apoptotic liver cell shows chromatin condensation, fragmentation and migration of the heterochromatin to the nuclear periphery (N, nucleus; M, mitochondria), typical features of apoptotic changes (see **Table 1** for more details). Liver injury and apoptosis was induced by a single hepatotoxic dose of acetaminophen (500 mg kg^{-1}, i.p.). (Reproduced from Ray SD, Mumaw VR, Raje RR, and Fariss MW (1996) Protection of acetaminophen-induced hepatocellular apoptosis and necrosis by cholesterge hemisuccinate pretreatment. *The Journal of Pharmacology and Experimental Therapeutics* **279**(3): 1470–1483, with permission from ASPET.)

Cytochrome *c* serves as the key regulator of apoptosis because once it is released from the intermembrane space, the cell is irreversibly committed to death. Either apoptosis occurs through the caspase-mediated process described above, or the cell undergoes a necrosis-like death due to the collapse of electron transport. Release of Cyt *c* interrupts the transfer of electrons between respiratory chain complexes III and IV, resulting in the generation of deleterious radical species (oxidative stress) and the

cessation of ATP synthesis. Cyt *c* in its holo form (i.e., attached with its heme group), associates with Apaf-1 (a molecule associated with Bcl-2), caspase-9, and ATP to form a complex called an 'apoptosome'. This apoptosome proteolytically activates caspase-3, which leads to the activation of the caspase cascade and the degradation phase of apoptosis. Interestingly, these proteins-Bcl-2, Apaf-1, caspase-9, Cyt *c*, and caspase-3 serve as the mammalian equivalent of the apoptosome (Ced-9, Ced-4, Ced-3) in *C. elegans*. Key to all these events is 'how Cyt *c* leaks out from mitochondria?' It has been suggested that a mitochondrial outer membrane protein, voltage-dependent anion channel 2 (VDAC2), interacts with BAK (Bcl-2 antagonist, a killer pro-apoptotic protein) to keep this potentially lethal apoptotic effector under control. When the death signal is received, products of the activation cascade – such as apoptosis promoters tBID, BIM, or BAD – displace VDAC2. Subsequently BAK and BAX are activated, and the mitochondrial outer membrane becomes permeable (MPT pores). This results in the release of caspase activators, including Cyt *c*. The whole process requires energy and a cell machinery not too damaged. If the cell damage is between certain levels, the cell can start the earliest events of apoptosis and then continue with necrosis (features of apocrosis or aponecrosis).

Oxidative stresses caused by the ROS/RNS family members are powerful necrogens. For example, the highly reactive hydroxyl radical is believed to be one of the most potent inducers of oxidative damage during necrotic cell death. In apoptosis, radicals still play a major role by determining the cellular redox status, and many forms of apoptosis involve ROS, RNS, and peroxynitrite (a potent anion oxidant generated by the reaction of nitric oxide with superoxide). Oxidants like superoxide and hydrogen peroxide can act as proapoptotic stimuli by changing the cellular redox status. And in most systems, antioxidants have been shown to be antiapoptotic. In fact there is overwhelming evidence to support antiapoptotic effect of natural (pycnogenol, epigallocatechin gallate, grape seed proanthocyanidins, quercetin, resveratrol, etc.) and synthetic antioxidants (*N*-acetyl cysteine, butylated hydroxytoluene). Oxidants indirectly induce apoptosis by changing cellular redox potentials, depleting reduced glutathione, and decreasing reducing equivalents such as NADH and NADPH. Similarly, agents that deplete intracellular glutathione render cells more vulnerable to oxidative stress-induced apoptosis. Redox potential of a cell is as critical as maintaining intracellular ion homeostasis or genomic integrity. An oxidative shift by ROS may altogether modify the nature of the stimulatory signal resulting in alteration of the direction of the program (apoptosis/necrosis or proliferation). Interestingly, spontaneous redox changes facilitate the formation of MPT pores, leading to the subsequent release of Cyt *c*. These MPT pores possess several redox-sensitive sites, including one in equilibrium with mitochondrial matrix glutathione, and one directly activated by oxidants. Antioxidants typically detoxify these noxious free radicals, antagonize their damaging influence, and protect cells. However, low levels of oxidants may provoke cells to proliferate, whereas mild oxidative stress conditions counteract apoptotic stimuli. Low cellular levels of superoxide and hydrogen peroxide are continually being produced from the mitochondrial respiratory chain and electron transport chains in the endoplasmic reticulum and nuclear membranes. In addition, low levels of H_2O_2 are produced as a by-product of the activity of γ-glutamyl transpeptidase (GGT). This enzyme is responsible for metabolizing extracellular reduced glutathione. Several studies suggest that the low levels of H_2O_2 generated by GGT protect cells against apoptosis and help maintain cell proliferation.

The free radical gas nitric oxide ($NO^{\bullet}$) is an important regulator of mitochondrial function, cell signaling, and gene expression. Production of $NO^{\bullet}$ can be a common denominator during xenobiotic metabolism. Exogenous application of high levels of $NO^{\bullet}$ donating compounds (sodium nitroprusside) are known to induce $NO^{\bullet}$-mediated apoptosis. Investigators believe the mechanism may involve an upregulation of ceramide levels by activating sphingomyelinases while concomitantly inhibiting ceramidases. In contrast, at physiological levels $NO^{\bullet}$ prevents apoptosis by interfering with the activation of the caspase cascade. In one experiment, proinflammatory cytokines were used to activate inducible nitric oxide synthase (iNOS), resulting in full protection for endothelial cells undergoing UV-A radiation. The mechanism involves $NO^{\bullet}$-mediated increases in Bcl-2 expression with a concomitant decrease in the expression of antiapoptotic Bax protein. Moreover, *in vitro* and *in vivo* experiments have shown that $NO^{\bullet}$ inhibits caspase-3 by S-nitrosation of the enzyme. Associated with inhibiting caspase 3, $NO^{\bullet}$ suppresses the self-amplification feed forward loop of apoptosis by inhibiting Bcl-2 cleavage and Cyt *c* release. However, at high levels oxidants can induce both apoptosis and necrosis.

Inactivation of DNA repair enzymes can also turn on apoptosis. A fascinating apoptotic process can result from stress or toxicity that culminates in genomic damage in the cell nucleus, triggered by a nuclear enzyme poly (ADP-ribose) polymerase

(PARP). This enzyme is instrumental in maintaining genomic integrity. Massive activation of PARP can deplete the cell of energy-providing molecules (such as NAD), an event that sends signals from the nucleus for the mitochondrion to start the apoptotic process. PARP was the first protein identified as a substrate for caspases. PARP is involved in repair of DNA damage and functions by catalyzing the synthesis of poly (ADP-ribose) and by binding to DNA strand breaks and modifying nuclear proteins. The ability of PARP to repair DNA damage is prevented following cleavage of PARP by caspase-3. Similarly, inactivation of enzymes involved in cell replication can also trigger apoptosis. DNA topoisomerase II is a nuclear enzyme essential for DNA replication and repair. Caspases can inactivate this enzyme leading to DNA damage. Overall malfunctioning of DNA repair enzymes may propagate signals for PCD.

Other biochemical determinants pivotal to apoptosis include intracellular deregulation of cAMP and ceramide. Ceramides are known stimuli of apoptosis and are released by activation of an acidic and/or neutral sphingomyelinase. Both enzymes are activated by the Fas receptor. Fas-mediated apoptosis can be partially inhibited by direct inhibition of acidic sphingomyelinase using the drug imipramine. Fas-triggering of early caspases (caspase-8/may be caspase-1) stimulate directly or indirectly the acidic sphinomyelinase resulting in the release of ceramide. The sphingomyelin pathway, initiated by hydrolysis of the phospholipid sphingomyelin in the cell membrane to generate the second messenger ceramide, is thought to mediate apoptosis in response to tumor necrosis factor-α (TNF-α), to FAS ligand and to X-rays. Generation of ceramide (hydrolysis product of sphingomyelin) through the sphingomyelin pathway results in the induction of apoptosis. At least two specific intracellular targets for ceramide have been identified: (1) a membrane ceramide-activated protein kinase and (2) a cytoplasmic ceramide-activated protein phosphatase. Ceramide promotes the formation and release of oligonucleosomal DNA fragments, produces corresponding loss of integrity of bulk DNA fragments, and elicits the expression of classical morphology of apoptosis.

Participation of Ca^{2+} in a well-known capacity (as activators) comes from studies during phospholipid-dependent protein kinase (protein kinase C: PKC) activation in intracellular signaling processes. One of the pathways of the transmembrane signaling system operates through the activation of PKC, whereas the other signal system involves receptor-mediated activation of cAMP-dependent protein kinase. Information concerning a role for PKC in apoptosis mostly comes from studies with phorbol esters (TPA: 12-*O*-tetradecanoyl-phorbol 13 acetate). However, recent reports suggest, depending upon the experimental system, PKC may either enhance or retard the apoptotic process. Protein kinase B (PKB or Akt is a PKC family member) is a serine/threonine kinase, that prevents apoptosis of neurons.

Genetic Control of Apoptosis

The balance between the withdrawal of positive signals (signals needed for continued survival) and the receipt of negative signals (signals needed to commit cellular suicide) may propel apoptosis in a particular direction. The continued survival of most cells requires that they receive continuous stimulation from other cells and, for many, continued adhesion to the surface on which they are growing. Some examples of positive signals are interleukin-2 (IL-2 is a cytokine), an essential factor for the mitosis of lymphocytes. Negative signals could be growth factor deprivation, redox imbalance, radiations (UV light, X-rays), chemotherapeutic drugs, and most importantly molecules that bind to specific receptors on the cell surface and signal the cell to begin the apoptosis program. The fundamental apoptosis signaling pathway and the involved proteins have been highly conserved throughout evolution. The basic apoptotic machinery mapped out in mammalian system mimics the genetic screens discovered in the nematode *Caenorhabditis elegans* (Ced-9, Ced-4, and Ced-3). Among these, anti-apoptotic molecule Ced-9 negatively regulates the activity of the proapoptotic molecule Ced-4 which in turn activates Ced-3. Bcl-2, Apaf-1, and the caspase protease family (see **Table 2**) have been identified as mammalian homologs of Ced-9, Ced-4, and Ced-3. Another breakthrough in apoptosis research was the discovery of a family of receptors that can specifically trigger apoptosis. This growing subfamily of death receptors (and death ligands) belongs to the TNF/NGF-receptor superfamily and is characterized by the presence of extracellular cysteine-rich domains. The death receptors have an intracellular death domain (DD), which couples receptors to the apoptosis-inducing machinery. Most noted members of this family are CD95 (APO-1/FAS), tumor necrosis factor receptor 1 (TNF-R1, 55 kDa protein), Death Receptor-3 (DR3; TNF receptor family member also known as Apo-3, WSL-1, TRAMP or LARD), lymphocyte-associated receptor of death (LARD), TNF-related apoptosis-inducing ligand (TRAIL-R1; has five members) and TRAIL-R2 (death receptor-5), TNF receptor I-associated death domain (TRADD) is somehow related to TRAIL-R1. Overexpression of TRADD leads to two major TNF-induced responses,

Table 2 List of the main caspases and some of their substrates

Caspase	*Common name*	*Substrate(s)*	*Function*
Caspase-1	ICE (Ced-3 homolog)	ILs, preinterleukin-1β, interleukin-18, Lamins	Processing of ILs (inflammation). Can also induce apoptosis depending on isoform and if overexpressed
Caspase-2	Ich-1 (human), Nedd2 (rat, mouse)	Golgin-160, Lamins (?)	Apoptosis (activity suppressed by serum deprivation)
Caspase-3	CPP32, Yama, apopain	PARP, SREBs, ICAD Gelsolin, Caspase-6, Caspase-7, Caspase-9, DNA-PK, MDM2, Gas2, Fodrin, β-Catenin, Lamins, NuMA, FAK, $p21^{Waf1}$, HnRNP proteins, Topoisomerase I, Calpastatin, Presenelin2	Apoptosis
Caspase-4	Ich-2, $ICE_{rel}II$	Caspase-1	Inflammation/apoptosis (note: this could be the human form of mouse caspase-11). Related to human caspase-5 and caspase-1
Caspase-5	$ICE_{rel}III$, TY	?	Inflammation/apoptosis (related to human caspase-4 and caspase-1)
Caspase-6	Mch2	PARP, Lamins, NuMA, FAK, Caspase-3, Keratin-18	Apoptosis
Caspase-7	Mch3, ICE-LAP3, CMH-1	PARP, Gas2, SREB1, EMAP II, FAK, Calpastatin, $p21^{Waf1}$	Apoptosis (activity blocked by cIAP1 and cIAP2). Similar in structure and substrate specificity to caspase-3
Caspase-8	FLICE, MACH, Mch5	Caspase-3, caspase-4, caspase-6, caspase-7, caspase-9, caspase-10, caspase-13, PARP, Bid	Apoptosis (death receptors)
Caspase-9	Apaf-3, ICE-LAP6, Mch6	Caspase-3, pro-caspase-9, caspase-7, PARP	Apoptosis
Caspase-10	FLICE-2, Mch4	Caspase-3, caspase-4, caspase-6, caspase-7, caspase-8, caspase-9	Apoptosis (death receptors)
Caspase-11	Ich-3, ICE-B	?	Murine caspase similar to human caspase-4. Belongs to the same family as caspase-3 of enzymes. May be involved in inflammation and apoptosis
Caspase-12	ICE-C	?	Involved in mediating apoptosis following ER stress. Related to mouse caspase-1 and caspase-11 and human caspase-4 and caspase-5
Caspase-13	ERICE (FLICE activatable caspase)	?	Member of the ICE family of caspases that include caspase-1 and caspases-4, -5 and -11. Involved in inflammation
Caspase-14	MICE		Cysteinyl aspartic acid-protease-14, also known as MICE. Overexpression of MICE induces apoptosis
Granzyme A	Granzyme A		A serine protease located in the granules of cytotoxic T cells and NK cells that are involved in the induction of target cell apoptosis (*Journal of Immunology* 1988, 141: 3471–3477)
Granzyme B	Granzyme B	Can activate caspases -3, -7, -8, and -10 (a chemical substrate 7-amino-4-methylcoumarin used for evaluation)	A serine protease located in the granules of cytotoxic T cells and NK cells that is involved in the induction of target cell apoptosis

apoptosis, and activation of NF-kβ. The death ligands have co-evolved as a death ligand family, corresponding to death receptors. Activation of death receptors is controlled in many cases by the inducible *de novo* expression of the respective death ligands such as CD95L, TNF, or TRAIL. Besides DD, there are also death effector domains (DEDs) involved downstream of the process.

Classic examples of death activators include: (1) TNF-α that binds to the TNF receptor; (2) lymphotoxin (also known as TNF-β) that binds to the TNF receptor, and (3) Fas ligand (FasL), a molecule that binds to a cell-surface receptor named Fas (also called CD95). Fas and the TNF receptor are integral membrane proteins with their receptor domains exposed at the surface of the cell. Binding of the complementary death activator (FasL and TNF, respectively) transmits a signal to the cytoplasm that leads to activation of caspase 8. Caspase 8 (like caspase 9) initiates a cascade of caspase activation leading to phagocytosis of the cell. When cytotoxic T cells recognize (bind to) their target, they produce more FasL at their surface. This binds with the Fas on the surface of the target cell leading to its death by apoptosis. The early steps in apoptosis are reversible – at least in *C. elegans*. In some cases, final destruction of the cell is guaranteed only invagination and digestion by a phagocyte.

Neurons, and perhaps other cells, have another way to self-destruct and that is independent of caspase activation but dependent on a factor known as apoptosis-inducing factor (AIF). AIF is a protein that is normally located in the intermembrane space of mitochondria. When the cell receives a death-inducing signal, AIF is released from the mitochondria, migrates into the nucleus, binds to DNA, which ultimately triggers the destruction of the DNA and cell death. The other straightforward mechanism is executed by caspases (mentioned at several places in this chapter), which are normally suppressed by inhibitor of apoptosis (IAP) proteins. When a cell receives an apoptotic stimulus, IAP activity is relieved after second mitochondria-derived activator of caspases (SMAC, also called DIABLO), a mitochondrial protein, is released into the cytosol in order for the proper execution of the death program. Activation-induced cell death (AICD) involves over expression of receptors for pro-apoptotic ligands (e.g., negative selection of T lymphocytes).

Bcl-2 Family of Proteins Regulate Apoptosis

The concept of active cell death or PCD being a genetically encoded process has stimulated an intense search for the genes involved in the cell-death program schemes. The first anti-apoptotic gene to be clearly identified in humans was Bcl-2 (B-cell lymphoma-leukemia-2 gene), cloned from the breakpoint of the 14:18 translocation found in the majority of follicular lymphomas. The discovery of Bcl-2 oncogene helped stimulate recognition of the concept that gene products which modulate the susceptibility of certain cell types to apoptosis, may play an important role in the process leading to malignant transformation. This is primarily due to the ability of survival of cells in inappropriate physiological situations. In both pathological as well as physiological conditions, the Bcl-2 gene has emerged as a critical regulator of apoptosis. Expression of the Bcl-2 protein directly prevents apoptosis by enhancing cellular antioxidant capacity possibly through scavenging reactive oxygen radicals, or indirectly by counteracting oxidative stress. The other potential mechanisms of this gene include a role in regulation of intracellular calcium ion, nuclear transport, and control of signal transduction pathways. Bcl-2 is no longer a single entity but one member of a growing multi-gene family. Included in this family are: Bcl-X (Bcl-XL and Bcl-XS), myeloid cell leukemia 1 (Mcl-1, Mcl-1 is a mitochondrial protein that enhances cell viability under apoptotic conditions), Bax (Bcl-2 associated $\times$ protein; pro-apoptotic; has four forms: α, β, γ, ω), A1, Bag (Bcl-2 associated athanogene-1; Bag is a Bcl-2-binding protein which provides protection from apoptotic cell death), Bak (Bcl-2 antagonist/killer, pro-apoptotic), Bad (Bad is a heterodimeric partner for Bcl-XL and Bcl-2 that displaces Bax and promotes cell death), Bcl-w (promotes cell survival), and Ced-9. The transcripts of bcl-X are alternatively spliced to form bcl-XL (long form; antiapoptotic property) and bcl-XS (short form; proapoptotic).

But how does the Bcl-2 family regulate apoptosis? Bcl-2 and Bcl-X_L prevent MPT pore opening in isolated mitochondria. Since Bcl-2 can prevent MPT pore opening in a system without Cyt *c*, this indicates that Bcl-2 can directly regulate MPT pores. However, Bcl-2 can also maintain mitochondrial $\Delta\Psi_m$ (membrane potential) under conditions that do not allow permeability transitions. This suggests that Bcl-2 regulates $\Delta\Psi_m$ rather than MPT pores through enhancing H^+ efflux in the presence of stimuli that collapse $\Delta\Psi_m$. Second, Bcl-2 inhibits the release of Cyt *c*. Third, Bcl-2 attenuates the MPT-pore promoting effects of atractyloside (an activating ligand of the adenine nucleotide translocator, ANT) and Bax. Moreover, Bcl-2 homologs can form ion channels or pores in artificial membranes. In fact, they have a crystal structure similar to colicins and the b-subunit of diphtheria toxin. The diphtheria b-subunit translocates the A-subunit across membranes; likewise

Bcl-2 and Bcl-X_L can potentially translocate molecules across membranes. Bcl-2 and Bcl-X_L may interact physically or functionally with the MPT pore or with non-MPT pore proteins that control volume regulation of the matrix. In several experimental paradigms, Bcl-2 further reduces the cellular redox potential. Moreover, free radical-induced cell death is accompanied by lipid peroxidation, which is attenuated with Bcl-2 overexpression. More research is needed to determine whether intracellular redox status is a key regulator in apoptosis-signaling pathways.

p53, The Guardian of Genome

Considerable focus has fallen upon another gene, known as the guardian of the genome, associated with this suicidal process – p53. This suppressor gene codes for a 53 kDa protein that helps organisms cope with DNA damage by either stalling cell division or inducing cell death. The p53 tumor suppressor limits cellular proliferation by inducing cell cycle arrest and apoptosis in response to cellular stresses such as DNA damage, hypoxia, and oncogene activation. Many apoptosis-related genes that are transcriptionally regulated by p53 have been identified. These are candidates for implementing p53 effector functions. In response to oncogene activation, p53 mediates apoptosis through a linear pathway involving bax transactivation, Bax translocation from the cytosol to membranes, Cyt *c* release from mitochondria, and caspase-9 activation, followed by the activation of caspase-3, -6, and -7. P53-mediated apoptosis can be blocked at multiple death checkpoints, by inhibiting p53 activity directly, by Bcl-2 family members regulating mitochondrial function, by blocking caspase-9 activation, and by caspase inhibitors. Understanding the mechanisms by which p53 induces apoptosis, and the reasons why cell death is bypassed in transformed cells, is of fundamental importance in cancer research, and has great implications in the design of anticancer therapeutics. For example, the genotoxic anti-cancer drugs such as etoposide and γ-radiation generate damage in chromosomal DNA. The signal seems to be transferred to mitochondria in a p53-dependent manner by as yet an identified mechanism. This releases Cyt *c* from mitochondria, and activates caspase 9 as described in this article.

Inappropriate Regulation of Apoptosis May Head to Cancer and Autoimmune Diseases

Aberrant regulation of apoptosis contributes to well-known pathologies such as autoimmune diseases, cancer, and viral infections. As discussed above, p53 serves as a checkpoint for cell cycle and apoptotic cell death. While massive apoptotic death may signal cell proliferation, uncontrolled cell division may lead to cancer. There are a number of proteins that regulate and control the cell cycle. They dictate the cell when is the proper time to grow and divide, and they stop the cell when the time is not right. The main families involved in cell cycle are the cyclins (especially cyclin D and cyclin E and cyclin A), the cyclin-dependent kinases (especially CDK4, CDK6, and CDK2), the CDK inhibitors (especially p16, p21, and p27), and the tumor suppressor genes (especially Rb and p53). There are two pathways which regulate (the Rb pathway and the p53 pathway) this complex event. One of the main clinical interests of cell cycle control is cancer. Therefore, research in understanding cell cycle control has many implications for cancer, especially for the development of therapeutics. A major breakthrough of the twenty-first century in the field of medicine may be select induction of PCD in cancerous cells in *in vivo* system including humans.

Another oncogene which was long thought to play a critical role in life and death processes of cells is *C-myc*. The *C-myc* gene is the cellular homolog of the viral oncogene v-myc, and it plays a prominent role in carcinogenesis. *C-myc* gene encodes nuclear phosphoprotein of $\sim$60 kDa size, a member of helix-loop-helix family of transcription factors abundant in proliferating cells. *C-myc* and *C-fos* (and cofactors *c-max* and *c-myb*) expressions appear transiently during castration-induced and postlactation breast regression-induced apoptotic death of prostate and mammary cells, respectively. Deregulated expression of *C-myc* not only promotes proliferation, but also can either induce or sensitize cells to apoptosis. Inappropriate expression of c-myc under conditions which inhibit growth and downregulate endogenous *C-myc* expression, including serum deprivation and exposure to cytotoxic agents including the anticancer agents vinblastine, etoposide, Ara-C, and nocodazole, usually results in PCD in many different cell types. Moreover, inappropriate *C-myc* expression is associated with an apoptotic response elicited by induction of differentiation. The proto-oncogene *C-myc* encodes a transcription factor *C-myc*, which is of great importance in controlling cell growth and vitality. The quantity of *C-myc* is carefully controlled by many mechanisms, and its actions to induce and repress genes modulated by interactions with other regulatory proteins. Understanding the kinetic and quantitative relationships that determine how and what genes *C-myc* regulates is essential to understanding how *C-myc* is involved in apoptosis.

Execution of apoptosis in mammalian cells requires the coordinated action of several aspartate-specific cysteine proteases, called caspases, which are responsible for the cleavage of key enzymatic and structural

substrates, resulting in the systematic and orderly disassembly of the dying cell. The caspases exist within the cell as inactive pro-forms or zymogens. zVAD-FMK, Benzyloxy-valine-alanine-aspartate-*O*-methyl-fluoromethylketone, is a well-known synthetic caspase inhibitor. These zymogens can be cleaved to form active enzymes following the induction of apoptosis. The finding that the product of the Ced-3 gene (*Caenorhabditis elegans*) is strongly related to mammalian interleukin1β-converting enzyme (ICE), together with the observation that overexpression of ICE induces apoptosis, prompted an intensive search for new family members, that has led to the identification of at least 14 related caspases. These proteins are characterized by an absolute specificity for Asp in the cleavage site's P1 position, and they all contain a conserved QACXG (where X is R, Q, or G) pentapeptide motif in the catalytic site. Phylogenetic analysis of the caspases revealed that they can be grouped into three subfamilies: an ICE subfamily, comprising caspases -1, -4, and -5 (ICE, TX, and TY, respectively), a CED-3/CPP32 subfamily, comprising caspases -3, -6, -7, -8, -9, and -10 (CPP32, Mch2, Mch3, FLICE, Mch6, Mch4), and an Ich-1/Nedd-2 subfamily. Accumulating evidence indicates that members of the ICE subfamily predominantly play a role in inflammation, whereas members of the CPP32 subfamily are largely involved in apoptosis. Induction of apoptosis via death receptors results in the activation of an initiator caspase such as caspase 8 or caspase 10. These caspases can then activate other caspases in a cascade. This cascade eventually leads to the activation of the effector caspases such as caspase 3 and caspase 6. These caspases are responsible for the cleavage of the key cellular proteins that leads to the typical morphological changes observed in cells undergoing apoptosis.

The interaction of toxicants and apoptosis is complex and the mechanisms of interaction are likely to differ among different toxicants. The apoptosis-inducing potential of a variety of drugs, chemicals, and carcinogens has been intensively investigated from a mechanistic standpoint. Decades of research have focused on the ability of carcinogens to induce cell transformation, however, although it is not entirely true, many investigators share the notion that carcinogens antagonize (or alter) or mutate the apoptotic pathway and improve the chances of cell survival. On the contrary, many carcinogens are powerful inducers of apoptosis. These issues have been addressed at great length during recent years. A short list of classic apoptogens has been provided in **Table 3**. Treatment of cells with certain pharmacologic agents may not result in either classic apoptosis or classic necrosis. The formation of micronuclei, aberrant mitoses, a mitotic arrest and other cellular perturbations can result in cell death that may be nonapoptotic/non-necrotic in nature (called apocrotic, aponecrotic, or oncotic necrosis; mechanism described elsewhere in this chapter).

Clinical Relevance

Overactivation of apoptosis causes tissue damage. For example, administration of Fas ligand, exposure to γ-irradiation, or treatment with a high dose of glucocorticoid kills the animals by causing massive apoptosis in the liver or thymus. Hepatitis, insulitis, graft-versus-host disease, and allergic encephalitis are due to the excessive apoptosis by Fas ligand

Table 3 Examples of interactions of drugs and toxicants with apoptosis

Drugs and chemicals	*Physical insults and free radicals*	*Microbes*	*Cytokines*	*Withdrawal from trophic factors*
Acetaminophen, ethanol, chloroform, CCl_4, furosemide, dimethylnitrosamine, doxorubicin, chemotherapeutic agents, glucocorticoids, glutamate, calcium, azide, hydrogen peroxide, propanolol, TCDD, okadaic acid, lead nitrate, vincristin, vinblastin, TPA, PMA, BAP, PAHs	Neutrons, X-rays, β-rays, γ-rays, UV-radiation, heat shock quinones $O_2^{\bullet}$, $NO^{\bullet}$, $OH^{\bullet}$	HIV-1, Sindbis Baculo virus, influenza virus-A, human papilloma virus, Reo virus, Epstein–Barr virus, *Escherichia* spp., *Yersinia* spp., *Salmonella* spp., *Propionibacterium* spp., fungal toxins (ochratoxin A and fumonisins)	TNF-α, TGF-β, some ILs	Glucose, growth factors (interleukin-2, interleukin-3, interleukin-10, interleukin-13, granulocyte-macrophage colony stimulating factor, granulocyte stimulating factor, fibroblast growth factor, transforming growth factor β1, neurotrophic factor), hormones (estrogen, androgen, progesterone, ACTH)

expressed on cytotoxic lymphocytes. Apoptotic cells are detected in the brain of ischemia or Alzheimer patients, suggesting that apoptosis is at least in part responsible for the disease manifestification of these patients. A proper dose of anti-cancer drugs or γ-irradiation can kill cancer cells by activating the apoptotic death program in the target cells. Some cancer cells are resistant to these drugs by an unknown mechanism. It is hoped that elucidation of the molecular mechanisms of apoptosis leads to development of an efficient cancer therapy.

A disadvantage to the organism of the mechanism that necessitates signaling to prevent apoptosis is that its failure by mutation can lead to the survival of unwanted cells, which – paradoxically – can lead to death of the organism itself. On the other hand, an opportunity is presented by such a mechanism to allow investigators to devise means for targeting unwanted cells for destruction. Prostate cancer is an example. The survival of prostate cells is dependent upon androgens; androgen depletion leads to a reduction in cell number by apoptosis. Recently, the dependence of prostate cells on androgens to avoid cell death has been exploited therapeutically by the use of androgen ablation to invoke apoptosis in prostate cancer cells and prolong survival in men with prostate cancer.

Another noteworthy example is nonvirally produced cancer cells perplexing host tissues to bypass apoptosis. Some B-cell leukemias and lymphomas express high levels of Bcl-2, thus blocking apoptotic signals they may receive. The high levels result from a translocation of the *BCL-2* gene into an enhancer region for antibody production. Melanoma (the most dangerous type of skin cancer) cells avoid apoptosis by inhibiting the expression of the gene encoding Apaf-1. Some cancer cells, especially lung and colon cancer cells, secrete elevated levels of a soluble 'decoy' molecule that binds to FasL, plugging it up so it cannot bind Fas. Thus, cytotoxic T cells (CTL) cannot kill the cancer cells. Other cancer cells express high levels of FasL, and can kill any cytotoxic T cells (CTL) that try to kill them because CTL also express Fas (but are protected from their own FasL). The hallmark of AIDS (acquired immunodeficiency syndrome) is the decline in the number of the patient's $CD4^+$ T cells. What causes the disappearance of $CD4^+$ T cells is a mystery! All T cells, both infected and uninfected, express Fas. Expression of a HIV gene (called *Nef*) in a HIV-infected cell causes the cell to express high levels of FasL at its surface while preventing an interaction with its own Fas from causing it to self-destruct. However, when the infected T cell encounters an uninfected one (e.g., in a lymph node), the interaction of FasL with Fas on the uninfected cell kills it by apoptosis. Exploration of a similar interaction may yield to new possibilities of preventing graft rejection and other chemotherapies.

Tools for Apoptosis Detection

There are several methods available for the detection of apoptosis *in vivo* and *in vitro*. *In vitro* methods may include but not limited to the following: (1) flow cytometry, (2) fluorescence microscopy, (3) DNA fragmentation assay (quantitatively by spectrophotometry or spectroflurometry, and qualitatively by agarose gel electrophoresis and single cell gel assay), (4) brightfield and electron microscopy, (5) Tdt-Utp Nick End Labeling assay (TUNEL), (6) Western blot analysis for gene expression, and (7) DNA microarray or apoptosis gene array analysis. *In vivo* methods may include but not limited to the following: (1) Brightfield microscopy (a minimum of X1000), (2) fluorescence microscopy, (3) DNA fragmentation assay (quantitatively by spectrophotometry or spectroflurometry, and qualitatively by agarose gel electrophoresis), (4) electron microscopy, (5) TUNEL assay on tissue sections, (6) Western blot analysis for gene expression, (7) study-specific monoclonal antibodies, and (8) DNA microarray or apoptosis gene array analysis.

Summary

The benefit of a comprehensive knowledge of cell death is not easily predicted but clearly stands to be immense. So, what has propelled apoptosis into the forefront of basic research? Widespread involvement of apoptosis in diverse normal physiological and disease conditions gives rise to numerous hopes suggesting that targeting this response will lead to the development of novel therapeutic regimens. The fact that apoptosis is present in tumors suggests that its induction could be used as a therapy. The ability to modify sensitivity to apoptosis through the regulatory pathways has clear implications for the treatment of malignancy. Potential strategies fall into three categories: direct induction of apoptosis by cytotoxic agents, enhancing vulnerability to apoptosis to increase the efficacy of other therapies, and boosting the resistance of normal cells to apoptosis. Among these direct antitumor therapies targeting apoptotic modulation may prove to be much less systemically toxic than standard chemotherapy and could also be used in an adjuvant manner, to increase the susceptibility of tumors at the time they are exposed to chemotherapy.

The field apoptosis has captured worldwide attention of biomedical scientists is attested by the fact

that the 2002 Nobel Prize in Physiology and Medicine was awarded to Sydney Brenner (Great Britain), H. Robert Horvitz (US), and John E. Sulston (GB) for their discoveries concerning genetic regulation of PCD.

See also: Cell Cycle; Cell Proliferation.

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Aquatic Ecotoxicology

See Ecotoxicology, Aquatic.

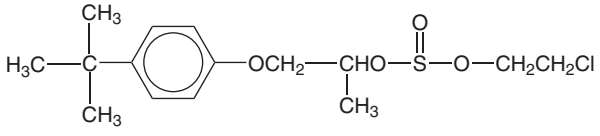
Aquatic Toxicity Testing

See Toxicity Testing, Aquatic.

Aramite

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 140-57-8
- Synonyms: 2-(*p*-Butyl phenoxy)-1-methylethyl-2-chloroethylsulfite; 2-(*p*-Butyl phenoxy) isopropyl-2-chloroethyl sulfite; Aracide; Aramit; Aratron; Ortho-mite
- Chemical Structure:



Uses

Aramite was formerly used as an antimicrobicide agent and as a miticide.

Exposure Routes and Pathways

Although exposure through oral consumption of contaminated fruits is possible, it should no longer be occurring since the use of aramite has been discontinued voluntarily on the basis of oncogenicity according to the US Environmental Protection Agency. Occupational exposure through dermal contact and inhalation of aerosols and dusts is possible.

Acute and Short-Term Toxicity (or Exposure)

Animal

A large oral dose causes central nervous system depression of long duration in laboratory mammals. The principal autopsy finding was hemorrhagic syndrome involving particularly the lung. Undiluted aramite and its concentrated solution are irritating to the skin and conjunctiva of experimental animals. Aramite has been found to give electroretinographic indications of intoxication of retinal photoreceptors when injected into mice and when applied to the eyeball.

Human

Acute exposure to aramite in an undiluted form may cause skin irritation.

Chronic Toxicity (or Exposure)

Animal

Increased incidence of liver tumors and/or neoplastic nodules in three strains of male and female rats and males of one strain of mice, and extrahepatic biliary system tumors were noted in dogs following chronic oral exposure.

Human

This compound is classified as a probable human carcinogen (classification B2) based on insufficient human data. No data are available on the number of workers who were actually or potentially exposed to aramite during its manufacture and formulation. The lowest published lethal dose/concentration in humans is $429\,mg\,kg^{-1}$.

Environmental Fate

Aramite can be released directly into the environment through its use as an acaricide; however, this use has been discontinued. If released to soil, aramite is not expected to leach. If released to water, it may sediment in water. Insufficient data are available to predict the relative importance of chemical or biological degradation processes in soil or water. If released into air, aramite is expected to be physically removed by deposition processes such as rainfall.

Miscellaneous

Aramite is a clear light-colored oil with a melting point of $-31.7^{\circ}C$ and a boiling point of $175^{\circ}C$ at 0.1 mmHg. It is noncorrosive and has a specific gravity of 1.145 at $20^{\circ}C$. It is practically insoluble in water and is miscible with many organic solvents. When heated to decomposition, it emits highly toxic fumes of chlorides and oxides of sulfur [SO(X)].

See also: Pesticides.

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Systems, National Library of Medicine. Search for Aramite.

Arsenic

Robert Kapp

This article is a revision of the previous print edition article by Arthur Furst, Shirley B Radding, and Kathryn A Wurzel, volume 1, pp. 80–82, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-38-2
- Synonyms: Arsen; Arsenic black; Arsenic-75; Arsenicals; Colloidal arsenic; Gray or grey arsenic; Metallic arsenic
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: As^{3+}; As^{5+}

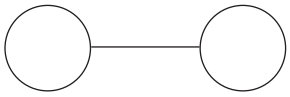
Uses

Arsenic was used as one of the earliest poisons and it is still used in some sheep dips, rat poisons, wood preservatives, weed killers, and other pesticides. It has been used in bronzing operations and is presently in use in pyrotechnics, in electronic devices, and as a laser material in converting electricity directly into coherent light. For many decades, arsenic was used medicinally to treat syphilis. Certain alloys of arsenic are used in making designer glass. The largest consumption of arsenic in the United States is in wood preservatives, especially in chrome copper arsenate (CCA), $CrO_3 \cdot CuO \cdot As_2O_5$ treated wood.

Background Information

In its elemental form, arsenic is an odorless, tasteless semimetallic compound, which appears steel gray in color, is brittle and is a crystalline solid. The symbol is As; the atomic number is 33. Arsenic compounds were mined by the early Chinese, Greek, and Egyptian

civilizations. There are many different forms of arsenic widely distributed in nature including the trivalent and pentavalent forms. It is believed that Albertus Magnus discovered the element in AD 1250. He obtained it by heating soap together with orpiment (arsenic trisulfide, As_2S_3). Arsenic is one of the elements that has an alchemical symbol, shown below (alchemy is an ancient pursuit concerned with, for instance, the transformation of other metals into gold):



Exposure Routes and Pathways

The primary exposure pathway for arsenic exposure is ingestion of water or food (including some wines). Inhalation exposure is a minor component and dermal absorption is negligible. Some food and wine exposures result from the use of arsenic-containing pesticides; however, the primary source is drinking water in places where the natural arsenic content of the water is high. Occupational exposure is generally associated with smelting industries and the manufacture of arsenic-containing compounds.

Arsenic is also still used for murder by poisoning, for which use it has a long and continuing history in literature and real life.

Toxicokinetics

Soluble forms of arsenic (such as arsenite) are readily absorbed from the gastrointestinal tract and the lungs. Less soluble compounds such as arsenic selenide, lead arsenate, and gallium arsenide are less efficiently absorbed. Absorbed arsenic is widely distributed in the body concentrating in the liver, kidneys, lungs and skin. Chronic exposure may result in hair, nail and skin accumulation, which is reflected in Mees' lines (transverse white bands across the fingernails). These lines can help estimate the time of onset of arsenic exposure based upon the rate of nail growth, which is $\sim$0.1 mm per day. In addition, arsenic tends to accumulate in the skin and can be found in sweat of exposed individuals. Metabolism of ingested arsenic compounds results in the excretion of methylated arsenic. The half-life of ingested inorganic arsenic is $\sim$10 h with up to 80% excreted in 3 days or less while the half-life of ingested organic arsenic is $\sim$30 h.

Mechanism of Toxicity

Arsenic affects mitochondrial enzymes and impairs tissue respiration. This appears to be related to the cellular toxicity of arsenic. Arsenic reacts primarily with enzymes containing two thiol groups. Some sulfhydryl-containing proteins and enzymes are functionally altered when exposed to arsenic. When arsenic accumulates in the mitochondria, the respiration mediated by the NAD-linked substrates results in a reaction between the arsenite ion and the dihydrolipoic acid cofactor, which is needed for the oxidation of the substrate. It is further shown that arsenic inhibits succinic dehydrogenase activity resulting in stimulation of ATPase activity by uncoupling oxidative phosphorylation. Studies have shown that the addition of glutathione and British anti-Lewisite (BAL) can reverse some of these arsenic changes. In addition, inhibition of mitochondrial respiration is shown to increase the production of hydrogen peroxide, which in turn, may cause the production of reactive oxidative species (ROS). ROS result in the induction of major stress protein families. Arsenical-induced oxidative stress and ROS may be critical factors in mediating DNA damage and initiation of carcinogenesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values for elemental arsenic in rats and mice are 763 and 145 mg kg^{-1}, respectively. Arsenic compounds can have vesicant effects, which means they can cause blisters, irritation, and necrosis in exposed tissues, whether internal or external.

Human

Ingestion of large concentrations ($>$70 mg) of arsenic may be fatal. Symptomology varies and may include fever, anorexia, nausea, vomiting, difficulty swallowing, cardiac arrhythmia, damage to the mucous membranes, respiratory tract distress, peripheral neuropathy, and hematopoietic effects. Sensory loss in the peripheral nervous system is the most common effect appearing 1–2 weeks postexposure. Symptoms of anemia, neuropathy and leukopenia are reversible once exposure is terminated.

Chronic Toxicity (or Exposure)

Animal

High doses of inorganic arsenic have produced various developmental malformations in animals. Animal data suggest that inorganic arsenite rather than methylated metabolites of arsenic caused the developmental abnormalities.

It has been difficult to demonstrate arsenic carcinogenicity in animal experiments. Oral and dermal administrations of arsenic trioxides and pentoxides have not resulted in carcinogenic outcomes in animal studies. Likewise, animal studies on organic arsenic compounds have been negative. Inorganic arsenic compounds induce deletion mutations and some chromosomal abnormalities, but no point mutations.

Human

Chronic exposure to arsenic may result in neuropathy in both the peripheral and central nervous system. The result is generally muscle weakness progressing from proximal to distal muscle groups. Chronic exposures produce much more gradual effects that can occur over many years and are clinically difficult to detect. Liver injury is also characteristic with chronic exposure first manifesting itself as jaundice and subsequently progression to cirrhosis and ascites. Peripheral vascular disease has been observed in some individuals exposed to high arsenic concentration in drinking water in Taiwan and Chile. These effects included Raynard's phenomenon and gangrene of the lower extremities (blackfoot disease) and are related to the cumulative dose of arsenic.

The skin also appears to be a critical target of arsenic toxicity. Dermatitis is observed with erythema followed by itching and swelling with a mottled appearance. Melanosis (abnormal pigmentation) subsequently appears at various points on the body often followed by hyperkeratosis (thickening of the skin).

Arsenic has also been shown to cross the placenta in pregnant women. Much of the arsenic was in the form of dimethyl arsenic, suggesting that there is an increase in methylation during pregnancy.

Hutchinson recognized the carcinogenic potential of arsenic long ago in 1887 when an unusual number of skin cancers occurred in patients treated with arsenicals. The International Agency for Research on Cancer, MAK, National Institute for Occupational Safety and Health, Occupational Safety and Health Administration (OSHA), National Toxicology Program (NTP), and the Environmental Protection Agency (EPA) have all classified arsenic as a human carcinogen based on sufficient evidence from epidemiological studies, that arsenic causes skin cancer and lung cancer. The hyperkeratosis that is seen in the skin of chronically exposed individuals can lead to basal cell carcinomas and/or squamous cell carcinomas. Angiosarcoma has been reported in vineyard workers chronically exposed to arsenic in drinking water, patients exposed to Fowler's solution, and agricultural workers exposed to various arsenic-containing pesticides. Lung cancers have been reported among copper smelter workers. The mode of action of arsenic carcinogenesis remains elusive.

Clinical Management

Syrup of ipecac (purging solution) and gastric lavage should be administered within 4–6 h of oral exposure to arsenic. Antidotes include 3–5 $mg\,kg^{-1}$ BAL (2,3-dimercaptopropanol) administered intramuscularly. Penicilamine has also been administered with optical neuritis as a side effect. Certain synthetic, water-soluble dimercapto compounds (DMSA – meso-2, 3-dimercaptosuccinic acid and 2,3-dimercaptopropane-1-sulfonate) have been found effective.

Environmental Fate

Arsenic is released to the atmosphere as a result of smelting of ores, incineration of arsenic containing materials and blowing of arsenic containing soils. It is usually found in air as a mixture of the trivalent and pentavalent forms. Arsenic can be transported significant distances and then will settle out or be carried to the earth's surface in rain or snow. Once arsenic reaches water from the atmosphere, runoff, discharges or other sources, it can be converted to a variety of forms; generally, arsenate is the predominant one. In addition, aquatic microorganisms can convert arsenate to arsenite and can methylate arsenic. Since arsenic is a natural component of the earth's crust, it is ubiquitous in soil. In some places, natural soil arsenic levels are high and this can lead to elevated groundwater arsenic levels.

Arsenic is found in a variety of foods, with seafood, meats, and grains generally showing the highest levels. Most of this arsenic is in the organic form and so does not pose a health risk. Arsenic is also found in plant tissues, often as a result of pesticide applications.

Exposure Standards and Guidelines

OSHA has established permissible exposure limits (PELs) for both inorganic and organic arsenic compounds. For the inorganic compounds, the PEL time-weighted average is $10\,\mu g\,m^{-3}$ and for the organic ones, it is $500\,\mu g\,m^{-3}$ ($0.05\,mg\,m^{-3}$). Although the current US EPA maximum contaminant limit (MCL) for arsenic in drinking water is $50\,mg\,l^{-1}$, the agency has recently adopted a new standard, an MCL of $10\,mg\,l^{-1}$, which will take effect on January 23, 2006.

See also: Metals; Neurotoxicity; Pollution, Soil; Pollution, Water; Sister Chromatid Exchanges; Skin.

Further Reading

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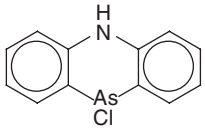
Arsenical Vomiting Agents

Harry Salem, Bryan Ballantyne, and Sidney A Katz*

Published by Elsevier Inc.

Adamsite (DM)

- Chemical Abstracts Service Registry Number: CAS 578-94-9
- Synonyms: Diphenylaminearsine; Diphenylaminochlorarsine; 10-Chloro-5,10-dihydrophenarsazine; White Cross Gas; Phenarsazine chloride
- Description: It was first synthesized in 1915 by a German chemist Weiland, and then again in 1918 by US chemist Robert Adams who named it adamsite. DM is a yellow-green, odorless crystalline solid that is not very volatile. It is insoluble in water and relatively insoluble in organic solvents
- Chemical/Pharmaceutical/Other Class: It is classified as a chemical warfare vomiting agent
- Chemical Formula: $C_{12}H_9AsClN$
- Chemical Structure:



Uses

Adamsite is used as a vomiting agent. It is considered insufficiently toxic for use in war, but too potent for control of civilian disturbances. Thus, it was banned in 1930 for use against civilians. Adamsite (DM) has found extensive use as a pesticide for treatment of wood against insects.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Exposure Routes and Pathways

Normally a solid, but upon heating DM first vaporizes and then condenses to form an aerosol. It is toxic through inhalation, ingestion, and skin contact. It irritates the eyes and respiratory tract, but not necessarily the skin.

Toxicokinetics

By any route of administration, the effects are slower in onset and longer in duration than typical riot control agents such as CS (*o*-chloro-benzylmalononitrile). Vomiting agents are irritants upon initial exposure. The slow onset for DM allows for the absorption of much more DM before a warning is perceived. The estimated threshold concentrations for irritation of the throat, lower respiratory tract, and initiation of the cough reflex are 0.38, 0.5, and 0.75 $mg\,m^{-3}$, respectively.

Mechanism of Toxicity

DM's primary action is on the upper respiratory tract, causing irritation of the nasal mucosa and nasal sinuses, burning in the throat, tightness and pain in the chest, and uncontrollable coughing and sneezing. It also causes eye irritation and burning, with tearing, blepharospasm, and injected conjunctiva.

DM is more toxic than other riot control agents; the LCt_{50} for humans has been estimated to be 11 000 $mg\,min\,m^{-3}$. The amount that is intolerable

for humans has been estimated by some to be 22 mg min m^{-3} and by others to be 150 mg min m^{-3}. The threshold for irritation in humans is about 1 mg m^{-3}, but some people have tolerated Ct exposures of 100–150 mg min m^{-3}.

This class of compounds is unique among the riot control agents because the effects do not appear immediately on exposure or seconds afterwards, but rather several minutes later.

The other characteristic of these compounds is that there may be more prolonged systemic effects, including headache, mental depression, chills, nausea, abdominal cramps, vomiting and diarrhea, which last for several hours after exposure. The Ct necessary to cause nausea and vomiting has not been established, but is estimated to be about 370 mg min m^{-3}.

DM is considered less effective as a riot control or incapacitating agent than CS and CN (chloroacetophenone), and it has been conjectured that there are greater differences in susceptibility among people to DM than to the other agents. DM, like CS, is considered to be a cholinesterase inhibitor, which may be responsible for its lacrimatory effect. DM also has a direct effect on gastric activity, but the evidence suggests that the lethal effect is respiratory related.

Toxicity (or Exposure)

Animal

Various animal species including monkeys have been exposed to DM. Following acute exposures, the animals exhibited ocular and nasal irritation, hyperactivity, salvation, labored breathing, ataxia, and convulsions.

Histopathology did not reveal any abnormalities at exposure dosages of below 500 mg min m^{-3}. At higher dosages, animals that died or were killed demonstrated hyperemia of the trachea, pulmonary congestion and edema, and pneumonia. These effects were consistent to exposure to pulmonary irritants. DM toxicity values are given in **Table 1**.

Monkeys have been exposed to varying concentrations and durations. At a Ct dosage of 2565 mg min m^{-3} only one animal responded, and that was with oral and nasal discharges and a diminished response to stimuli. A Ct of 8540 mg min m^{-3} resulted in ocular and nasal conjunctival congestion, facial erythema, and decreased responses, all of which were resolved within 24 h. Exposure to the high dosage of 28 765 mg min m^{-3} resulted in hyperactivity, copious nasal discharge, conjunctival congestion, marked respiratory distress, as well as gasping and gagging in all the exposed monkeys. Eight of these exposed monkeys died within 24 h of exposure. Necropsy of these animals revealed congestion and extremely edematous lungs. Microscopic examination revealed ulceration of the tracheobronchial tree and pulmonary edema.

Table 1 DM toxicity values

Species	*LCt_{50} (mg min m^{-3})*	*Intravenous LD_{50} (mg kg^{-1})*
Mouse	22 400	17.9
2Rats	3 700	14.1
Guinea pig	7 900	2.4

Theoretical dose calculated from respiratory volume, LCt_{50}, and estimated percent retention.

Studies were also conducted in which monkeys were exposed to low target concentrations of 100 and 300 mg m^{-3} DM for 2–60 min and 2–40 min, respectively. The signs of toxicity increased as the duration increased, characteristic of exposure to irritants. At the maximum dosage of 13 200 mg min m^{-3}, the animals exhibited nausea and vomiting, oral and nasal discharge, and conjunctival congestion. Only blinking was noted below 1296 mg min m^{-3}.

The effects of DM on the gastrointestinal tract were suggested as a possible cause of death. Dogs were dosed both intravenously and orally with lethal doses of DM, while central venous pressure, right ventricular pressure, cortical electric activity, alveolar CO_2, respiratory rate, heart rate electrocardiogram, and gastric activity were monitored. DM caused a marked elevation of both amplitude and rate of gastric activity for 15–20 min, and then returned to normal. Pretreatment with trimethobenzamide, an effective antiemetic for peripheral and centrally acting emetics, did not prevent DM gastric activity; however, pretreatment with chlorpromazine was effective. The authors concluded that DM affects the stomach directly, and that the primary cause of death following exposure to DM is its effects on the lungs.

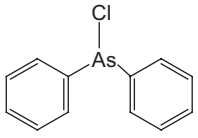
The effects of DM on the eyes and skin of rabbits were studied. DM was suspended in corn oil and instilled into the eyes of rabbits in doses of 0.1, 0.2, 0.5, 1.0, and 5.0 mg. No effect was observed at 0.1 mg, but mild conjunctivitis was observed at 0.2 mg. At 0.5 mg, mild blepharitis was also seen. Corneal opacity persisted over the 14 day observation period in rabbit eyes that were dosed with 1.0 and 5.0 mg. Corn oil suspensions of DM (100 mg ml^{-1}) were placed on the clipped backs of rabbits at doses of 1, 10, 50, 75, and 100 mg. Necrosis of the skin was observed at 10 mg and higher. The skin sensitization potential of DM in guinea pigs was negative.

Other Arsenical Vomiting Agents

The other arsenical vomiting agents include: diphenylchlorarsine (DA) and diphenylcyanoarsine (DC).

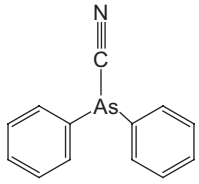
Diphenylchlorarsine (DA)

- Chemical Abstracts Service Registry Number: CAS 712-48-1
- Synonyms: Clark I
- Chemical/Pharmaceutical/Other Class: It is classified as a chemical warfare vomiting agent
- Chemical Formula: $C_{12}H_{10}AsCl$
- Chemical Structure:



Diphenylcyanoarsine (DC)

- Chemical Abstracts Service Registry Number: CAS 23525-22-6
- Synonyms: Clark II
- Chemical/Pharmaceutical/Other Class: It is classified as a chemical warfare vomiting agent
- Chemical Formula: $C_{13}H_{10}AsN$
- Chemical Structure:



See also: Arsenic; Riot Control Agents.

Relevant Websites

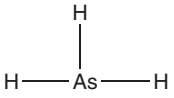
http://www.bt.cdc.gov – (US) Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – (US) National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Arsine

Felix Ayala-Fierro

- Representative Chemicals: Arsine; Monomethyl arsine; Dimethylarsine; Trimethylarsine
- Chemical Abstracts Service Registry Number: CAS 7784-42-1
- Synonyms: Arsenic hydride; Arsenic trihydride; Arsenous hydride; Hydrogen arsenide
- Chemical/Pharmaceutical/Other Class: Inorganic compounds
- Chemical Formula: AsH_3
- Chemical Structure:



Uses

Arsine is used commercially by the electronics industry for epitaxial growth of gallium arsenide and as a dopant applied to ultrapure crystals to increase electrical conductivity for silicon-based electronic devices.

Background Information

Arsine gas is a potent hemolytic agent. It was first identified in 1775 and since then recognized as one of the most potent and least inherently detectable gaseous toxicants. It can be formed when acid or base contacts inorganic arsenic and elemental metals like aluminum, zinc, and others. This makes possible the formation of arsine in the environment in such places as hazardous waste dumpsites. The current occupational exposure limit for arsine is 50 ppb; however, American Conference of Governmental Industrial Hygienists (ACGIH) placed arsine in the '2003 under study' list. In 2004, it listed arsine in the 'notice of intended changes' list, with a proposed (trial value) of 5 ppb.

Exposure Routes and Pathways

Arsine is highly poisonous, colorless, nonirritating, flammable, and with slight garlic odor at levels 10-fold greater than Occupational Safety and Health Administration (OSHA)/ACGIH limit values. Human exposure occurs via inhalation. Since arsine is used in the semiconductor industry, human exposure to arsine can occur from accidental release of the gas during these manufacturing processes or from accidental generation

from arsenic-contaminated substances, such as wastes from mining, smelting, refining, soldering, galvanizing, painting, and during application of herbicides. The reaction occurs when caustic or acidic solutions are used to clean arsenic-containing material in the presence of metals such as zinc or aluminum. The continuous inhalation of 250 ppm ($800\,mg\,m^{-3}$) is fatal, although lower levels (10 ppm) for longer time periods (up to 2 h) can be also lethal.

Toxicokinetics

Arsine gas is quickly absorbed through the lungs. Since arsenic in arsine is the fully reduced form of arsenic ($-$III), it will be oxidized in the presence of oxygen. Arsine may oxidize in several ways. One is to produce superoxide and arsenous acid (As(III)) as illustrated in this equation: $AsH_3 + 6O_2 + 2H_2O \rightarrow 6O_2^- + 6H^+ + HAsO_2$. The standard reduction potential, E'_0, for this reaction at physiological pH is $+0.31$ V. From the lungs arsine rapidly diffuses and dissolves in the blood, where it produces hemolysis. If dissolved in the plasma, it is possible that arsine reacts with water in the plasma (low probability) as depicted in this reaction: $AsH_3 + 2H_2O \rightarrow HAsO_2 + 3H_2$; $E_0 = +0.189$ V. In blood, it binds to hemoglobin. This reaction results in heme oxidation and may probably happen in a series of two electron steps: As($-$III) to As($-$I) to As($+$I) to As($+$III). As arsine oxidizes, oxygen, water, or biological molecules could be the electron acceptors in cell systems. Blood distributes arsine, hemolytic products, and arsine metabolites to other tissues, such as the liver and kidney. The mechanism of toxicity in these organs is not completely known. The resulting arsenicals from arsine exposure are eliminated in urine.

Mechanism of Toxicity

Hemolysis constitutes the main effect for arsine toxicity, a process which requires oxygen, hemoglobin containing the reduced iron, and access to the heme–ligand binding. This effect is due to a loss of membrane integrity that is characterized by a loss of intracellular potassium and influx of extracellular sodium (reversible) and calcium (irreversible). The influx of calcium is responsible for the morphologic changes within the erythrocyte. The mechanism of toxicity seems to be via oxidation of key sulfhydryl-containing ion gradients (transport pathways) across the cell membrane altering ion permeability. A single transport pathway has not been identified. In liver and kidney, arsine is oxidized to very small amounts of arsenite and arsenate. Liver toxicity seems to be caused by a similar mechanism as in blood; that is, disruption of the cell membrane, a process that may be related in part to an unknown metabolite and may involve hemoproteins. In the kidney, arsine produces early toxicity on endothelial cells from glomerular capillaries and peritubular microvessels, causing compromised filtration and edema. At a later point the presence of insoluble hemolytic products along with arsine metabolites would lead to oliguric renal failure.

Acute and Short-Term Toxicity (or Exposure)

Animal

The 10 min median lethal concentrations (LC_{50}s) reported in the literature for rats and rabbits are 120–210 and 200–300 ppm, respectively. The lethal effect of arsine is dependent on exposure concentration and duration. The rat LC_{50} at 0.5, 1 and 4-h exposures is 240, 178, and 45 ppm, respectively. Female rats have slightly greater mortality than males. The effects in animals include dyspnea, hematuria, dark material around the head or anogenital area, and pallor of ears and eyes. During necropsy the animals showed red, yellow, or orange fluid in the bladder, stomach, or intestine, and discoloration of the kidneys, lungs, and liver. Most of the available data come from experiments in rats; however, some authors state that the rat is not a suitable model for arsine toxicity because of differences in arsenic methylation and excretion compared to humans.

Human

The human literature on arsine primarily consists of case reports; therefore, data on arsine effects versus concentration in air do not exist. At the time of exposure there is no discomfort due to the nonirritating characteristics of arsine. Concentrations of arsine as low as 3–10 ppmv (parts per million volume) have been associated with symptoms. Within 24 h these symptoms include headaches, dizziness, weakness, dyspnea, abdominal cramping, nausea, vomiting, and hematuria (dark red urine). After 2 weeks of exposure renal effects are evident (morphological and functional changes) due to a direct arsine effect and the presence of a massive quantity of hemoglobin in the nephron. In severe cases oliguric renal failure is the final effect. Other effects normally reported include abnormalities of the nervous system (central and peripheral), cardiovascular (electrocardiographic changes due in part to electrolyte disturbances), pulmonary (edema), and immune system. Arsine is oxidized to inorganic arsenicals, which results in elevated levels of arsenic in the body and eventually elevated urinary excretion.

Chronic Toxicity (or Exposure)

Animal

The main chronic toxic effect in animals exposed to arsine for 28–90 days is in the hematopoietic system, including a decrease in packed erythrocyte volume and a peripheral erythrocyte regenerative response. The reproductive and developmental toxicity has not been completely studied. Rats exposed to 2.5 ppm arsine 6 h day^{-1} on gestation days 6–15 exhibited an increase in fetal body weight.

Human

The main chronic effect of arsine exposure (low dose) in humans is anemia. Repeated exposure to arsine may also damage the kidneys, liver, heart, and nervous system. There is a concern about the formation of carcinogenic arsenic from long-term exposure to arsine.

In Vitro Toxicity Data

In vitro toxicity studies in the rat indicate that arsine toxicity is tissue-specific. Red blood cells are very susceptible to arsine toxicity, followed by the primary hepatocytes and renal cortical epithelial cells. In blood arsine is the only factor responsible for hemolysis whereas in other tissues it is only responsible for the early signs of toxicity. At later points the toxicity effect is a combination of many other factors, including formation of inorganic arsenicals and hemolysate (kidney toxicity).

Clinical Management

In acute exposure prompt medical attention is critical. The victim should be immediately removed to fresh air and away from the source of exposure. Oxygen should be provided if there is a respiratory distress. Initial therapy should be directed at stopping the ongoing hemolysis by performing exchange transfusion. Currently there is no other treatment to decrease arsine hemolysis; however, studies *in vitro* have shown that some dithiol chelators (meso-2,3-dimercaptosuccinic acid, DMSA; 2,3-dimercapto-1-propanesulfonic acid, DMPS; and 2,3-butanedithiol) are effective (see Further Reading). This should be followed by aims to restore renal function or compensate for lost renal function (hemodialysis). This process does not remove any formed arsenic from the exposed body. Administration of dimercaprol (British Anti-Lewisite, BAL) has no effect on arsine hemolysis, but it lowers blood arsenic levels resulting from arsine exposure. The use of chelators must be carefully evaluated due to potential side effects. Other treatment that may be considered includes urine alkalinization (pH > 7.5 using sodium bicarbonate), monitoring of serum electrolytes, hemoglobin, and creatinine, and supportive care to improve oxygenation of the body.

Environmental Fate

Arsine accidentally released in air or water will be rapidly diluted and oxidized to other arsenicals. The final oxidized state would be arsenate (thermodynamically more stable). A small percentage would remain in water whereas the rest would be distributed along the sediment zones.

Ecotoxicology

There is no aquatic toxicity information available for arsine. However, one predicts arsine oxidation to small quantities of inorganic arsenicals in water.

Other Hazards

Arsine and arsine gas/air mixtures are flammable and explosive. The explosive limits (% by volume in air) are 4.5% and 78% for lower and upper, respectively. The gas is heavier than air and may ignite at distant ignition sources and flash back. Poisonous gases are produced during a fire. Arsine is incompatible with oxidants and oxidizing agents.

Exposure Standards and Guidelines

Arsine was placed under revision for the 2003 ACGIH threshold limit values (TLVs) because

Table 1 Summary of exposure criteria for arsine

Agency	*Criteria*		*Averaging time*
ACGIH	TLV – TWA	0.05 ppm	8 h/40 h week
NIOSH	IDLH	3 ppm	NA
NIOSH	Ceiling	0.002 mg m^{-3}	15 min sampling period
OSHA	PEL (TWA)	0.05 ppm (0.2 mg m^{-3})	8 h/40 h week

Conversion: 1 ppm = 3.19 mg m^{-3}.
OSHA, Occupational Safety and Health Administration; NIOSH, National Institute of Occupational Safety and Health; ACGIH, American Conference of Governmental Industrial Hygienists; IARC, International Agency for Research on Cancer; NTP, National Toxicology Program; TLV – TWA, threshold limit value – time-weighted average; IDLH, immediately dangerous to life or health; PEL, permissible exposure limit.

OSHA, National Toxicology Program (NTP), and International Agency for Research on Cancer (IARC) classify its metabolites (arsenic and arsenic inorganic compounds) as human carcinogens. The 2002 ACGIH proposal requested a new arsine TLV – TWA of 3 ppb and a designation as A1 confirmed human carcinogen. However, in 2004 ACGIH listed arsine in the 'notice of intended changes', with a proposed (trial value) of 5 ppb and a designation A4 not classifiable as a human carcinogen. Arsine is also reported in the List of Highly Hazardous Chemicals, Toxics, and Reactives (Mandatory), OSHA 1910.119 App A. This list contains all chemicals which present a potential for a catastrophic event if present at or above the threshold quantity (TP). The TP for arsine is 100 lbs. The current exposure standards and guidelines are summarized in **Table 1**.

See also: Arsenic; Arsenical Vomiting Agents.

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Arts and Crafts Materials and Processes

Angelique Dosh

Introduction/Background

Art and craft materials include those substances that are used to create works of visual or graphic art. Art processes include activities such as painting, printmaking, photography, pottery, and sculpting. The Consumer Product Safety Commission (CPSC) has defined two main categories of art materials. The first category includes products that become a component of the work of art such as crayons, paint, clay, ink, etc. The second includes products that are closely associated with the creation of art such as solvents, brush cleaners, mold making materials, and photo developing chemicals. Many of these products are composed of or contain substances that have the potential to cause both acute and chronic health hazards. Users may be exposed through the skin, via inhalation (volatile or particulates) or by accidental/incidental ingestion. Engaging in art processes may pose certain health risks, particularly in children. Use of these materials may pose a greater risk to children for several reasons: first, because of their age and size (smaller body mass), they may be more susceptible to the effects of a particular toxicant than adults; second, they may have a greater likelihood of ingestion because of behavior patterns (such as putting their hands in their mouths, not washing hands, or not washing hands thoroughly before eating); and lastly the widespread use of art materials in schools, which leads to more frequent exposures. Proper labeling of art materials is required by law to notify consumers about the risks associated with them.

Toxicity of Art Materials

Paint

Paints often contain solvents such as toluene, xylene, halogenated aromatic hydrocarbons, and methylene chloride, as well as heavy metals in their pigments including: chromium yellow, lemon yellow (barium chromate), vermilion red (cadmium and mercuric sulfides), and flake white (lead). Both acute and chronic exposures to toluene and xylene are associated with neurotoxicity and can also damage the liver and kidneys.

Toluene and xylene have inhalation reference concentrations (RfCs) of 0.4 and 0.1 $mg\,m^{-3}$, respectively. The RfC is an estimate of a daily inhalation exposure of the human population that is likely to be without an appreciable risk of harmful effects over a

lifetime. Methylene chloride is a liver toxicant with a no-observed-adverse-effect level (NOAEL) of $5.85\,mg\,kg^{-1}\ day^{-1}$ (rat). The NOAEL is the highest concentration of a substance that has been shown through testing to cause no-adverse-observed health effects in humans or animals. Barium and chromium are confirmed human carcinogens and cadmium is a probable carcinogen. Mercury and lead are highly neurotoxic and their effects on humans have been well documented.

Ceramics

Clay used in ceramics is usually composed of powdered aluminum silicates. It has been found that long-term exposures from inhaling silica dust can result in silicosis. Potters may handle dry clay and respirable dust may accumulate in areas where clay is routinely used. Ceramic glazing components usually consist of silica and a flux. The flux that is often used in ceramics may include heavy metals such as lead or barium. Glazing components are mixed with water and then brushed onto a piece of pottery prior to firing it. Exposure to the fumes from firing pottery with lead glazes may result in lead poisoning, particularly among children. Glazing components may also contain metal oxides such as arsenic, beryllium, cadmium, chromium, and nickel for color. Arsenic, nickel (dust), and chromium(VI) are all classified as known human carcinogens and beryllium and cadmium are probable human carcinogens.

Printmaking

Printmaking (lithography, intaglio, photoetching, relief printing, and screen printing) involves printing or transferring images onto various media (metal plates, wood, etc.). Pigments that are often used in creating the images include cadmium, zinc chromate, lead chromate, and strontium. Acids such as hydrochloric, acetic, hydrofluoric, tannic, phosphoric, and nitric, which are all corrosive, are used to etch the plates. Toluene, xylene, trichloroethylene, and kerosene are often used to clean printmaking equipment.

Other Art Materials

There are many other art materials that contain potentially hazardous substances. For example: magic markers often contain volatile organic compounds; glues and adhesives can contain solvents such as toluene, methyl ethyl ketone, acetone, and hexane; and instant papier-mâché and some modeling compounds may contain asbestos. In addition, many products pose an inhalation hazard due to the fine powders they are composed of, or the dusts they generate. Examples of these are powdered dyes, wood, and fibers from materials used in spinning and weaving.

Legislation

Prior to the early 1980s, the Federal Hazardous Substances Act (FHSA) was the only regulation governing art materials. The FHSA required consumer products (which included art materials) to be tested only for acute toxicity and labeled according to their associated hazard. Those products which are considered acutely hazardous must have warning labels with the following information: name and address of manufacturer, packer, distributor, or seller; common or chemical name of the substance; the signal word 'DANGER' for extremely flammable, corrosive, or highly toxic substances; the signal word 'WARNING' or 'CAUTION' on other hazardous substances; a statement of major hazards; precautionary measures; first-aid instructions; the word 'POISON' for highly toxic substances; special handling and storage instructions; the statement 'Keep out of reach of children'.

During the early 1980s, a group which included art material manufacturers, of artist organization representatives, and health experts came together to develop what is now the American Society of Testing and Materials (ASTM) D-4236: Standard Practice for Labeling Art Materials for Chronic Health Hazards. This was a voluntary standard, which to comply with, the formulation of an art material would need to be evaluated by a toxicologist for potential chronic hazards and then labeled accordingly.

On November 18, 1988 the Labeling of Hazardous Art Materials Act (Public Law 100-695, also known as LHAMA) was passed by Congress and signed into law by the President. This law, which is enforced by the CPSC, amended the FHSA to require manufacturers of art and craft materials to determine if their products pose any chronic health hazards and identify those hazards. If a chronic hazard exists, appropriate warning labels must be placed on the product including the ingredients causing the hazard, and directions for safe use.

Benefits to Consumers

When art materials are labeled with appropriate warning statements, consumers are made aware of the hazards associated with them, if any exist. Labeling is especially helpful for parents so that they are able to determine if an art material is suitable for their children to use. If an art material is not labeled

with the statement, 'Conforms to ASTM D-4236', the consumer may want to obtain additional information on product ingredients and potential risks associated with its use.

See also: Cadmium; Chromium; Consumer Product Safety Commission; Lead; Methylene Chloride; Toluene; Xylene.

Further Reading

California Office of Environmental and Health Hazard Assessment. Guidelines for the Safe Use of Art and Craft Materials. California Office of Environmental and Health Hazard Assessment (OEHHA), December 2003.

Consumer Product Safety Commission. Law Requires Review and Labeling of Art Materials Including Children's Art and Drawing Products. Consumer Product Safety Commission – CPSC Document #5016.

Arts and Toxicology *See* Toxicology in the Arts, Culture, and Imagination.

Arum

Susan M Stejskal

This article is a revision of the previous print edition article by Rita Mrvos, volume 1, p. 82, © 1998, Elsevier Inc.

Uses

Historically, some of the plants in the Arum family have been used to treat respiratory illnesses due to some properties as an expectorant, to treat dermal corns due to its irritant properties, and, after repeated processing, as a starch for thickening foods.

Background Information

The name Arisaema is derived from the Greek word Aris (arum) and Haema (blood). Arum constitutes a large family of plants that includes ~2000 species and includes members from the Arum, Arisaema, Calla, Dieffenbachia, and Philodendron genera. Common names included in this family include calla lilies, dumb cane, jack-in-the-pulpit, taro, and skunk cabbage.

Exposure Routes and Pathways

Routes of exposure include accidental ingestion and dermal contact.

Mechanism of Toxicity

Calcium oxalate crystals are commonly found in all members of this family. It is postulated that needle-like calcium oxalate monohydrate crystals with grooved ends are located within specialized cells known as idioblasts. Inside each idioblast is a bundle of raphides, which are ejected from the plant when pressure such as squeezing is applied. These raphides are then embedded in the skin or mucous membranes. There are also other proteolytic enzymes found in various species.

Acute and Short-Term Toxicity (or Exposure)

Animal

Feline ingestion of philodendron results in central nervous system excitability, seizures, renal failure, and encephalitis.

Human

Symptoms include skin irritation consisting of erythema and vesiculation. Ingestion of plant material may result in local mouth or throat irritation and resultant swelling. Salivation and dysphagia may also be present. Systemic effects are extremely rare. Ocular exposure will result in pain and photophobia, followed by eyelid edema and corneal disturbances such as abrasions and chemosis.

Clinical Management

Treatment for dermal exposure should include irrigation of the contaminated area followed by cool compresses. Treatment for oral exposures should consist of removing any plant material from the oral cavity and administering cool liquids. Significant toxicity is rare. Irrigation should be performed in instances of ocular contamination. Further care should be symptomatic and supportive.

See Also: Oxalates.

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http://www.botanical.com – Botanical.com. A Modern Herbal. Mrs. M. Grieve. Botanical: Arum maculatum. Family: N.O. Araceae.
http://earthnotes.tripod.com – Earthnotes Herb Library.

Asbestos

Xuannga Mahini

- Chemical Abstracts Service Registry Number: CAS 1332-21-4 (Other Registry Numbers: CAS 12413-45-5; CAS 77641-59-9)
- Synonyms: Asbestos fiber; Actinolite; Amianthus; Amosite; Amphibole; Anthophylite; Ascarite; Chrysotile; Crocidolite; Fibrous Grunerite; Serpentine; Tremolite
- Chemical/Pharmaceutical/Other Class: Mineral fibers

Uses

Asbestos is a generic name that refers to a group of six naturally occurring fibrous silicate minerals (actinolite, amosite, anthophylite, chrysotile, crocidolite, and tremolite). Asbestos minerals are characterized by fibers or bundles of fine single crystal fibrils. Chrysotile (curved, flexible fibers that can be woven) belongs to the serpentine family, while all others (straight, brittle fibers) belong to the amphibole family. It should be noted that serpentine and amphibole minerals also occur in nonfibrous form and are not asbestos. Chrysotile, known as white asbestos, is the predominant commercial form of asbestos (99% in the United States); amphiboles are of minor commercial importance. Because of its insensitivity to heat and chemical attack, asbestos is widely used in textiles, electrical and sound insulation, ceiling and floor tiles, dry wall, roof shingles, reinforced cement, industrial water filters, gaskets, and automotive brake and clutch linings. Consumptions of asbestos in the United States have been declining for two decades. Roofing products, gaskets, and friction products will continue to be the only significant domestic markets for asbestos in the foreseeable future. Only chrysotile is presently used for manufacturing in the United States. Ninety-four percent of chrysotile consumed was grade 7, a short 3 μm fiber.

A particle visible under phase contrast microscopy (PCM) is counted as a fiber if it has a length greater than 5 μm, a diameter less than 3 μm, and length/diameter ratio greater than 3:1, equivalent to one fiber per millimeter ($f\,ml^{-1}$). Based on the 1984 NRC suggestion, both 1 PCM $f\,ml^{-1}$ and 60 transmission electron microscopy (TEM) $f\,ml^{-1}$ are approximately equal to a mass concentration of 30 $\mu g\,m^{-3}$.

Exposure Routes and Pathways

Epidemiological studies of asbestos-exposed workers and supporting animal studies indicate that inhalation of asbestos is the principal route of exposure of public health concern. Other routes of exposure to asbestos include incidental ingestion or dermal exposure. Asbestos fibers may be ingested through food or drink or by swallowing of inhaled asbestos cleared from the lungs.

Toxicokinetics

The most common route of entry into the body is by inhalation. When asbestos is inhaled, larger fibers (10–20 μm in length) tend to be filtered out in the upper airways or collide with the walls of the conducting airway walls in the lungs where they are captured in the respiratory mucous. These fibers are then removed by cilia of the tracheobrocheal tree and are swallowed. Asbestos fibers less than 10 μm in length may eventually reach the alveoli. In autopsy lung specimens, asbestos fibers of 5–200 μm have been found in alveoli. Very small fibers may be engulfed by alveolar macrophages and transported to lymph nodes. In human autopsies, asbestos fibers have also been found in the thoracic diaphragm and chest wall. Once deposited in alveoli, asbestos fibers remain permanently embedded as asbestos bodies (ferruginous bodies) and are not excreted. Thus, asbestos fibers build up in lung tissues over time, but

some fibers, particularly chrysotile fibers, can be removed or degraded in the lung with time.

Asbestos fibers may be deposited in the gastrointestinal tract via ingestion. There is no systemic absorption of asbestos fibers and they do not appear to stimulate an inflammatory reaction or any other adverse effect in the gastrointestinal tract. Nearly all ingested asbestos fibers pass along the intestines within a few days and are excreted in the feces. Asbestos fibers may penetrate the skin but are not absorbed and metabolized in the body.

Mechanism of Toxicity

Asbestos produces its toxic effects by direct contact with lung tissue or by stimulating an acute or chronic inflammatory reaction in the tissue (via active oxygen mechanism or other cell-mediated mechanisms). The important determinants of asbestos toxicity are fiber size, fiber durability, and iron content.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, mesothelioma developed in two rats exposed to high concentrations of amosite or crocidolite for only 1 day. These data are not extensive enough to define the dose–response but data indicate that short-term exposures should not be regarded.

Human

It has been noted that workers exposed to asbestos for 1–12 months had an increased risk of developing lung cancer a number of years later. There is some evidence that acute oral exposure may induce precursor lesions of colon cancer.

Chronic Toxicity (or Exposure)

Animal

Animals exposed to asbestos over a long period of time can develop lung tumors (adenomas, Aden carcinomas, and squamous cell carcinomas) and mesothelioma. Animals given very high doses of asbestos in food did not get significantly increased fatal cancers compared to the control group, although some extra nonfatal tumors did occur in the intestines of rats in one study. A few studies in rats have reported some alterations in cells of the gastrointestinal tract after chronic exposure to chrysotile.

Human

Chronic inhalation of asbestos produces a disease called asbestosis, which is characterized by interstitial fibrosis of lung parenchyma. All types of asbestos fibers can cause asbestosis, but crocidolite is most potent. The first symptoms of asbestosis are dyspnea with exertion and reduced exercise tolerance. Lung function abnormalities can include decreases in vital capacity, residual volume, functional residual capacity, and lung compliance. The disease can progress to massive pulmonary fibrosis. In these cases, the diffuse fibrosis and contraction of lung tissue causes constriction of the pulmonary vasculature, leading to pulmonary hypertension, which may lead to death. Asbestos can cause a fibrous pleuritis in which the pleural membrane thickens to encase the lung in a rigid fibrous capsule. There is formation of pleural plaques. Radiologic evidence of asbestos-induced lung damage is not present at least until 5 years after exposure. The most important physical sign is the presence of high-pitched fine crepitations (crackles) at full inspiration, which persist after coughing. As the total lung volume is decreased, especially the forced vital capacity, blood flows to the lungs may also decrease, and this causes the heart to enlarge. Asbestosis is a serious disease and can eventually lead to disability or death in people exposed to high amounts of asbestos over a long period. However, asbestosis is not usually of concern to people exposed to low levels of asbestos.

Chronic exposure to asbestos can also cause lung cancer, bronchogenic carcinoma, and mesothelioma (cancer of the thin membrane that surrounds the lung and other internal organs). The latency period for lung cancer is 20–30 years. Symptoms of lung cancer may include chest pain, chronic cough, hemoptysis, and decreased exercise tolerance. Mesothelioma is another malignant disease associated with asbestos. The latency period is 35–40 years. Asbestos is the only known cause of this tumor. The first symptoms of mesothelioma are those associated with pleural irritation such as cough and chest pain. Lung cancer is usually fatal, while mesothelioma is almost always fatal, often within a few months of diagnosis. For lung cancer, the magnitude of risks appear to be a complex function of a number of parameters, the most important factors are: (1) the level and duration of exposure; (2) the time since exposure occurred; (3) the age at which exposure occurred; (4) the cigarette smoking history; and (5) the type and size distribution of the asbestos fibers. There is some evidence from animal studies that asbestos-induced cancer stems from regions in the lung with advanced fibrosis (asbestosis); however, lung cancer caused by chrysotile was also produced at fiber concentrations that did not lead to detectable fibrosis. With respect to lung cancer, some studies have indicated that the interaction between asbestos and smoking is greater

than additive. Asbestos may increase the risk of cancer at other sites, but the evidence is not strong. Significant effects on other tissues have not been detected.

There is some evidence that chronic oral exposure to asbestos may lead to an increased incidence risk of gastrointestinal tumors. However, the health effects from ingesting asbestos are unclear. Although some groups of people who have been exposed to asbestos fibers in drinking water have higher-than-average death rates from cancer of the esophagus, stomach, and intestines, it is very difficult to determine whether this is caused by asbestos or by other causes. Handling asbestos without gloves can cause corns (asbestos warts), which are areas of thickened skin surrounding implanted fibers.

In Vitro Toxicity Data

Studies of exposed asbestos workers, residentially exposed Turkish villagers, mesothelioma patients, and lung cancer patients suggest that asbestos is genotoxic. The number of chromosomal aberrations and the rate of sister chromatid exchange were significantly elevated in the peripheral blood lymphocytes of the exposed individuals compared with the nonexposed control group. Tests of asbestos for gene mutations have been mixed, in both *in vivo* and *in vitro* toxicity data.

Clinical Management

A chest X-ray cannot detect the asbestos fibers themselves, but it can detect early signs of lung disease caused by asbestos from relatively heavy exposure. The most reliable test for asbestos exposure is the detection of microscopic asbestos fibers in pieces of lung tissue removed by surgery (invasive test). The use of biological markers, such as tissue polypeptide antigen, may play a useful role in the early detection of mesothelioma in individuals at risk.

There is no effective treatment for asbestosis. The only preventive measure is to keep asbestos fibers out of the lungs. Once asbestosis has started, further inhalation of asbestos fibers causes acute inflammatory reactions, which can worsen the disease. At the time of diagnosis, lung cancer is usually too advanced for successful treatment. Long-term survival rates are low following surgery and treatment with chemotherapeutic agents or radiation. There is no effective treatment for mesothelioma, and death usually occurs within 1 year after diagnosis. Early detection, radiation therapy, and chemotherapy may prolong survival.

Environmental Fate

Asbestos minerals are widespread in the environment. Asbestos fibers are chemically inert – they do not evaporate, dissolve, or undergo significant degradation in the environment. They enter the air and water from wearing down or disturbance of natural deposits or manufactured asbestos products. Small diameter fibers can remain suspended in the air and water for a long time and be carried long distances by wind or water. Asbestos fibers are not able to move through soil and are generally not broken down to other compounds in the environment. Chrysotile may undergo some dissolution in the aquatic environment, especially at low pH.

Other Hazards

Transplacental transfer of asbestos may occur, but this has not been linked with any adverse reproductive outcomes in humans. Results of animal studies do not indicate that exposure to asbestos is likely to result in birth defects. A recent study of brake workers, who are typically exposed to short chrysotile fibers, indicates that brake work does not increase the risk of mesothelioma. The results add to the evidence that fiber type and size are important determinants of mesothelioma risk. Along with asbestos, Simian virus 40 (SV40), a DNA monkey virus, has recently been implicated in the etiology of mesothelioma. It was proposed that SV40 and asbestos are possibly cocarcinogens.

Exposure Standards and Guidelines

Asbestos is considered by the US Department of Health and Human Services (DHHS), the US Environmental Protection Agency (EPA), and International Agency for Research on Cancer to be a known human carcinogen. EPA established a ban on new uses of asbestos on July 12, 1989. EPA also established regulations that require school systems to conduct asbestos inspection and abatement. They also regulate the release of asbestos from building demolition/renovation and management of waste containing asbestos. The 2000 American Conference of Governmental Industrial Hygienists threshold limit values, time-weighted average (8 h exposure), for asbestos is $0.1\,\mathrm{f\,ml^{-1}}$. The National Institute for Occupational Safety and Health recommended exposure limit and Occupational Safety and Health Administration permissible exposure limit (8 h exposure) is also $0.1\,\mathrm{f\,ml^{-1}}$. EPA has proposed a maximum contaminant level of 7 million fibers (greater than or equal to 10 μm) per liter in drinking water.

US Food and Drug Administration currently regulates the use of asbestos in preparation of drugs and restricts the use of asbestos in food-packaging materials.

Miscellaneous

Asbestos fibers do not have any detectable odor or taste.

See also: Carcinogenesis; Respiratory Tract.

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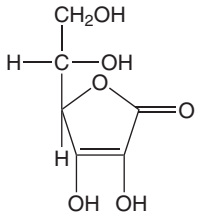
Ascorbic Acid

John Sanseverino

This article is a revision of the previous print edition article by Dennise L Kurta, volume 1, pp. 84–85, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 50-81-7
- Synonyms: Vitamin C; Acidum; Antisorbutic vitamin; Ascurbicum; Cevitamic acid; 2,3-Didehydro-L-threo-hexono-1,4-lactone; E300; L-Ascorbic acid; L-Xyloascorbic acid; L-3-Ketothreohexuronic acid lactone
- Chemical/Pharmaceutical/Other Class: Organic acid; Vitamin
- Chemical Formula: $C_6H_8O_6$
- Chemical Structure:



Uses

Ascorbic acid, a water-soluble vitamin widely distributed in the plant and animal kingdoms, is used for the growth and repair of bodily tissues. It is essential for the formation of collagen, skin, tendons, ligaments, and blood vessels as well as wound repair, and the repair and maintenance of cartilage, bones, and teeth. Deficiencies in ascorbic acid lead to dry and splitting hair, gingivitis and bleeding gums, dry skin, decreased wound healing, easy bruising, nosebleeds, weakened tooth enamel, swollen and painful joints, anemia, decreased ability to ward off infection, and possibly weight gain due to a slowed metabolism. Ascorbic acid is used as a nutritional supplement during deficiency states (scurvy). Ascorbic acid needs may increase during chronic illness, infection, trauma, pregnancy, and lactation. It has also been used as a urinary tract acidifier and purportedly is a cure for the common cold. Ascorbic acid and α-tocopherol may alleviate arsenic-induced alterations in mitochondria. It may also have a protective role in lead toxicity; its derivative ascorbyl palmitate may alleviate gastric disease (gastritis, duodenal ulcer, and carcinoma) by direct action on *Helicobacter pyroli*; and it may relieve symptoms of aflatoxin B1 toxicity.

Background Information

Ascorbic acid, or vitamin C, was discovered after scientists had searched for centuries for a cure for the disease known as scurvy. The name ascorbic acid comes from word 'anti-scurvy' acid, because it was known to dramatically cure this disease. This disease was caused by a serious deficiency of vitamin C, and it caused its victim's small blood vessels to rupture, bones to weaken, and joints to swell, among other symptoms. These symptoms were due to the fact that without a source of vitamin C one developed severe problems concerning the body's connective tissues, which is found in bones, skin, muscles, teeth, blood vessels, and cartilage. This disease would eventually lead to death if it went untreated, and was not uncommon, especially during the winter months of the year. The disease often plagues armies, explorers, and crusaders, since these men's diets normally consisted of biscuits and salted meat that could easily be stored and kept unspoiled on a ship.

Exposure Routes and Pathways

Routes of exposure are oral, intravenous, intramuscular, and subcutaneous. Dietary sources of ascorbic acid include citrus fruits, tomatoes, potatoes, cantaloupe, raw peppers, and green leafy vegetables.

Toxicokinetics

Ascorbic acid is readily absorbed from the gastrointestinal tract; however, absorption may be delayed with large doses. It is metabolized hepatically, reversibly oxidized to dehydroascorbic acid, and metabolized to inactive ascorbate-2-sulfate and oxalic acid. Protein binding is 25%, and ascorbic acid is widely distributed into body tissue. It is renally excreted. Elimination increases with higher doses. Vitamin C is an antioxidant, which means that it quenches free radicals that can damage organs, tissues, and cells. Free radicals are believed to be one of the causes of the degenerative changes seen with aging, but it is not yet known whether consumption of additional antioxidants like vitamin C can help.

Mechanism of Toxicity

Metabolism of ascorbic acid can lead to deposition of oxalate crystals in kidney tissue. Reduction of carcinogenic Cr(VI) by ascorbic acid generates ascorbate–Cr(III)–DNA cross-links that have been linked to mutagenicity and the formation of DNA lesions. Uranyl acetate–ascorbate has also been shown to nick plasmid DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity is not expected; however, the oral LD_{50} dose for rats is $11.9\,g\,kg^{-1}$ body weight.

Human

Toxicity is unlikely following acute ingestions of even 100 times the recommended daily allowance. The most common manifestations of vitamin C toxicity are kidney stones, and in very rare circumstances, anemia (caused by interference with vitamin B_{12} absorption).

Chronic Toxicity (or Exposure)

Human

Chronic megadoses of vitamin C may precipitate formation of calcium oxalate renal stones, oxalate nephropathy, and renal failure. The amount required to cause this is variable from 2 to $8\,g\,day^{-1}$. Bone oxalate deposits have also been reported. Esophageal and dental erosion are possible with tablet ingestion. Heinz body hemolytic anemia has been seen in premature infants.

Clinical Management

Acute ingestions seldom require treatment. Dilution is recommended to reduce the risk of esophageal and gastrointestinal irritations. During chronic excessive use, patients should be instructed to discontinue the supplement and observe for signs of rebound scurvy. Any toxic symptom should be treated symptomatically.

Exposure Standards and Guidelines

Recommended dietary allowances are defined as the levels of intake of essential nutrients that, on the basis of scientific knowledge, the Food and Nutrition Board judges to be adequate to meet the known nutrient needs of practically all healthy persons. Vitamin C should be consumed every day, since it is not a fat-soluble vitamin and cannot be stored for later use. Specific recommendations for each vitamin depend on age, gender, and other factors (such as pregnancy). There has been much debate regarding the use of vitamin C in cancer and heart disease prevention. Although the evidence is mixed regarding a definitive benefit of vitamin C in this regard, it is still encouraged that individuals maintain adequate intake. On the other hand, the majority of current evidence does not support vitamin C's role in the prevention or treatment of the common cold.

See also: Dietary Supplements; Vitamin A; Vitamin D; Vitamin E.

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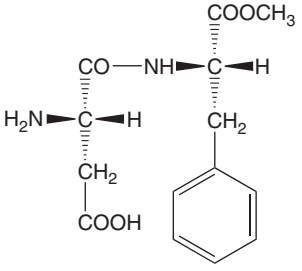
Aspartame

Robin C Guy

This article is a revision of the previous print edition article by Arvind K Agarwal, volume 1, pp. 85–86, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 22839-47-0
- Synonyms: L-α-aspartyl-L-phenylalanine 1-methyl ester; NutraSweet
- Chemical/Pharmaceutical/Other Class: Dipeptide methyl ester
- Chemical Formula: $C_{14}H_{18}N_2O_5$
- Chemical Structure:



Uses

Aspartame is a synthetic sweetener commonly used in soft drinks and many foods. It is a high-intensity sweetener and a flavor enhancer.

Background Information

More than 20 years have elapsed since aspartame was approved by regulatory agencies as a sweetener and flavor enhancer. The safety of aspartame and its metabolic constituents was established through extensive toxicology studies in laboratory animals, using doses much higher than people could possibly consume. The safety profile was further confirmed in studies of several human subpopulations, including healthy infants, children, adolescents, and adults, and in obese individuals and diabetics. Further, the studies included individuals heterozygous for the genetic disease phenylketonuria (PKU) who have a decreased ability to metabolize the essential amino acid, phenylalanine. In total, prior to marketing, the safety of the high-intensity sweetener aspartame for its intended uses as a sweetener and flavor enhancer was demonstrated by the results of over 100 scientific studies in animals and humans.

Aspartame has been a noteworthy example of a high-profile chemical with many years of risk perception issues. The scientific issues continued to be raised after approval, including concern for theoretical toxicity from aspartame's metabolic components. The metabolic components include the amino acids, aspartate and phenylalanine, and methanol, even though dietary exposure to these components is much greater than from aspartame. In the postmarketing period, the safety of aspartame was further evaluated through extensive monitoring of intake, postmarketing surveillance of anecdotal reports of alleged health effects, and additional research to evaluate these anecdotal reports and other scientific issues.

The results of the extensive intake evaluations in the United States and in other countries demonstrated that intakes which were well below the acceptable daily intakes (ADIs) set by the Food and Drug Administration (FDA) and regulatory bodies in other countries, as well as the Joint FAO/WHO Expert Committee on Food Additives (JFECFA). The studies have also included evaluations of possible associations between aspartame and headaches, seizures, behavior, cognition, and mood as well as allergic-type reactions and use by potentially sensitive subpopulations, has continued after approval. Evaluation of the anecdotal reports of adverse health effects were the first ones done for a food additive, and revealed that the reported effects were generally mild and also common in the general population,

and that there was no consistent or unique pattern of symptoms that could be causally linked to consumption of aspartame. Finally, the results of the extensive scientific research done to evaluate these allegations did not show a causal relationship between aspartame and adverse effects. Recent reviews have stated that when all the research on aspartame, including evaluations in both the pre- and postmarketing periods, is examined as a whole, it is clear that aspartame is safe, and there are no unresolved questions regarding its safety under conditions of intended use.

Exposure Routes and Pathways

The mode of aspartame exposure is oral.

Toxicokinetics

Aspartame is hydrolyzed entirely in the gastrointestinal tract to its constituent amino acids, aspartate and phenylalanine, and methanol. These are absorbed by the body and utilized via the same metabolic pathways as when these same constituents are derived from common foods; they are found in common foods in much larger quantities than from aspartame in foods or beverages.

Mechanism of Toxicity

Individuals with the rare, genetic disease PKU cannot properly metabolize phenylalanine. These individuals are placed on special low-phenylalanine diets to control their blood phenylalanine concentrations, and need to be aware that aspartame is a source of phenylalanine.

Acute and Short-Term Toxicity (or Exposure)

Animal

The bone marrow cells isolated from mice exposed to blends of aspartame and acesulfame-K via gavage were analyzed for chromosome aberrations, and the results show that aspartame in combination with acesulfame-K is not genotoxic. In a study examining the effect of aspartame on the cytogenetic effects of dioxydin and cyclophosphan, aspartame was found to possess antimutagenic properties in relation to chromosome aberration counts in the bone marrow cells of mice. The antimutagenic activity of aspartame was manifested more when it was injected for 5 days before the administration of a mutagen, while joint administration of aspartame with the mutagens did not change the clastogenic effect of dioxydin and cyclophosphan.

No micronuclei were formed in bone marrow cells isolated from mice (rats) exposed to an acute dose of aspartame. Aspartame is not toxic, as tested in acute animal studies in mice, rats, and rabbits.

Human

Aspartame is not toxic when administered in acute doses. When humans were administered aspartame at dosages up to 200 mg kg^{-1} body weight as a single bolus dose, the blood concentrations of aspartic acid, phenylalanine, and methanol were well below levels considered potentially harmful. The toxic effects of methanol in humans are due to accumulation of its metabolite, formate, and the blood formate concentrations did not increase after this high dose of aspartame (equal to the amount in ~28 l of beverage with aspartame consumed at once or ~65–70 times the amount of aspartame people consume daily at the 90th percentile). Urinary excretion of formate increased significantly in samples collected 0–4 and 4–8 h after aspartame ingestion. Therefore, the rate of formate formation did not exceed the rate of formate excretion, even after this very large bolus dose. Studies have also shown that when aspartame is consumed at levels within the ADI-limit of 40 mg kg^{-1} body weight, there is no significant risk for an aspartate-induced neurotoxic effect in the brain. Further, the available behavioral studies in humans with acute dosing found no adverse effects.

Chronic Toxicity (or Exposure)

Animal

Aspartame is not toxic, carcinogenic, mutagenic, or teratogenic, and has no effect on reproduction.

Human

As noted above, the available evidence suggests that consumption of aspartame by normal humans is safe and is not associated with serious adverse health effects. Specific chronic studies included studies up to 27 weeks duration in healthy adults, children, and adolescents, obese subjects, individuals with diabetes, and individuals heterozygous for PKU. The results of these studies show that there was no accumulation of plasma aspartate, phenylalanine, or methanol in humans following long-term exposure.

Further, a 6 month study in healthy adult volunteers aged 18–62 years used 75 mg kg^{-1} body weight per day of aspartame or placebo (provided as three divided doses daily), approximately the same amount of aspartame per day as 10 l of a soft drink

sweetened with 100% aspartame. There was no accumulation of blood or plasma aspartate, phenylalanine, methanol, or formate over the course of the study. In addition, urinary formate excretion did not increase, indicating no significant increase of formate formation. There were no adverse experiences and no effects on physical examinations, including vital signs, electrocardiograms, ophthalmologic examinations, or biochemical parameters after aspartame compared to placebo.

In Vitro Toxicity Data

The nonnutritive sweeteners acesulfame-K, aspartame, cyclamate, saccharin, and sucralose were tested for DNA damaging activity in the rat hepatocyte/DNA repair assay using hepatocytes from rats. The results found no evidence of genotoxic potential.

Exposure Standards and Guidelines

Based on the lack of toxicity observed in animal studies, a no-observed-effect level of at least $4000\,mg\,kg^{-1}$ body weight per day was established by the JECFA, the Scientific Committee on Food, and the Health Protection Branch of Health and Welfare Canada. As a result, an ADI of $40\,mg\,kg^{-1}$ body weight was set by these agencies. The (US) FDA set the ADI at $50\,mg\,kg^{-1}$ body weight based on both animal and human studies. Further, it is the position of The American Dietetic Association that consumers can safely enjoy a range of nutritive and nonnutritive sweeteners, including aspartame, when consumed in a diet that is guided by current (US) federal nutrition recommendations, such as the Dietary Guidelines for Americans and the Dietary References Intakes, as well as individual health goals.

Aspartame has also been considered by other bodies including the UK Committee on Toxicity and the European Commission's Health and Consumer Protection Directorate-General's Scientific Committee on Food (SCF). In December, 2002, the SCF concluded that, on the basis of its review of all the data in animals and humans available to date, there is no evidence to suggest that there is a need to revise the outcome of the earlier risk assessment or the $40\,mg\,kg^{-1}$ body weight ADI previously established for aspartame.

See also: Food Additives; Food and Drug Administration, US; Redbook.

Further Reading

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Relevant Website

http://europa.eu.int – European Commission. Health and Consumer Protection Directorate-General. Scientific Committee on Food (SCF). 2002. Opinion of the Scientific Committee on Food. Update on the Safety of Aspartame. December 10, 2002.

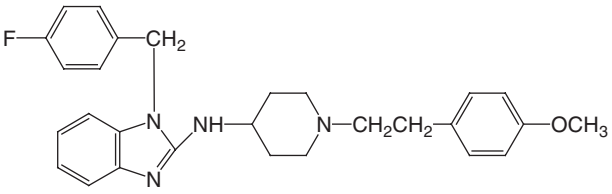
Aspirin *See* Acetylsalicylic Acid.

Astemizole

Michael D Reed

This article is a revision of the previous print edition article by Carla M Goetz, volume 1, pp. 86–87, © 1998, Elsevier Inc.

- Synonyms: Hismanal
- Chemical/Pharmaceutical/Other Class: Antihistamine; Nonsedating antihistamine; H-1 receptor antagonist
- Chemical Formula: NyNHOCH
- Chemical Structure:



Uses

Astemizole is indicated for the symptomatic relief of seasonal allergic rhinitis and chronic idiopathic urticaria. The drug is used in any medical condition in which histamine-1 (H-1) receptor antagonism is beneficial.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to astemizole.

Toxicokinetics

The drug is available (has been removed from the US market due to cardiotoxicity) as a tablet formulation. Astemizole is rapidly and very well absorbed after oral administration; reaching peak plasma concentrations within 1 h (0.5–1 h) of administration. Concurrent administration with food will decrease the rate but not the overall extent of intestinal absorption. The drug is ~97% bound to plasma protein and is extensively distributed within the body; astemizole V_d is $\sim 250\,l\,kg^{-1}$ in adults.

Astemizole undergoes extensive first-pass hepatic metabolism via cytochrome P450 (CYP 450) enzymes (primarily 3A4) to three primary, active, metabolites, desmethylastemizole, norastemizole, and 6-hydroxydesmethylastemizole. The major metabolites of astemizole, desmethylastemizole and norastemizole, possess antihistaminic activity approaching that of the parent compound. The $t_{1/2}$ of astemizole is ~1.1 days whereas the estimated $t_{1/2}$ for desmethylastemizole is ~9.5 days. Drugs that interact, that is, stimulate or antagonize CYP 3A4 activity, will modulate astemizole systemic exposure and toxic potential.

Mechanism of Toxicity

The primary and life-threatening toxicity associated with astemizole administration is cardiotoxicity; torsades de pointes arrhythmia. The exact mechanism(s) of the cardiotoxic effects of astemizole is not well understood though is believed to be via a similar, and possibly identical pathway as observed with terfenidine. Both astemizole and its primary desmethylastemizole metabolite appear to inhibit cardiac delayed potassium rectifier (Ik) channels in a manner similar to terfenidine. The Ikr (delayed potassium rectifier – rapid acting) channel is the potassium channel involved in repolarization of cardiac cells. Blockade of the cardiac Ikr channel produces a depressed peak in the voltage and a decrease in potassium cellular outflow predisposing the myocardium too early after repolarization, which, when sizeable, results in dysrhythmia and most notably torsades de pointes. Further, the drug's remaining two metabolites, norastemizole and 6-hydroxyastemizole, can also inhibit Ikr channels but to a much lesser extent than the parent astemizole or desmethylastemizole. Nevertheless, the additive or synergistic inhibition of Ikr channel activity under conditions of high dose astemizole administration, administration of excessive astemizole doses in patients with hepatic and/or renal dysfunction leading to accumulation of astemizole and/or its metabolites, and in the case of overdose, all predispose the patient to a very high likelihood of astemizole-induced cardiotoxicity.

Acute and Short-Term Toxicity (or Exposure)

Human

Unlike traditional, first-generation (e.g., diphenhydramine, hydroxyzine) antihistamines, astemizole is considered 'nonsedating' and lacks the anticholinergic properties noted with first-generation H-1 receptor antagonists. Patients presenting with astemizole overdose are usually fully awake or only slightly sedated. Serious cardiac effects, including prolongation of the QT interval, arrhythmias (i.e., ventricular tachycardia, torsades de pointes, ventricular fibrillation, and heart block), arrest, hypotension, palpitations, syncope, dizziness, and death have

been described in patients receiving astemizole. As noted above, these cardiotoxic effects are usually associated with higher than recommended doses and/or increased plasma concentrations of the drug and its active metabolites. Although rarely reported, cardiotoxic effects may occur at the recommended dose and at doses two or three times the recommended dose (10 mg daily). Concomitant administration of drugs known to inhibit CYP 3A4 activity including the azole antifungals (e.g., itraconazole, ketoconazole, fluconazole) and macrolide antibiotics (e.g., erythromycin, clarithromycin), cimetidine, metronidazole, certain selective serotonin re-uptake inhibitors (SSRIs), and anti-retroviral drugs substantially increase the risk of astemizole-induced cardiotoxicity.

Chronic Toxicity (or Exposure)

Animal

Varying effects were seen in a rat model of teratogenicity. At lower doses (50 times the recommended human dose), no toxicity was observed in the mothers or pups. At higher doses of 100 times the recommended human dose, toxic effects were noted on the unborn rat pups as well as in the mothers.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Activated charcoal will adsorb astemizole following oral ingestion. The effectiveness of activated charcoal and/or gastric lavage will depend upon the time these therapies are instituted after the ingestion, as the drug is rapidly absorbed from the intestine. Further, the effectiveness of multidose (e.g., q4 h, etc.) activated charcoal would appear limited considering the drug's extensive V_d ($250\,l\,kg^{-1}$) and high protein binding (97%). Close EKG monitoring should be instituted immediately upon presentation to a healthcare facility, including equipped ambulances and medical helicopters, and continued for a minimum of 24 h.

See also: Cimetidine; Diphenhydramine; Erythromycin.

Further Reading

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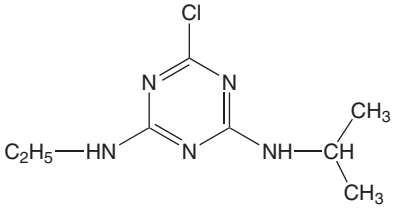
Atrazine

Jing Liu

- Chemical Abstract Service Registry Number: CAS 1912-24-9
- Synonyms: Atrasol; Atranex; Atratol; Gesaprim; Primatol; Crisazine; 2-Chloro-4-ethylamino-6-isopopylamine-*s*-triazine
- Chemical/Pharmaceutical/Other Class: Triazine herbicide
- Chemical Formula: $C_8H_{14}ClN_5$
- Chemical Structure:



Uses

For decades, atrazine has been the most heavily used herbicide in the United States. Atrazine is used for selective and nonselective weed control in various field crops and industrial applications.

Exposure Routes and Pathways

The ocular and dermal routes are the primary exposure pathways. Ingestion and inhalation of atrazine are other possible routes of exposure. In all cases, atrazine falls into toxicity category III or IV.

Toxicokinetics

Atrazine has the potential to be absorbed through the gastrointestinal tract, through the intact skin, and by inhalation. The percentage absorbed

through dermal application increased with time and decreased with dose. However, the majority (65–95%) of atrazine applied on the skin was recovered in the water used for washing or was found associated with the skin at the site of exposure. Once absorbed, it follows first-order distribution kinetics and undergoes *N*-dealkylation and dechlorination of the triazine ring. The highest level of atrazine is noted in the red blood cell followed by lungs, liver, spleen, and kidneys. The half-life of atrazine in the tissues is ~31–39 h, indicating that atrazine does not bioaccumulate. Urinary excretion is the major route of elimination in mammals. A small amount is also excreted in the feces. The major metabolite in both urine and feces is diaminochlorotriazine.

Mechanism of Toxicity

The triazine herbicides are selective inhibitors of the Hill reaction in plant photosynthesis. In mammals, atrazine disrupts luteinizing hormone and prolactin secretion through direct action on the hypothalamus–pituitary axis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Atrazine has low acute toxicity in mammals. The oral LD_{50} in rats is ~2 g kg^{-1}. The dermal LD_{50} and inhalation LC_{50} (1 h) values in rats are ~3 g kg^{-1} and 700 mg m^{-3}, respectively. The oral LD_{50} values in mice and rabbits are ~1.8–4.0 g kg^{-1} and 750 mg kg^{-1}, respectively. Atrazine was negative in primary skin irritation and dermal sensitization tests. Rats exposed to high dosages of atrazine showed changes in arousal and motor function, dyspnea, hypothermia, and spasms. With lethal oral dosages, death occurred rapidly (within 12–24 h).

A 90 day subchronic oral study in rat and 21 day dermal study in rabbit provided no-observed-adverse-effect levels (NOAELs) of 3.3 and 100 mg kg^{-1} day^{-1}, respectively. Prenatal developmental toxicity study in female Sprague–Dawley rats exposed to atrazine during gestation day 6 through day 15 demonstrated maternal and developmental NOAELs of 25 mg kg^{-1} day^{-1}.

Using both Long–Evans and Sprague–Dawley female rats, atrazine was found to disrupt the hypothalamic control of pituitary-ovarian function as indicated by alteration in luteinizing hormone and prolactin serum levels. Females treated with atrazine (75, 150, and 300 mg kg^{-1} day^{-1} for 21 days by gavage) showed irregular cycles and repetitive pseudopregnancies. Maternal exposure to atrazine during lactation may result in prostatitis in adult male offspring due to atrazine's suppressive effect on suckling-induced prolactin release.

Human

There have been only 65 recorded cases of human poisonings among occupationally exposed workers during 1966–81 in the United States. One death was reported following extensive dermal exposure. Dermal exposure to atrazine can cause skin rash, erythema, blisters, and edema. Ocular irritation, chest pains, and a feeling of tightness in the chest, nausea, and dizziness have also been reported after dermal, oral, or inhalation exposures.

Chronic Toxicity (or Exposure)

Animal

About 40% of rats died with signs of respiratory distress and paralysis of the limbs following oral administration of 20 mg kg^{-1} day^{-1} atrazine for 6 months. Structural and chemical changes were noticed in various organs including heart, liver, ovaries, etc. Dogs treated with 33.65 mg kg^{-1} day^{-1} of atrazine in the diet for 52 weeks showed various treatment-related cardiac changes including EKG alterations, moderate to severe atrial dilation, and enlarged hearts. Histopathology revealed cardiac myolysis and focal atrophy. The NOAEL for atrazine in dogs of both sexes was established at 4.97 mg kg^{-1} day^{-1}.

When CD-1 mice of both sexes were treated with atrazine in the diet at dose levels of 10–3000 ppm daily for 91 weeks, no treatment-related increase in tumor incidence was noted when compared to controls. Neither male and female Fischer 344 rats nor male Sprague–Dawley rats given atrazine at a maximum tolerated dose in the diet for 24 months exhibited any increase in the incidence of tumors of any type. However, mammary tumors were observed in female Sprague–Dawley rats after 24 months of dietary administration of high levels of atrazine. The differences in response to the carcinogenic effect of high levels of atrazine observed in mice versus rats and male versus female Sprague–Dawley rats is because of differences in endocrine control mechanisms affecting reproductive senescence and the development of the mammary tumors during aging.

Based on evidence derived from a large array of assays such as bacterial reverse mutation test, mammalian bone marrow chromosome aberration test, dominant lethal assay, and UDS assay, atrazine was concluded to lack mutagenic potential.

Human

The carcinogenic effect of high doses of atrazine noted in female Sprague–Dawley rats is a strain-, sex-, and tissue-specific response that may not have biological relevance to humans due to the differences in the endocrine control of reproductive senescence.

In Vitro Toxicity Data

Atrazine was found to strongly potentiate arsenic trioxide-induced cytotoxicity and transcriptional activation of stress genes in transformed human hepatocytes, while atrazine itself did not show any significant effects.

Clinical Management

Treatment is symptomatic.

Environmental Fate

Atrazine is highly persistent in the environment due to its resistance to abiotic hydrolysis (stable at pHs 5, 7, and 9) and to direct aqueous photolysis (stable under sunlight at pH 7). Moreover, the compound has a limited volatilization potential and is only moderately susceptible to aerobic biodegradation, which is the main route of dissipation of atrazine. A colder climate makes atrazine even more persistent in the environment. Atrazine does not get adsorbed to soil particles strongly and therefore has a relatively high potential to contaminate ground and surface waters despite its moderate solubility in water.

Ecotoxicology

Atrazine, with acute oral LD_{50} values of $>900\,mg\,kg^{-1}$, is practically nontoxic to birds. The compound is slightly toxic to aquatic animals. Rainbow trout and midge, the most sensitive freshwater species tested, have 96 and 48 h LC_{50} values of 5.3 and $0.72\,mg\,l^{-1}$, respectively. The most sensitive marine animals tested were the spot fish (*Leiostomus xanthurus*) with a 96 h LC_{50} value of $8.5\,mg\,l^{-1}$ and the copepod (*Acartia tonsa*) with a 96 h LC_{50} value of $88\,\mu g\,l^{-1}$. Atrazine is not toxic to bees (oral LD_{50} $>97\,\mu g$ per bee).

Exposure Standards and Guidelines

Oral RfD (reference dose) is $0.035\,mg\,kg^{-1}\,day^{-1}$. ACGIH TLV – TWA for atrazine is $5\,mg\,m^{-3}$.

See also: Pesticides; Pollution, Water.

Further Reading

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Relevant Websites

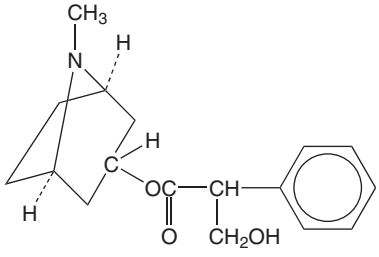
http://ace.orst.edu – Extension Toxicology Network.
http://www.epa.gov – US Environmental Protection Agency.

Atropine

Amanda Lofton

This article is a revision of the previous print edition article by Bridget Flaherty, volume 1, pp. 87–88, © 1998, Elsevier Inc.

- Synonyms: AtroPen auto injector; Atropine sulfate injection; Atropisol; Atrosulf-1; Ocu-tropine; Ocean-A/S; Sal-tropine
- Chemical/Pharmaceutical/Other Class: Anti-muscarinic agent; Anticholinergic agent
- Chemical Formula: $C_{17}H_{23}NO_3$
- Chemical Structure:



Uses

Atropine is used in the management of sinus bradycardia with hemodynamic instability and in the treatment of peptic ulcer disease, irritable bowel syndrome, urinary incontinence, and organophosphate and carbamate poisoning. It is also present in ophthalmic preparations to induce mydriasis and cyclopegia. Atropine is often administered preoperatively to decrease secretions.

Background Information

Atropine is the racemic mixture of L- and D-hyoscyamine and possesses 50% of the antimuscarinic potency of L-hyoscyamine. Atropine is derived from components of the Belladonna plant and is also present in other plants from the Solanaceae family. Women in ancient times often dripped the plant's juices into their eyes, causing mydriasis and thereby enhancing their beauty. In Italian, Belladonna translates to 'beautiful lady'. In the United States, the atropine autoinjector has been in use since 1973 for the treatment of exposures to chemical warfare nerve agents and insecticides.

Exposure Routes and Pathways

Ingestion is the most frequent route of exposure. Exposure can also occur following instillation of eye solutions and via subcutaneous, intramuscular, intravenous, and inhalation routes. Accidental overdosage may occur when atropine is administered for the treatment of organophosphate or carbamate insecticide poisoning.

Toxicokinetics

In therapeutic doses, atropine is well absorbed. In toxic doses, absorption may be prolonged secondary to decreased gastric motility. Atropine is $\sim$18% bound to plasma protein and its volume of distribution ranges from 2 to 4 l kg^{-1}. Atropine is metabolized in the liver to tropic acid, tropine, esters of tropic acid, and glucuronide conjugates. Elimination follows first-order kinetics. Approximately 30–60% is excreted unchanged in the urine. Drug clearance is dependent on glomerular filtration. The elimination half-life is 2–3 h in adults but may be longer in children.

Mechanism of Toxicity

Atropine competitively antagonizes acetylcholine at the neuroreceptor site. Atropine prevents acetylcholine from exhibiting its usual action but does not decrease acetylcholine production. Cardiac muscle, smooth muscle, and the central nervous system are most affected by the antagonism of acetylcholine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals are at risk for anticholinergic poisoning from atropine. Toxicity is similar to that in humans. Gastrointestinal decontamination and supportive care should be employed.

There is interspecies variability and variability based on route of exposure to atropine. The rat LD_{50} oral is 500 mg kg^{-1}; the LD_{50} IP is 280 mg kg^{-1}, and the LD_{50} IV is 73 mg kg^{-1}.

Human

Overdosage of atropine results in signs and symptoms consistent with the anticholinergic toxidrome. Signs and symptoms have been reported following the ingestion of as few as four to five drops of 4% ocular atropine solution. Patients exhibit warm, flushed, and dry skin as a result of peripheral vasodilitation. Mydriasis occurs due to antagonism of acetylcholine in the muscles of the iris. Urinary retention, thirst, delirium, hallucinations, and decreased bowel sounds may occur. Tachycardia with ensuing hypertension can appear secondary to vagal blockade. The anticholinergic toxidrome may be delayed and can occur in cycles. Severe intoxications may progress to seizures, coma, and arrhythmias.

Chronic Toxicity (or Exposure)

Animal

A juvenile pygmy sperm whale (*Kogia breviceps*) was treated with several doses of atropine to relieve symptoms of pyloric stenosis. The animal developed signs and symptoms of anticholinergic toxicity including hyperexcitability, ascending weakness, vomiting, and aspiration of seawater. Symptoms resolved after administration of physostigmine.

Human

Chronic ingestion of greater than therapeutic amounts of atropine may produce symptoms of the anticholinergic toxidrome.

Clinical Management

Basic and advanced life support measures should be utilized as necessary for atropine exposure. Gastric decontamination procedures should be employed based on the patient's history and current symptomatology. Activated charcoal can be given to adsorb atropine. The mainstay of treatment is supportive care. Physostigmine, a cholinesterase inhibitor, can be given to patients to reverse signs and symptoms of

the anticholinergic toxidrome. However, the administration of physostigmine may be contraindicated in the patient who has also been exposed to a tricyclic antidepressant, or another agent known to cause QRS interval widening on the EKG. Extracorporeal elimination measures are ineffective.

See also: Anticholinergics; Carbamate Pesticides; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates; Poisoning Emergencies in Humans.

Further Reading

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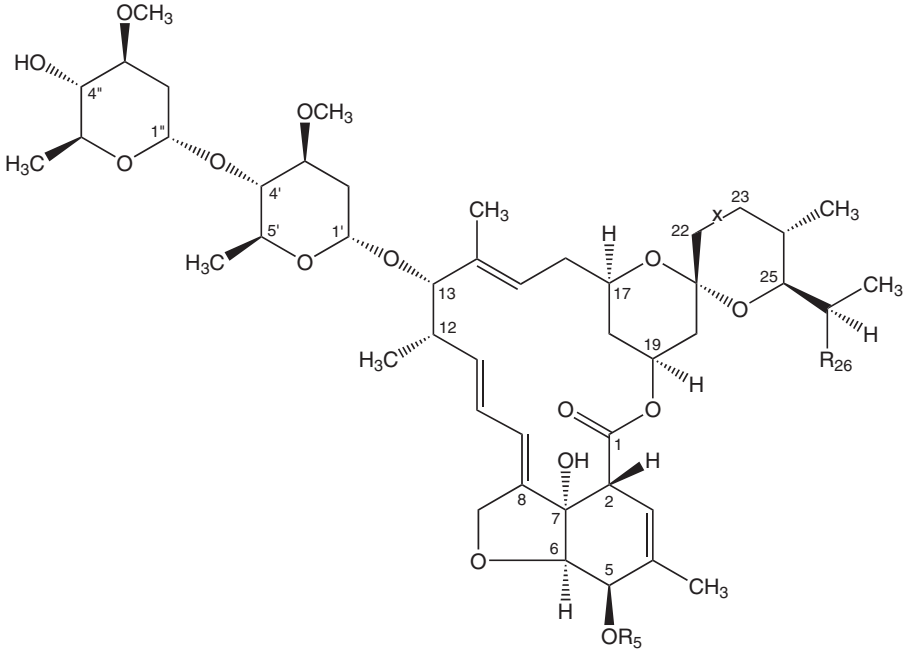
Avermectins

Katherine K Williamson

This article is a revision of the previous print edition article by Arvind K Agarwal, volume 1, pp. 89–90, © 1998, Elsevier Inc.

- Chemical/Pharmaceutical/Other Class: Avermectins are a group of chemically related natural and semisynthetic macrocyclic lactones (macrolide endectocides) produced from the fermentation products of *Streptomyces avermitilis*. The mechanism of action of macrocyclic lactones was long believed to be due to an increased release of GABA. However, recent information indicates that avermectins produce flaccid paralysis of parasites via selective, high-affinity binding to glutamate-gated chloride channels in invertebrate neural and muscle cells. These compounds also interfere with the reproductive cycle of nematodes and arthropods through a poorly understood process.
- Chemical Structure:



Components A: $R_5 = CH_3$ Components a: $R_{26} = C_2H_5$ Components 1: X = –CH═CH–

Components B: $R_5 = H$ Components b: $R_{26} = CH_3$ Components 2: X = –CH_2═$\overset{\overset{OH}{\vdots}}{C}$H–

Uses

Avermectins have a broad spectrum of activity against arthropods, endoparasites, and ectoparasites. However, avermectins have no significant anticestodal (i.e., efficacy against tapeworms), antifungal, or antimicrobial activity. Currently available avermectins include abamectin, selamectin, eprinomectin, doramectin, and ivermectin. Avermectins do not readily cross the blood–brain barrier of mammals, which accounts for the wide margin of safety associated with these products. The recommended antiparasitic dose of ivermectin in cattle and horses is $0.2\,mg\,kg^{-1}$. The dose used to prevent heartworm infection in dogs is $0.006\,mg\,kg^{-1}$ and to treat intestinal parasites is $0.2–0.4\,mg\,kg^{-1}$. The wide range in dosage recommendations between and within a given species demonstrates the wide therapeutic index of ivermectin. Avermectins (most commonly ivermectin) are routinely used as antiparasitics in humans and domestic animals. They are also used in horticulture and agronomy as pesticides and are useful in combating fire ants (abamectin).

Ivermectin is the most widely used and studied member of the avermectin family. It is a semisynthetic derivative of avermectin B1. Ivermectin is produced as an off-white photolabile powder which is very lipophilic, hydrophobic, and poorly soluble in water. Ivermectin has a very broad spectrum of activity against adult, larval, and microfilarial stages of nematodes and arthropods. It is approved for a wide variety of uses in a number of species including humans, domestic and wild ruminants, horses, swine, dogs, and cats. In humans, ivermectin is used in the treatment of *Strongyloides stercoralis*, microfilarial stages of *Onchocerca volvulus* and *Wuchereria bancrofti*, as well as *Ascaris lumbricoides*, *Trichuris trichiura*, and *Enterobius vermicularis* parasitisms. The list of susceptible organisms for which ivermectin is used in domestic animals is extensive and includes gastrointestinal nematodes, ticks, lice, mites, cattle grubs, and lungworms. Ivermectin is perhaps most commonly used in the prevention of heartworm (*Dirofilaria immitis*) infection in dogs and cats. It is safe for use in pregnant animals and in animals as young as 4 weeks of age. Care should be taken to properly dose animals less than 6 months of age as they are more sensitive to acute toxicity following overdose.

Exposure Routes and Pathways

Accidental exposure via skin contact, inhalation, ingestion, or injection stab are all possible.

Toxicokinetics

The pharmacokinetics of ivermectin depends greatly upon the species, formulation of the product, and route of administration. The half-life following a single intravenous dose in cattle is 2.8 days whereas in dogs it is approximately 1.7 days. In ruminants receiving subcutaneous ivermectin the half-life increases to 8 days. In humans, the plasma half-life of ivermectin is 16 h. The volume of distribution of ivermectin ranges from $4.6\,l\,kg^{-1}$ in sheep to $1.9\,l\,kg^{-1}$ in cattle. Oral bioavailability is 95% in simple stomached species and 25–33% in ruminants; as a result, ruminants often receive an injectable or topical formulation. In horses the bioavailability of ivermectin is greatly increased when an aqueous micelle formulation is administered as compared to a paste. Ivermectin is metabolized in the liver through oxidative pathways, 98% is excreted in the feces and the remainder in the urine. In lactating females however, up to 5% of the original dose may be excreted in the milk. Residues may remain in the liver of food animal species for up to 14 days following administration; therefore, labeled withdrawal times should be closely followed.

Mechanism of Toxicity

In humans, the mechanism of toxicity following accidental exposure to veterinary products is poorly understood but may include the penetration of ivermectin into the central nervous system (CNS). Humans undergoing treatment with ivermectin may suffer from anaphylactic-type reactions as microfilaria die off. Toxicity in animals is usually associated with extreme overdosing. Purebred and mixed breed Collies and Australian Shepherds exhibit greater distribution of ivermectin into the CNS resulting in ataxia, tremor, and often death. Use of ivermectin should be avoided in these animals.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ivermectin has an extremely wide margin of safety in all species (with the exception of Collie and Australian Shepherd dogs). The LD_{50} in mice is $25–40\,mg\,kg^{-1}$. In ruminants, swine, dogs, and horses ivermectin is generally considered to have a $10\times$ safety margin. The LD_{50} for dogs has been estimated at $80\,mg\,kg^{-1}$. The margin narrows slightly to $7\times$ label dose in cats. Although extremely rare at labeled doses, toxic reactions may result from

extreme overdose resulting in CNS exposure. Signs of ivermectin overdose include mydriasis, depression, ataxia, tremors, and occasionally death. Much more commonly, toxicity is a result of anaphylaxis and tissue damage associated with the death of the parasite. As stated earlier, use of ivermectin is not advised in Collie and Australian Shepherd dogs due to an increased penetration of the compound through the blood–brain barrier (avermectin derivatives such as milbemycin oxime are safe to use in these animals). Young animals may be more sensitive than adults to avermectin toxicity.

Human

Acute toxicity in humans leads to a variety of clinical signs including rash, edema, headache, dizziness, nausea, vomiting, diarrhea, seizure, dyspnea, ataxia, paresthesia, abdominal pain, and urticaria. Toxicity following treatment with ivermectin is often the result of a hypersensitivity reaction known as the Mazzotti reaction. Signs of the Mazzotti reaction include fever, pruritus, arthralgia, myalgia, postural hypotension, edema, lymphadenopathy, gastrointestinal upset, sore throat, and headaches.

Chronic Toxicity (or Exposure)

Animal

Toxicity may be increased with repeated lower doses as opposed to a single high dose of ivermectin. Dogs given oral doses of 0.5 or 1 mg kg^{-1} day^{-1} for 1 year showed pupillary dilation, weight loss, lethargy, tremors, and recumbency. Rats fed avermectin for 2 years at 0.75, 1.5, or 2 mg kg^{-1} day^{-1} exhibited body weight gains significantly higher than the controls and some tremors in the high-dose group. Male mice fed 8 mg kg^{-1} day^{-1} for 94 weeks showed dermatitis while females exhibited tremors and weight loss. Reproductive toxicity was noted at 0.4 mg kg^{-1} day^{-1} in rats. Avermectins are not considered carcinogenic.

Human

Little is known about the chronic toxicity of avermectins in humans.

In Vitro Toxicity Data

Ivermectin was negative in Ames mutagenesis assays.

Clinical Management

Accidental exposure – supportive care, emesis, and gastric lavage. Treatment toxicity – analgesics and antihistamines.

Environmental Fate

Avermectins and the breakdown products are nearly insoluble in water and bind strongly to soil. Thus they have little mobility and are unlikely to leach into groundwater. Avermectins are rapidly degraded in soil, sensitive to rapid photodegradation. When applied to the soil surface, its soil half-life was about 1 week. Under dark, aerobic conditions, the soil half-life is somewhat extended (2 weeks to 2 months). Microbial degradation also contributes to rapid loss from soils. Avermectins are also rapidly degraded in water (half-life $\sim$12–24 h), principally due to photodegradation.

Ecotoxicology

Avermectins are relatively nontoxic to birds. The LD_{50} for avermectin in Bobwhite quail is 2 g kg^{-1}. Mallard ducks appear more sensitive than quail to the acute toxicity of avermectin. However, avermectin caused no reproductive problems in mallards fed dietary doses of 3, 6, or 12 ppm for 18 weeks. Avermectin is highly toxic to fish and aquatic invertebrates. The 96 h LC_{50} in rainbow trout, bluegill, sheepshead minnow, catfish, and carp was 3.2, 9.6, 15, 24, and 42 ppb, respectively. The 48 h LC_{50} in Daphnia was 0.34 ppb. Avermectin did not bioaccumulate in bluegill sunfish exposed for 28 days. Avermectin is highly toxic to bees, with a 24 h LC_{50} of 2 ng per bee and an oral LD_{50} of 9 ng per bee. The 28 days LC_{50} for avermectin in earthworms was 28 ppm.

See also: LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Selamectin; Veterinary Toxicology.

Further Reading

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Richard Adams H (ed.) (2001) *Veterinary Pharmacology and Therapeutics*, 8th edn., pp. 964–967, 1029. Ames, IA: Iowa State University Press.

Relevant Website

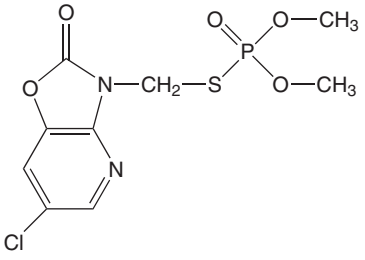
http://pmep.cce.cornell.edu – Extension Toxicology Network, Cornell University.

Avian Ecotoxicology *See* Ecotoxicology, Avian.

Azamethiphos

Jason R Richardson

- Chemical Abstracts Service Registry Number: CAS 35575-96-3
- Synonyms: *S*-(6-Chlorooxazolo(4,5-*b*)pyridine-2(3*H*)-on-3-ylmethyl)-*O*,*O*-dimethyl phosphorothioate; Ciba-Geigy 18809; Snip; Alfacron 10
- Chemical/Pharmaceutical/Other Class: Synthetic organophosphorus insecticide in the phosphorothiolate class
- Chemical Formula: $C_9H_{10}ClN_2O_5PS$
- Chemical Structure:



Uses

Azamethiphos is used as a pesticide spray for control of flies and cockroaches primarily in Europe, as it is not available for use in the United States. It has been used in commercial aquaculture to control external parasites (sea lice) in salmon. In addition, locally procured granular azamethiphos was used as a fly bait by US troops in the first Gulf War.

Exposure Routes and Pathways

Dermal, oral, and inhalation routes are all primary exposure pathways.

Toxicokinetics

Azamethiphos is well absorbed following oral administration to rats but much less effectively by the dermal route. Unlike many other organophosphorus insecticides, azamethiphos does not undergo bioactivation through the P450 monooxygenase pathway, as it is already in its active oxon form. Following oral administration, azamethiphos is rapidly excreted, primarily in the urine, with the major metabolite being 2-amino-3-hydroxy-5-chloropyridine, which is then conjugated to glucuronic or sulfuric acid for excretion. Hepatic and serum carboxylesterases have also been suggested to detoxify azamethiphos.

Mechanism of Toxicity

Similar to other organophosphorus insecticides, azamethiphos elicits toxicity through the inhibition of acetylcholinesterase.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral toxicity of azamethiphos is low to moderate, with rat oral LD_{50} values of 1040–1180 mg kg^{-1}. Azamethiphos is much less toxic when applied dermally, with LD_{50} values greater than 2150 mg kg^{-1}. Azamethiphos is much more toxic to birds, with an acute oral LD_{50} of 91 mg kg^{-1} in quail. Skin sensitization was observed in guinea pigs administered azamethiphos.

Human

No human toxicity data are available for azamethiphos. For experimental animals, toxicity is low for a single oral dose. Prolonged skin exposure may cause skin irritation. Ocular contact may cause eye irritation and pain.

Chronic Toxicity (or Exposure)

Animal

Azamethiphos has not been found to be carcinogenic, teratogenic, or to result in reproductive toxicity in rodent studies. The no-observed-adverse-effect level established in a 52 week study with beagles fed azamethiphos was 2.7–2.9 mg kg^{-1} day^{-1} in males and females, respectively. Azamethiphos did not cause delayed neuropathy in hens given two LD_{50} dosages 21 days apart.

Human

Cholinesterase inhibition may persist for a period of days to weeks. Therefore, repeated exposure to azamethiphos over a period of time may result in the accumulation of enzyme inhibition and onset of acute toxicity. Azamethiphos does not appear to be capable of eliciting organophosphate-induced delayed neuropathy. Likewise, azamethiphos does not appear to be carcinogenic.

In Vitro Toxicity Data

Azamethiphos has been reported to be mutagenic in several *in vitro* assays. However, it was negative in follow-up *in vivo* mutagenicity assays.

Clinical Management

For dermal contact, hands and exposed skin should be washed immediately. For ocular exposure, eyes should be flushed with clean water for a period of 15–20 min. If irritation develops and persists from either dermal or ocular exposure, the victim should seek medical attention.

In the case of inhalation exposure, the victim should be moved to fresh air and medical attention sought immediately. Artificial ventilation is indicated in the case of diminished respiratory function.

If exposure is through ingestion, the victim should seek medical help immediately. Emesis should not be induced. Initial management involves establishment of adequate ventilation and maintenance of adequate respiratory function. Activated charcoal therapy may be used to retard absorption from the gastrointestinal tract. Atropine sulfate alone, or in combination with pralidoxime chloride, can be administered as an antidote. Atropine is initially administered intravenously at a dosage of 1–2 $mg\,kg^{-1}$ every 5–10 min until cholinergic signs decrease. Pralidoxime is preferably administered by slow intravenous infusion at a maximum rate of 8–10 $mg\,kg^{-1}\,h^{-1}$ until full recovery. Seizure activity may be treated with anticonvulsants such as diazepam.

Environmental Fate

Azamethiphos degrades rapidly in seawater and does not bioaccumulate. A study evaluated movement of azamethiphos after application in salmon aquaculture. Dye was added to aid tracking of plumes, and samples were analyzed for azamethiphos content as well as toxicity to a small crustacean. The results suggested that azamethiphos used under recommended conditions posed little contamination or nontarget toxicity potential.

Ecotoxicology

Azamethiphos is toxic to several aquatic species. Biotransformation of azamethiphos in salmon is similar to that in rats, with formation of 2-amino-3-hydroxy-5-chloropyridine and conjugation to glucuronic or sulfuric acid.

Exposure Standards and Guidelines

The accepted daily intake for azamethiphos is 0.025 $mg\,kg^{-1}\,day^{-1}$. As this pesticide is not used in the United States, no reference dose is available.

See also: Carboxylesterases; Cholinesterase Inhibition; Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphates.

Relevant Websites

http://www.ec.gc.ca – Environment Canada.
http://www.scotland.gov.uk – Scottish Executive Publications.

Azathioprine

Eric M Silberhorn

- Chemical Abstracts Service Registry Number: CAS 446-86-6
- Synonyms: 6[(1-Methyl-4-nitro-1*H*-imidazol-5-yl) thio]-1*H*-purine; 6-(1-Methyl-4-nitro-5-imidazolyl) mercatopurine; Azamune; Azanin; Azothioprine; Imuran; Imurek; Imurel; Zytrim
- Chemical/Pharmaceutical/Other Class: Immunosuppressive antimetabolite; Disease modifying antirheumatic drug (DMARD)
- Chemical Formula: $C_9H_7N_7O_2S$

Uses

Azathioprine is an immunosuppressive and antiproliferative agent used in the treatment of several

different indications and conditions. It is effective as an adjuvant for protection against rejection of human organ transplants and in the treatment of immune-mediated and inflammatory diseases (e.g., inflammatory bowel disease including Crohn's disease, severe rheumatoid arthritis, chronic active hepatitis, acute leukemia). It has also been found to be an effective steroid sparing agent.

Background Information

Azathioprine was first introduced in 1961 and helped make allogenic kidney transplantation possible. It was originally designed as a prodrug of 6-mercaptopurine (6-MP), which had previously been found to produce remissions in acute childhood leukemia as a result of its immunosuppressive properties.

Exposure Routes and Pathways

Exposure as a therapeutic drug is oral, intravenous (IV), or colonic depending on the indication. IV administration is not as common as it once was because it does not reduce response time. The adult therapeutic dose for organ transplant is typically 3–5 $mg\,kg^{-1}\,day^{-1}$ for several days, then reduced to ~1–3 $mg\,kg^{-1}\,day^{-1}$ for maintenance. For inflammatory bowel disease, the adult dosage ranges from 1.5 to 3.0 $mg\,kg^{-1}\,day^{-1}$.

Toxicokinetics

In healthy individuals, anywhere from 16% to 50% of the ingested dose of azathioprine is absorbed. This percentage may be significantly less in individuals with bowel problems such as Crohn's disease. Maximum blood levels of azathioprine peak within 1–2 h after administration and rapidly drop as it is converted to 6-MP. The plasma half-life of azathioprine is ~12–15 min, while that for 6-MP is from 30 min to 4 h. Other metabolites have much longer half-lives. Binding of azathioprine to plasma proteins is low, a maximum of 30%.

Approximately 88% of azathioprine is converted to 6-MP, presumably in the liver. Conversion takes place nonenzymatically by sulfhydryl-containing compounds such as glutathione, which cause the imidazole group to split off, but also may occur enzymatically. 6-MP is further metabolized enzymatically to (1) the active compounds 6-thioguanine nucleotides (6-TGN) and 6-metilmercaptopurinic ribonucleotides (6-MMPR), or (2) inactive metabolites 6-thiouric acid (6-TU; the major urinary metabolite) and 6-methylmercaptopurine (6-MMP). Enzymes resulting in inactivation include xanthine oxidase (conversion to 6-TU) and thiopurine methyltransferase (TPMT; conversion to 6-MMP).

Up to 50% of the dose is excreted in the urine within 24 h of administration; however, only a small amount (<10%) of azathioprine is excreted unchanged. A further 12% of the dose is excreted unchanged in the feces.

Mechanism of Toxicity

Azathioprine is classified as an antiproliferative and immunosuppressive agent. Primarily through its metabolites, azathioprine antagonizes purine metabolism and may inhibit synthesis of DNA, RNA, and proteins. It may also interfere with cellular metabolism and inhibit mitosis. Following exposure to nucleophiles (e.g., glutathione, cysteine), azathioprine is cleaved nonenzymatically to 6-MP, an analogue of hypoxanthine. This conversion is believed to contribute to many, but not all, of the pharmacological and toxicological effects of azathioprine. The toxicity of azathioprine/6-MP has been attributed to at least three different mechanisms including the following:

1. Azathioprine/6-MP are a source of thioguanine nucleotides, which incorporate into DNA, yielding abnormal DNA that, in turn, interferes with the function of DNA polymerases, ligases, and endonucleases.
2. Aathioprine/6-MP are catalyzed to inhibitors of enzymes that are important in *de novo* purine synthesis.
3. Azathioprine and 6-MP promote rapid cell death by apoptosis and produce additional changes in B lymphocytes that favor apoptotic processes in those cells.

A feature of the pharmacologic action of azathioprine is its delayed onset, which may take 8–12 weeks to become apparent, possibly due to the slow accumulation of 6-TGN within the cells. The same is not necessarily true for the toxic effects of azathioprine, some of which may occur at any time during treatment (e.g., bone marrow suppression). Azathioprine appears to be a more potent immunosuppressive agent than does 6-MP itself, which may reflect differences in the pharmacodynamics and pharmacokinetics of the two compounds, as well as the relative abundance of different metabolites which are formed after their administration. Studies with hepatocytes have found that azathioprine toxicity involves depletion of reduced glutathione leading to mitochondrial injury with profound depletion of ATP and cell death by necrosis. Cell death was

prevented by potent antioxidants, glycine, and blocking the mitochondrial permeability transition pore.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} in the rat after oral administration is $535\,mg\,kg^{-1}$. Oral toxicity to the mouse is less, with an LD_{50} of $1389\,mg\,kg^{-1}$. The rat LD_{50} after intraperitoneal administration is $300\,mg\,kg^{-1}$, while that for the mouse is $272\,mg\,kg^{-1}$. In dogs, a dose of $10\,mg\,kg^{-1}$ for 10 days produced death from agranulocytosis. In general, animal studies have shown the hemopoietic system to be particularly sensitive to the effects of azathioprine with depression of granulopoiesis, magakaryocytes and, as a result, platelet formation. Dogs are very susceptible to hepatotoxicity, which is reversible, when given doses of $5\,mg\,kg^{-1}$ daily. The lymphatic system is also affected in monkeys, with atrophy of the lymphoid tissue at levels as low as $1\,mg\,kg^{-1}\,day^{-1}$.

Human

Signs of acute overdose include vomiting, diarrhea, and bone marrow depression in the form of mild leucopenia (in 2–3 days).

Chronic Toxicity (or Exposure)

Animal

There is limited evidence of carcinogenicity of azathioprine in experimental animals. Studies with rats produced squamous cell carcinomas of the ear duct after administration at $150\,mg\,kg^{-1}$ in the diet for 52 weeks and lymphomas of the thymus gland under other conditions. In mice, intraperitoneal injections at $40\,mg\,kg^{-1}$ in 1–4-day-old offspring produced leukemia and long-term studies produced lymphoma and hemangiodothelioma of the uterus.

Animals studies have show adverse effects of azathioprine (and 6-MP) on different stages of embryonic and fetal development. These include cleft palate, open eye, and skeletal anomalies in the offspring of mice injected intraperitoneally during gestation with 4–13 times the human therapeutic dose of azathioprine. Similar findings have been found in the offspring of rabbits injected with doses equivalent to two to six times those used in humans, although no malformations were observed in rat fetuses at the same doses. Doses within the human dose range did not produce anomalies in mice or rat offspring, although increased frequencies of fetal loss and growth retardation were observed.

Human

The toxic side effects of azathioprine and 6-MP may be classified into two categories: (1) idiosyncratic or allergic, and (2) direct toxicity. The first group includes pancreatitis, hepatitis, rash, pain in the joints, and other symptoms that are not dose-dependent. Direct toxicity results in increased susceptibility to infection, hematological toxicity (e.g., leucopenia), and increased development of tumors. Overall, side effects that necessitate stopping azathioprine/6-MP treatment occur in 10–25% of patients. Among the most frequent secondary effects are infections (7.4%), pancreatitis (3.3%), hematological toxicity (2–5%), and cutaneous allergic reactions (2%). Other less frequent side effects include fever, nausea, vomiting, diarrhea, headaches, and pellagra. There are also reports of long-term azathioprine treatment resulting in chronic liver disease (hepatitis) and portal hypertension. Most side effects improve or resolve with dose reduction or withdrawal of the drug.

Of the toxic side effects, a major concern among clinicians is for dose-dependent bone marrow suppression (myelotoxicity), which occurs in 2–4.6% of patients and can be fatal if not addressed properly. Study data suggest that a high incidence of secondary acute myeloid leukemia or brain cancer is correlated with low TPMT activity and high 6-TGN levels in children under immunosuppressive therapy. TPMT activity is subject to wide interindividual and interethnic variability due to TPMT gene polymorphism. In the Caucasian population, $\sim$0.3% of all individuals have no TPMT activity and 11% have intermediate activity, leading some to advocate additional monitoring of this activity in patients to help prevent unnecessary bone marrow toxicity from azathioprine treatment.

Azathioprine is classified as carcinogenic to humans (group 1) by the International Agency for Research on Cancer and known to be a human carcinogen by the National Toxicology Program based on sufficient evidence in humans. Several studies, including two large prospective epidemiological studies, have shown that renal transplant patients are at increased risk for several types of cancers as a result of azathioprine treatment. Cancers produced by azathioprine include non-Hodgkin's lymphoma, squamous cell cancers of the skin, hepatobilliary carcinomas, and mesenchymal tumors. Patients who have received azathioprine treatment for other conditions, including rheumatoid arthritis, systemic lupus and other collagen disorders, inflammatory bowel disease, and certain skin and renal diseases

have also been studied. Some of these same malignancies have been found in these patients, although to a lesser extent than in renal transplant patients. However, two recent large studies in patients with inflammatory bowel disease have not confirmed the increase in neoplasia with extended azathioprine treatment, so the picture is not entirely clear and may depend on a multitude of factors.

Recent studies have indicated that exposure of pregnant women to azathioprine may adversely affect the human embryo and fetus. The US Food and Drug Administration classifies azathioprine in category D, which indicates positive evidence of risk. Effects associated with antenatal exposure to azathioprine include spontaneous abortions and prematurity (40–52%), intrauterine growth reduction (19–40%), and low birth weight. The frequencies of prematurity and fetal growth retardation appear to be increased in pregnancies of renal transplant recipients treated with azathioprine, particularly if the woman requires a high dose therapy or has reduced renal function.

Exposure to azathioprine during pregnancy is associated with a slight increase in the frequency of congenital malformations, which varied from 0% to 11.8% in a total of 27 different clinical studies of infants of renal transplant patients. This compares with a background incidence of 3–5% in children born in developed countries. Malformations include microencephaly, hydrocephalus, anencephaly, hypospadias, malformed hand and face, polydactyly, cleft palate, and congenital heart disease. The incidence of malformations has also been shown to increase in the offspring of fathers who took azathioprine/6-MP within 3 months before conception. In contrast, the evidence is much weaker, or absent, for malformations caused by treatment with azathioprine during pregnancy for other conditions such as rheumatoid arthritis and inflammatory bowel disease.

Prenatal exposure to azathioprine/6-MP during the second trimester has been associated with chromosomal abnormalities in offspring, including chromatid breaks, deletions and extra fragments, translocations, and bridging fusions. The significance of these aberrations is unknown at this time.

In Vitro Toxicity Data

Azathioprine gave positive results in the Ames *Salmonella typhimurium* mutagenicity assay, both with and without metabolic activation, in the TA100 strain. Negative results were found for the TA98 strain under similar test conditions. Azathioprine has been shown to induce chromosomal aberrations in human and rabbit lymphocytes *in vitro*, however, it did not induce sister chromatid exchanges in either human lymphocytes or Chinese hamster bone marrow cells.

The presence of azathioprine affected the development of 9.5–11.5-day-old rat embryos cultured *in vitro* during organogenesis, producing alterations of the brain, caudal trunk, the heart and forelimb regions, and vesicular structures.

Clinical Management

There is no antidote for azathioprine toxicity. Treatment for an overdose entails ipecac within 30 min or lavage within 1 h, followed by activated charcoal. Side effects may be minimized with adequate monitoring of peripheral blood count and liver enzymes. Asymptomatic leucopenia, as well as most other side effects, may be treated with dose reduction or drug cessation (and changing to 6-MP); however, a life-threatening leucopenic episode may require administration of granulocyte colony-stimulating factor as well as other supportive care.

Other Hazards

Xanthine oxidase, an enzyme involved in the catabolism of metabolites of azathioprine, is blocked by allopurinol. If azathioprine and allopurinol are used in the same patient, the dose of azathioprine should be reduced to 25–33% of the usual dose, although it is best not to use these two drugs together.

See also: Blood; Carcinogenesis; Liver.

Further Reading

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Azinphos-Methyl

Subramanya Karanth

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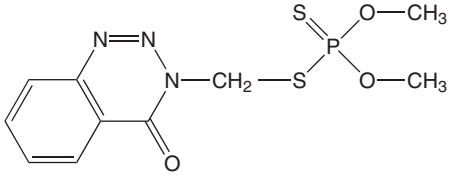
- Chemical Abstracts Service Registry Number: CAS 86-50-0
- Synonyms: Chrysthyon; Gusathion; Gusathion-M; Guthion; Methyl Guthion; Metiltrizotion
- Chemical/Pharmaceutical/Other Class: Organophosphorus (phosphorodithioate) insecticide
- Chemical Formula: $C_{10}H_{12}N_3O_3PS_2$
- Chemical Structure:



Uses

Azinphos-methyl is a broad-spectrum nonsystemic insecticide and acaricide commonly used on a number of fruit, vegetable, and nut crops. It is not used in residential and public health pest control.

Exposure Routes and Pathways

Dermal, inhalation, and ingestion are primary routes of exposure for azinphos-methyl.

Toxicokinetics

Azinphos-methyl is readily absorbed and distributed throughout the body following exposure. Mixed-function oxidase-mediated oxidative desulfuration of the parent compound produces the active metabolite azinphos methyloxon. Other major metabolites include dimethylphosphorothioic and dimethylphosphoric acids and desmethyl azinphosmethyl.

Mechanism of Toxicity

Azinphos-methyl requires bioactivation for its action. The parent compound is activated to the potent 'oxon' by microsomal mixed-function oxidase enzymes, which in turn elicits toxicity by inhibiting acetylcholinesterase in synapse and neuromuscular junctions. AChE inhibition leads to overstimulation of cholinergic receptors on postsynaptic neurons, muscle cells, and/or end-organs and consequent signs and symptoms of cholinergic toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity studies in laboratory animals have shown that azinphos-methyl is highly toxic to mammals. Oral and dermal LD_{50} values in laboratory rats are 4–16 and 88–220 mg kg^{-1}, respectively.

Human

Because of its high acute toxicity, low doses of azinphos-methyl ($\sim$1.5 mg day^{-1}) can lead to severe poisoning. Most common signs and symptoms of acute poisoning include salivation, excessive sweating, stomach pain, vomiting, and diarrhea. Inhalation of dust or aerosol containing azinphos-methyl can lead to wheezing, tearing of the eyes, blurred vision, and tightness in the chest. Eye contact with concentrated solutions of azinphos-methyl can be life threatening.

Chronic Toxicity (or Exposure)

Animal

Laboratory rats can tolerate a dietary dose of 0.5 mg kg^{-1} day^{-1} for 2 months without any adverse effects. Repeated long-term exposure to azinphos-methyl can lead to memory loss and irritability. While it is not mutagenic, the carcinogenic

potential of azinphos-methyl is not clearly understood due to lack of sufficient data.

Human

Acetylcholinesterase inhibition caused by azinphos-methyl can persist for a long time (2–6 weeks). Repeated chronic exposure may therefore result in prolonged acetylcholinesterase inhibition that may lead to flu-like illnesses.

Clinical Management

General decontamination procedures should be immediately initiated in case of azinphos-methyl exposure. For skin decontamination, the exposed area should be washed with plenty of water or soap and shampoo can be used during showering. The eyes are flushed with water repeatedly for several minutes. The contaminated clothing is removed and the airway cleared. In case of ingestion, vomiting should be induced. Atropine treatment should be initiated immediately to counteract muscarinic effects. Atropine (adults and children >12 years: 2–4 mg; children <12 years: 0.05–0.1 mg) treatment should be repeated every 15 min until oral and bronchial secretions are controlled and atropinization is achieved. The duration and dosage of atropine treatment is slowly reduced as the condition of the patient improves. Pralidoxime (2-PAM) should be administered slowly at the recommended dosage (adults and children >12 years: 1–2 g; children <12 years: 20–50 mg by IV infusion in 100 ml saline at $\sim 0.2\,\mathrm{g\,min^{-1}}$). This dosage can be repeated every 1–2 h intervals initially and at 10–12 h intervals later depending on the condition of the patient.

Environmental Fate

Persistence in the soil is generally low (half-life under aerobic and anaerobic conditions is 21 and 68 days, respectively). In sterile soil, the half-life is almost 1 year. Azinphos-methyl adsorbs strongly to soil particles and has low solubility in water. Biodegradation and evaporation are the primary routes of elimination from soil but azinphos-methyl is also degraded by ultraviolet light. Degradation is more rapid at higher temperatures. Azinphos-methyl has a short half-life in surface waters (2 days). Hydrolysis is more prominent under alkaline conditions but the compound is relatively stable in water below pH 10. The half-life on crops is 3–5 days under normal conditions.

Ecotoxicology

Azinphos methyl is highly toxic to fish and other aquatic organisms and moderately toxic to birds.

Exposure Standards and Guidelines

The chronic reference dosage is $0.0015\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ and the acceptable daily intake is $0.005\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.

See also: Acetylcholine; Biotransformation; Neurotoxicity; Organophosphates; Pesticides.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

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Bacillus cereus

Lee R Shugart

This article is a revision of the previous print edition article by Vittoria Werth, volume 1, p. 93, © 1998, Elsevier Inc.

Description

Bacillus cereus is a gram-positive, facultatively aerobic sporeformer whose spores do not swell the sporangium. These characteristics, along with specific biochemical features, are used to differentiate *B. cereus* from other species of the genus *Bacillus* (i.e., *B. thuringoemsos* and *B. anthracis*). The organism is widely distributed in nature and in food. It is commonly found in soil, milk, cereals, starches, herbs, spices, and other dried food stuffs.

Mechanism of Toxicity

B. cereus can cause two distinct types of food-borne intoxicants (as opposed to infections): (1) an emetic (vomiting) illness with a short incubation time of a few hours; and (2) a diarrheal illness with an incubation time of 8–6 h. The emetic form is caused by a preformed heat-stable enterotoxin of molecular weight less than 5000 Da. The long incubation form of the illness is mediated by a heat-labile enterotoxin of molecular weight of ~50 000 Da, which activates intestinal enzymes and causes intestinal fluid secretion.

Nature of Disease

B. cereus food poisoning occurs year-round without any particular geographic distribution and all people are believed to be susceptible. The emetic type of food poisoning is most often associated with rice products that have been cooked and then held at warm temperatures for several hours; other starchy foods such as potato, pasta, and cheese products have also been implicated. The emetic form is characterized by nausea and vomiting with 0.5–6 h after consumption of contaminated foods, symptoms that parallel those of *Staphylococcus aureus* food poisoning. The diarrheal type of food poisoning is frequently associated with foods (meats, milk, vegetables, and fish) after cooking (i.e., prepared food held above room temperature for a prolonged period). The onset of watery diarrhea, abdominal cramps, and pain occurs 6–15 h after consumption of contaminated foods. Nausea may accompany diarrhea, but vomiting rarely does. These symptoms resemble food poisoning caused by *Clostridium perfringens*. Nonanthrax *Bacillus* species are occasionally implicated in local infections especially those involving the eye. *B. cereus* is one of the most destructive organisms to infect the eye and can cause conjunctivitis, keratitis, iridocyclitis, dacryocystitis, orbital abscess, and panophthalmitis.

Control

B. cereus bacteria are common and widespread. Preventing contamination of food with spores is virtually impossible and because *B. cereus* are naturally present in some soil, their presence on fresh produce is not rare. Treatment of produce with chlorinated water reduces populations of microorganisms but cannot eliminate them. Effective prevention and control measures depend on inhibiting spore germination and preventing growth of vegetative cells in cooked, ready-to-eat foods. Freshly cooked food eaten hot, immediately after cooking is safe. Temperatures under 100°C will allow for the survival of some *Bacillus* spores, thus steaming under pressure, thorough roasting, frying, and grilling are most likely to destroy cells and spores.

See also: Clostridium perfringens; Staphylococcus aureus.

Further Reading

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Salyers AA and Whitt DD (1994) *Bacterial Pathogenesis: A Molecular Approach*. Washington, DC: American Society for Microbiology.

Relevant Website

http://vm.cfsan.fda.gov – *Bacillus cereus* and other *Bacillus* spp. (from the US Food and Drug Administration).

Bacillus thuringiensis

Eric M Silberhorn

- SYNONYMS: Bt; Acrobe; B 401; Bactimos; Bactospeine; Berliner (variety kurstaki); Biotrol 4K; Certan (variety aizawai); Dipel; Foray; Gnatrol; Javelin; Leptox; Novabac; Teknar (variety israelensis); Thuricide; Vectobac; Victory
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Bacterium

Uses

Bacillus thuringiensis (Bt) is a microbial insecticide.

Background Information

Bt is a group of strains or isolates of naturally occurring soil bacteria that are used to control insect pests on agricultural crops, stored food crops, ornamental plants, in bodies of water, and around homes. Different strains of Bt have specific toxicity to particular types of insects depending on the specific crystalline protein (delta-endotoxin) that they produce. For example, Bt *aizawai* (Bta) is effective against wax moth larvae; Bt *israelensis* (Bti) is effective against mosquitoes, blackflies, and some midges; Bt *kurstaki* (Btk) controls various types of lepidopterous species including the gypsy moth and cabbage looper; and Bt *san diego* is effective against certain beetle species and the boll weevil. Bt is considered an almost ideal agent for pest management because of its combination of insecticidal specificity and lack of toxicity to humans and nontarget organisms. Most Bt-based insecticides are formulated mixtures of delta-endotoxin crystals and Bt spores. The Bt spores synergize the toxicity of the crystalline proteins.

Exposure Routes and Pathways

The most likely routes of exposure to Bt for the general public are oral, dermal, and inhalation.

In addition to these routes of exposure, accidental parenteral or ocular exposures may occur in workers that apply Bt in the field.

Mechanism of Toxicity

Bt is ineffective against adult insects and must be eaten by feeding larvae in order to be toxic. During sporulation, Bt produces a parasporal inclusion body referred to as a crystal, which is made up of proteins also known as delta-endotoxins. When eaten, the delta-endotoxins (crystalline proteins) produced by Bt dissolve and act as poisons in the target species, paralyzing cells in the midgut, interfering with normal digestion, and triggering the insect to stop feeding and eventually die. The time until death may range from a few hours to several weeks depending on the insect species and the amount of Bt ingested. Gut paralysis is caused by toxins that bind to specific receptors present on the membranes of epithelial midgut cells. These toxins are formed during proteolytical processing of solubilized Bt protein crystals (the protoxin). Once membrane-bound, the toxin induces formation of pores or open channels in the midgut epithelial cell membrane, which causes paralysis and cell death, and later death of the larvae.

Acute and Short-Term Toxicity (or Exposure)

Animal

To date, numerous laboratory studies have been conducted on the infectivity and toxicity of Bt isolates and these studies have demonstrated that the isolates of Bt used in commercial products are safe. In several acute oral toxicity/pathogenicity studies, no adverse effects, infectivity, or pathogenicity has been observed in laboratory animals at doses up to 4.7×10^{11} spores kg^{-1}. In acute pulmonary toxicity studies, no adverse toxic effects have been seen at doses up to 2.6×10^{7} spores kg^{-1}. Similarly, Bt is nontoxic and not pathogenic in acute studies that administered Bt intraperitoneal to mice at dose levels below 10^{8} colony forming units (cfu) per animal. Repeated oral exposures for 21 days did not produce mortality or changes in weight gain in rats (1.2×10^{11} cfu) or mice (4.7×10^{10} cfu). Dermal exposures of several different Bt strains at levels up to 2500 mg kg^{-1} were not toxic or pathogenic to rabbits, but did produce mild irritation in some cases.

Human

A short-term study with human volunteers has not demonstrated any adverse health effects of Bt. Eight subjects ingested 1 g of Bt formulation (3×10^{9} spores g^{-1} of powder) daily for 5 days. Five of these volunteers also inhaled 100 mg of the Bt powder daily for 5 days. No adverse effects were observed in comprehensive medical examinations conducted before or after (including 4–5 weeks after)

the exposures, and clinical chemistry data were also negative.

Due to its mode of action, acute exposures to Bt via routes other than oral ingestion are not expected to produce toxicity. However, contact with Bt formulations in high enough concentrations may still potentially cause irritation of the skin, eyes, and respiratory tract due to the physical nature of these materials. For example, dermatitis was reported by one worker after contact with Bt solution.

Chronic Toxicity (or Exposure)

Animal

In rats, no toxicity or infectivity was associated with dietary exposure to Bt at a level of $4\,g\,kg^{-1}\,day^{-1}$ for 3 months. Sheep exposed repeatedly to commercial Bt formulations for 60 days showed no clinically significant effects. Rats fed a Bt product for 2 years in the diet at $8400\,mg\,kg^{-1}\,day^{-1}$ experienced a decrease in body weight gain (females only) during weeks 10–104 of the study, but no other significant effects.

Human

Bt microbial products have a long history (greater than 40 years) of safe use. Reports of serious adverse effects in humans from the use of these products are rare and none were considered to be casually related to Bt itself. Two detailed epidemiology studies have been carried out on the exposure of humans to Bt. In a Canadian study on ground spray operators in a control program for the gypsy moth, researchers found that workers without protective clothing developed minor irritations of the skin, eyes, and respiratory tract, but no serious health problems. Symptoms were reported at two to three times the rate for the control group. These symptoms were transient and frequently occurred during the beginning of a spray run and when Bt spray concentrations were increased. Mean exposure values ranged from 3.0×10^3 to 5.9×10^6 Bt spores m^{-3} of sampled air. The exposure rates for the spray operators were up to 500 times greater than that estimated for the general population. In a passive surveillance study conducted in Oregon during 1985–86, there was only one health complaint that could be attributed to Bt: dermatitis and eye irritation in a spray operator who was splashed in the face and eyes with a spray solution. More recently, the presence of specific IgE and IgG antibodies has been demonstrated in farm workers who picked vegetables treated with Bt products. The incidence of antibodies was higher in workers in the high exposure group; however, the significance of these finding is unknown as there was no increase in the incidence of asthma or other occupationally related clinical diseases in these workers.

Environmental Fate

Bt is moderately persistent in soil with a half-life of ~4 months. It is rapidly inactivated in soils that have a pH below 5.1. Bt is relatively short-lived on foliage due to rapid photodegradation. Its half-life under normal sunlight conditions is 3.8 h. In general, Bt loses 50% of its insecticide activity in 1–3 days after spraying.

Ecotoxicology

To date based on extensive laboratory and field data, there is little evidence that commercial Bt formulations cause any significant ecological impacts when used for insect control. Bt strains are classified as practically nontoxic to birds based on acute toxicity studies conducted for the US Environmental Protection Agency as part of the pesticide registration process. In general, field studies have not shown effects on bird populations after aerial spraying of Bt formulations, although effects on avian reproductive parameters (e.g., nesting attempts, fledgling success) have been measured in two instances. Field studies have also not shown any significant adverse effects of Bt products on mammals or plants exposed at typical application rates. Some Bt strains are highly toxic to certain aquatic invertebrates such as *Dipteran* (mosquitoes and blackflies) larvae; however, most aquatic species are quite tolerant to the effects of Bt. Field monitoring studies after application of Bt for control of the spruce budworm found no measurable effects on a wide variety of aquatic insects and similar results have been found in other studies. Bt is practically nontoxic to fish with acute LC_{50} values greater than $8.7 \times 10^9\,cfu\,l^{-1}$ for bluegill sunfish, rainbow trout, and the sheepshead minnow. There has been no documented evidence of fishes killed as a result of the many forestry, agriculture, and urban Bt spraying programs conducted in Canada and the United States over the past 30 years or more. In contrast, temporary reductions in populations of nontarget Lepidoptera and some other susceptible insects have been documented in the field after aerial applications of Bt. Because of the lack of persistence of Bt in the environment, the effects are primarily limited to the period of use.

See also: Genetically Engineered Foods; Pesticides.

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Joung K-B and Côté J-C (2000) A review of the environmental impacts of the microbial insecticide *Bacillus thuringiensis*. Technical Bulletin No. 29. Horticulture Research and Development Centre, Agriculture and Agri-Food Canada.

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Siegel JP (2001) The mammalian safety of *Bacillus thuringiensis*-based insecticides. *Journal of Invertebrate Pathology* 77: 13–21.

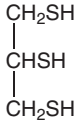
US Environmental Protection Agency (1998) *Reregistration Eligibility Decision (RED) Bacillus thuringiensis*. Washington, DC: Office of Prevention, Pesticides and Toxic Substances.

BAL (British Antilewisite)

Sharmilee P Sawant and Harihara M Mehendale

This article is a revision of the previous print edition article by Arvind K Agarwal, volume 1, pp. 93–94, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 59-52-9
- Synonyms: 2,3-Dimercaptopropanol; Dimercaprol; Dicaptol; Sulfactin; 1,2-Dithioglycerol; Dimercaptol; 2,3-Dimercaptopropan-1-ol; USAF ME-1
- Chemical Structure:



Uses

British antilewisite (BAL) is a chelating agent used as an antidote for treatment of metal poisoning, especially arsenic (organic and inorganic), gold salts, and mercury. BAL is more effective when given soon after toxic exposure because it is more effective in preventing inhibition of sulfhydryl enzymes than in reactivating them.

Background Information

Dimercaprol is a synthetic therapeutic substance developed during World War II as an antidote against the vesicant arsenic war gases (lewisite). The first experiments were based on the fact that arsenic products react with SH radicals. Among all the compounds originally tested, BAL was the most effective and the least toxic. In 1951, BAL was used by a renowned neurologist, Derek Denny-Brown, to treat patients suffering with Wilson's disease (hepatolentricular degeneration), which results from excessive copper accumulation, especially in the brain and liver. The intrinsic toxicity of BAL later led to the development of its water-soluble and less toxic derivatives dimercaptosuccinic acid and dimercaptopropanesulfonic acid.

Exposure Routes and Pathways

BAL is given only by deep intramuscular (i.m.) injection (never intravenous (i.v.) or subcutaneous (s.c.)). Oral ingestion is only accidental or intentional. Dimercaprol can be applied to the skin to heal local effects caused by arsenic vesicant substances.

Toxicokinetics

Peak concentrations in blood are obtained in about 30–60 min after intramuscular injection of dimercaprol. It is readily absorbed through the skin after topical application. Because it is a lipophilic drug, dimercaprol rapidly penetrates the intracellular spaces. The highest concentrations are found in the liver, kidneys, brain, and small intestine. BAL's biological half-life is short and metabolic degradation and renal excretion is complete within 6–24 h according to animal studies. The renal excretion is most often cited as its major elimination route but there appears to be a significant contribution from its conjugation with glucuronic acid. The major portion of the drug is excreted rapidly in the urine, and part of it is eliminated in the feces (via bile). The dimercaprol–metal complexes dissociate rapidly in the body, especially in an acid internal medium; alkalinization of the urine may prevent this dissociation and protect the kidneys from metal and BAL nephrotoxicity.

If the BAL–metal complex is oxidized, the metal is released and can exert its toxic effect again; therefore, the dosage of dimercaprol must be high enough to ensure the excess of free BAL in body fluids until the metal is completely excreted.

Mechanism of Toxicity

BAL is believed to compete with tissue sulfhydryl groups and interferes with cellular respiration. It also competes with metallic cofactors of metabolic enzyme systems and increases capillary permeability.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} in rabbits and rats is in the range of 0.6–1.0 mmol kg^{-1} by i.m., i.p., or s.c. absorption. In another study, the LD_{50} in rats after i.m. injection was 105 mg kg^{-1}. In animals, a lethal dose of dithiols causes convulsions and severe spasm of the abdominal muscles shortly before death occurs. Sublethal injection of dimercaprol to animals results in lacrimation, edema of the conjunctiva, salivation, and vomiting. With increasing doses, they develop ataxia, analgesia, tachypnea, and hyper-excitability. Nystagmus and muscle tremor develop; tonic and clonic convulsions occur at the final stages. Death occurs during coma. The most important acute toxic effect of dimercaprol is cardiovascular depression as judged by a fall in systemic and pulmonary artery pressure following i.v. injection in cats.

Human

A common side effect of BAL is an increase in systolic and diastolic arterial pressures with tachycardia. About 50% of patients who receive high therapeutic doses (4–5 mg kg^{-1}) have minor reactions: nausea, vomiting, fatigue, restlessness, apprehension, headache, burning sensation of the mouth, throat, and eyes, lacrimation, salivation, tingling of extremities, a feeling of constriction in the chest muscle, diffuse pain, and muscle spasm. Large doses may cause convulsions and coma. There may be pain at the injection site. BAL may cause hemolytic anemia in individuals with a glucose-6-phosphate dehydrogenase (G6PD) deficiency. When applied locally to skin, it produces redness and swelling. It is an irritant to eyes and mucous membrane.

Chronic Toxicity (or Exposure)

Animal

Very few chronic toxicity studies have been reported. After repeated local applications in animals, sensitization dermatitis may develop. Chronic parenteral administration increases the white blood cell count by 30%.

Human

Long-term exposure of BAL is unnecessary. There are no reports on the long-term toxic effects of BAL.

Clinical Management

There is no specific treatment, but symptomatic measures can be taken to improve the clinical course. Dimercaprol is stopped immediately if adverse reactions are observed. No antidote is available. If there has been dermal exposure, the skin should be washed with a nonirritating soap and water. If the eyes have been exposed, they must be irrigated with tap water. If ingested, activated charcoal must be given. Convulsions should be treated as usual with benzodiazepines and barbiturates. If cardiovascular collapse develops, fluids should be given according to the patient's hydroelectrolytic balance. Dopamine can be used, if necessary. Bicarbonate solution is useful, not only to correct acidosis but also to increase renal elimination of BAL–metal complexes, but also in preventing their dissociation and decreasing their toxicity. Some symptoms can be relieved by administration of an antihistamine. Alkalinization of urine may prevent kidney damage.

Miscellaneous

BAL should be administered carefully and under strict clinical control in patients with hypertension, hepatic, or renal impairment, and G6PD deficiency.

See also: Arsenic; Mercury; Metals.

Further Reading

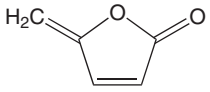
Vilensky JA and Redman K (2003) British anti-lewisite (dimercaprol): An amazing history. *Annals of Emergency Medicine* 41: 378–383.

Waters LL and Stock C (1945) Bal (anti-lewisite). *Science* 102: 601–606.

Baneberry

Rebeca Gracia

- Chemical Name: 4-Methylenebut-2-en-4-olide
- Representative Chemical: Protoanemonin
- Synonyms: *Actaea pachypoda* (white baneberry); *Actaea rubra* (red baneberry); *Actaea spicata* (grape wort, herb-Christopher); *Actaea erythrocarpa*; Doll's eyes; Cohosh; Snakeberry; Coral berry; Toadroot; Bugbane
- Chemical/Pharmaceutical/Other Class: *Actaea* species, of the order Ranunculaceae; Gastrointestinal irritants
- Chemical Formula: $C_5H_4O_2$
- Chemical Structure:



Uses

Topical compress for arthritis or frostbite, antispasmotic, anti-infective.

Background Information

Baneberry is a tall perennial herb that grows in most woodlands throughout the United States and Canada. It has large compound leaves, small white flowers, and either red or white berries (depending on the region).

There is substantial overlap between baneberry and black cohosh, a plant from the same order and more significant toxicity. Black cohosh is most likely to be associated with the pungent plant properties purported to be used as an antivenom and vermin repellant.

Exposure Routes and Pathways

The leaves, berries, and small white flowers may be ingested or applied dermally.

Toxicokinetics

Protoanemonin is released through enzymatic cleavage when the plant is crushed but is poorly absorbed due to low solubility. Large amounts may cause systemic toxicity. Ingestion of six or more berries may result in toxicity. Protoanemonin is excreted primarily by the kidneys.

Mechanism of Toxicity

The toxic effects of baneberry result from the irritant and vesicant effect of protoanemonin on mucous membranes.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxic effects in animals are similar to those in humans after ingestion of baneberry. Reports describe seizure activity and paralysis in livestock that ingest large amounts of the fresh plant. Protoanemonin is rapidly polymerized to the inactive anemonin if dried.

Human

The ingestion of the berries of the plant results in an initial burning sensation, increased salivation, and mucosal irritation, resulting in oral ulcerations. This is followed by acute stomach cramps and vomiting within 30 min. Dizziness, headache, and delirium were noted 1 h after ingestion. The symptoms usually disappear 3 h after ingestion. Prolonged dermal contact with the juice of berries or leaves may result in severe burning and skin irritation. The irritant properties persist even as protoanemonin is excreted, resulting in inflammation of the urinary tract, hematuria, and dysuria.

Clinical Management

Treatment of baneberry exposure is supportive. Careful evaluation and management of fluid and electrolytes is required. Gastric decontamination is effective if performed within the first hour after exposure. Tissue damage in the oral area from the effect of protoanemonin should be assessed before any gastrointestinal decontamination is performed. Monitoring renal output is necessary.

See also: Plants, Poisonous.

Relevant Website

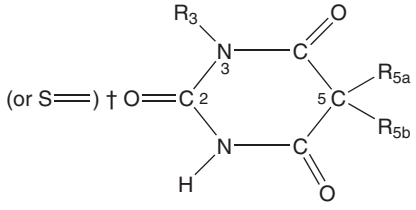
http://www.botanical.com – The electronic version of 'A Modern Herbal' by Maud Grieve.

Barbiturates, Long-Acting

Alexander B Baer and Christopher P Holstege

This article is a revision of the previous print edition article by Gregory P Wedin, volume 1, pp. 95–97, © 1998, Elsevier Inc.

- Representative Chemicals: Barbital (CAS 57-44-3); Mephobarbital (CAS 115-38-8); Phenobarbital (CAS 50-06-6)
- Synonyms:
  - Barbituratess – Courage pills; Downers; F-40s; Goof balls; Gorilla pills; Mexican yellows; Pink ladies
  - Barbital – Diethylbarbituric acid; Diethylmalonyl urea; Barbitone; DEBA
  - Mephobarbital – Methylphenobarbital; Mebaral
  - Phenobarbital – Phenylethylmalonylurea; Barbenyl; Barbiphenyl; Dormiral; Phenylbarbital; 5-Ethyl-5-phenylbarbituric acid; Solfoton
- Chemical/Pharmaceutical/Other Class: Barbituric acid derivative
- Chemical Structure:



Uses

The long-acting barbiturates are used for insomnia, anxiety, psychosis, preoperative sedation, and control of seizures. Long-acting barbiturates are utilized as drugs of abuse. This abuse peaked in the 1970s, but has since declined with the increased use of other sedatives.

Exposure Routes and Pathways

The most common route of exposure to the long-acting barbiturates is ingestion of oral dosage forms. Phenobarbital is also available for parenteral administration.

Toxicokinetics

Approximately 50–90% of the long-acting barbiturates are slowly absorbed from the gastrointestinal tract. Absorption is more rapid when ingested on an empty stomach and in the presence of alcohol. The onset of action varies: 30–60 min for mephobarbital and 8–12 h for oral phenobarbital. Mephobarbital is primarily metabolized by *N*-demethylation to form phenobarbital. Phenobarbital is metabolized by the hepatic microsomal enzyme system to an inactive metabolite. Long-acting barbiturates induce the hepatic microsomal enzyme system, especially CYP3A. This can lead to numerous drug interactions. Long-acting barbiturates, compared to short-acting barbiturates, are less lipid soluble, accumulate more slowly in tissue, are excreted more readily by the kidney as active drug, and have an elimination half-life longer than 40 h. The long-acting barbiturates are extensively distributed to all body tissues and fluids with highest concentrations achieved in the brain, liver, and kidneys. The apparent volume of distribution for phenobarbital is 0.5–1.01 kg^{-1}. Approximately 20–45% is bound to plasma proteins. A minimal amount of mephobarbital is eliminated unchanged in the urine. Phenobarbital has a long elimination half-life of ~2–6 days. Approximately 25% of a dose is eliminated unchanged in the urine with the remainder eliminated as inactive metabolites. The pK_a of phenobarbital (7.24) is similar to physiologic pH. As a result, the elimination of unchanged drug is significantly influenced by changes in the urine pH. Alkalinization of the urine can enhance the elimination of phenobarbital. This is referred to as ion trapping.

Mechanism of Toxicity

Barbiturates bind to specific sites on γ-aminobutyric acid (GABA)-sensitive ion channels found within the central nervous system (CNS). By binding to these sites, barbiturates allow an influx of chloride into cell membranes and, subsequently, hyperpolarize the postsynaptic neuron. GABA is the major inhibitory neurotransmitter in the CNS. Barbiturates enhance GABA-mediated chloride currents by binding to the GABA-A receptor–ionophore complex and increasing the duration of ionophore opening. At high doses, barbiturates stimulate GABA-A receptors directly in the absence of GABA. Barbiturates also block glutamate (excitatory neurotransmitter) receptors in the CNS. The CNS is particularly sensitive to the effect of barbiturates; however, with intoxication, the cardiovascular system and other peripheral functions are also depressed.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may be affected by the long-acting barbiturates much in the same way as humans. Lethargy,

coma, shallow respirations, incoordination, and depressed reflexes may occur. Standard supportive measures should be employed.

Human

Doses of 8 mg kg^{-1} or greater of phenobarbital will likely cause signs and symptoms of toxicity. The estimated potentially fatal dose in nondependent adults is 6–10 g. Overdose will produce CNS depression ranging from drowsiness to profound coma with a suppressed electroencephalogram. There have been reports of patients who achieved full neurological recovery after having isoelectric electroencephalograms for several days. Therefore, it is important to obtain drug levels on these patients before declaration of anoxic brain death. Patients who overdose with long-acting barbiturates may be comatose for several days. Severe intoxication may result in cardiovascular depression and vasodilation leading to hypotension, cardiovascular collapse, and cardiac arrest. Apnea and respiratory arrest also may occur. Depression of the gastrointestinal tract may cause an ileus. Horizontal gaze nystagmus may be seen. Comatose patients may develop bullous skin lesions primarily, but not always, over areas of pressure that are commonly called 'barb burns'. Barbiturate plasma concentrations aid in diagnosis and help determine whether to institute methods to enhance elimination. Barbiturate plasma concentrations are not accurate for predicting the duration or severity of toxicity.

Chronic Toxicity (or Exposure)

Animal

Phenobarbital is considered a carcinogen in experimental animals. Mice treated while pregnant developed a dose-related increase in the numbers of pups born with cleft-palate (0.6% of fetus in the 50 mg kg^{-1} diet vs. 3.9% in the 150 mg kg^{-1} diet).

Human

Chronic use of high doses of the long-acting barbiturates may produce psychological and physical dependence. Abrupt discontinuation of therapy may result in withdrawal signs and symptoms. Mild withdrawal may include weakness, anxiety, muscle twitching, insomnia, nausea, and vomiting. Severe withdrawal may consist of hallucinations, autonomic instability, delirium, and seizures. Unlike opioid withdrawal, long-acting barbiturate withdrawal may be life threatening. Barbiturates induce hepatic microsomal enzymes and can increase the metabolism of certain drugs, like acetaminophen, to their toxic metabolites potentially increasing the risk for adverse effects in polydrug overdoses.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Gastrointestinal decontamination procedures should be used as appropriate based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used to adsorb the long-acting barbiturates. Multiple-dose activated charcoal therapy (every 2–6 h for 24–48 h) enhances the nonrenal elimination of phenobarbital, but has not been shown to improve outcomes. It may be effective with other long-acting barbiturates. The patient's level of consciousness and vital signs should be monitored closely. Obtunded patients with reduced gag reflex should be intubated to reduce the risk of aspiration. Respiratory support including oxygen and ventilation should be provided as needed. There is no antidote for the long-acting barbiturates. If hypotension occurs it should be treated with standard measures including intravenous fluids, Trendelenburg positioning, and vasopressor support by intravenous infusion in refractory cases. Urine alkalinization may enhance elimination. Hemodialysis is effective for removing long-acting barbiturates but should be reserved for severe cases when standard supportive measures are inadequate. In chronic use of long-acting barbiturates, a gradual taper rather than abrupt discontinuation of barbiturates is appropriate to prevent the occurrence of life-threatening withdrawal.

See also: Anxiolytics; Neurotoxicity; Poisoning Emergencies in Humans.

Further Reading

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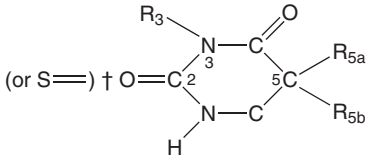
Lindberg MC, Cunningham A, and Lindberg NH (1992) Acute phenobarbital intoxication. *Southern Medical Journal* 85: 803–807.

Barbiturates, Short-Acting

Alexander B Baer and Christopher P Holstege

This article is a revision of the previous print edition article by Gregory P Wedin, volume 1, pp. 97–99, © 1998, Elsevier Inc.

- Representative Chemicals: Amobarbital; Aprobarbital; Butabarbital sodium; Cyclobarbital; Heptabarbital; Hexobarbital; Methohexital sodium; Pentobarbital; Secobarbital sodium; Talbutal; Thiamylal; Thiopental sodium
- Chemical Abstracts Service Registry Number: CAS 309-36-4 (methohexital sodium)
- Synonyms:
  - Amobarbital – Amytal; $C_{11}H_{18}N_2O_3$; 5-Ethyl-5-(3-methylbutyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Aprobarbital – Alurate; $C_{10}H_{14}N_2O_3$; 5-(1-Methylethyl)-5-(2-propenyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Butalbital – Sandoptal; $C_{11}H_{16}N_2O_3$; 5-(2-Methylpropyl)-5-(2-propenyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Butabarbital – Butisol; Butolan; Sarisol; $C_{10}H_{15}N_2NaO_3$; 5-Ethyl-5-(1-methylpropyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione sodium salt
  - Cyclobarbital – $C_{12}H_{16}N_2O_3$; 5-(1-Cyclohexen-1-yl)-5-ethyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Heptabarbital – $C_{13}H_{18}N_2O_3$; 5-(1-Cyclohepten-1-yl)-5-ethyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Hexobarbital – $C_{12}H_{16}N_2O_3$; 5-(1-Cyclohexen-1-yl)-1,5-dimethyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Methohexital – Brevital; Compound 25398; $C_{14}H_{17}N_2NaO_3$; 1-Methyl-5-(1-methyl-2-pentynyl)-5-(2-propenyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione sodium salt
  - Pentobarbital – Nembutal; $C_{11}H_{18}N_2O_3$; 5-Ethyl-5-(1-methylbutyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Secobarbital – Seconal; $C_{12}H_{17}N_2NaO_3$; 5-(1-Methylbutyl)-5-(2-propenyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione sodium salt
  - Talbutal – Lotusate; $C_{11}H_{16}N_2O_3$; 5-(1-Methylpropyl)-5-(2-propenyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Thiamylal – Surital; $C_{12}H_{18}N_2O_2S$; dihydro-5-(1-Methylbutyl)-5-(2-propenyl)-2-thioxo-4,6-(1*H*,5*H*)-pyrimidinetrione
  - Thiopental – Pentothal; $C_{11}H_{17}N_2NaO_2S$; 5-Ethyldihydro-5-(1-methylbutyl)-2-thioxo-4,6-(1*H*,5*H*)-pyrimidinetrione sodium salt
- Chemical/Pharmaceutical/Other Class: Barbituric acid derivative
- Chemical Structure:



Uses

The short-acting barbiturates are used for short-term treatment of insomnia, anxiety, psychosis, preoperative sedation, control of seizures, and anesthetics. Short-acting barbiturates are also used as drugs of abuse. Barbiturate use has dramatically decreased since the 1970s with the introduction of benzodiazepines.

Exposure Routes and Pathways

The most common route of exposure to the short-acting barbiturates is ingestion of oral dosage forms. Several of these agents are also available for parenteral administration (intramuscular or intravenous) and have been used as rectal suppositories.

Toxicokinetics

The short-acting barbiturates are rapidly and completely absorbed from the gastrointestinal tract. The sodium salts are absorbed more rapidly than the acids by all routes. Absorption is more rapid when ingested on an empty stomach and also in the presence of alcohol. The onset of action varies from 10 to 30 min. The short-acting barbiturates are all extensively metabolized by the hepatic microsomal enzyme system. The short-acting barbiturates are rapidly distributed to all body tissues and fluids with the highest concentrations achieved in the brain, liver, and kidneys. The apparent volume of distribution ranges from 0.6 to 1.9 l kg^{-1}. Inactive metabolites of the short-acting barbiturates are eliminated in the urine. Only aprobarbital, which is less lipid soluble, has a significant fraction (13–24%) that is eliminated unchanged in the urine. The elimination half-life ranges from 1 to 48 h.

Mechanism of Toxicity

These barbiturates are known to decrease the excitability of postsynaptic membranes by binding to the γ-aminobutyric acid (GABA) receptor and increasing the duration of time these chloride channels are open. Enhanced GABA receptor-mediated chloride conductance also occurs through a stronger receptor affinity for other ligands like GABA and benzodiazepines in the presence of some barbiturates. The central nervous system (CNS) is particularly sensitive to these effects, but with intoxication the cardiac and vascular smooth muscle tone can also be depressed.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may be affected by the short-acting barbiturates much in the same way as humans. Lethargy, coma, respiratory depression, ataxia, hypothermia, and hypotension have been described in poisoning. Some short-acting barbiturates are utilized as veterinary euthanasia agents. The treatment of overdose is similar to that in humans.

Human

There is a broad spectrum of signs and symptoms associated with acute short-acting barbiturate toxicity. Lethargy, ataxia, nystagmus, diplopia, amnesia, slurred speech, confusion, hypotonia, hypotension, hypothermia, hypoglycemia, coma, respiratory depression, and death have been reported. Comatose patients may develop erythematous or hemorrhagic bullous skin lesions primarily over areas of pressure (e.g., elbows and knees). These lesions are commonly referred to as 'barb burns'. Doses of 3–5 mg kg^{-1} of most short-acting barbiturates will cause toxicity in children. The estimated potentially fatal dose in nondependent adults is 3–6 g.

Hypersensitivity to barbiturates can result in a life-threatening syndrome called the Drug, Rash with Eosinophilia and Systemic Symptoms (DRESS) Syndrome with a mortality of 10%. In persons developing hypersensitivity to barbiturates, there is a potential of cross-sensitivity with other aromatic antiepileptics, such as phenytoin and carbamazepine.

Chronic Toxicity (or Exposure)

Animal

Dogs fed amobarbital chronically developed CNS depression, slowed reaction times, and incoordination.

Human

Tolerance and physical dependence may develop in persons who chronically use short-acting barbiturates. Abrupt discontinuation of chronic barbiturate therapy may result in a withdrawal syndrome consisting of anxiety, agitation, insomnia, tremors, headache, myalgias, nausea, vomiting, diaphoresis, hyperpyrexia, psychosis, seizures, and death. Chronic barbiturates use may result in induction of the hepatic microsomal enzyme system.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Activated charcoal can be used to adsorb the short-acting barbiturate drug as long as the patient's airway is protected. The patient's level of consciousness and vital signs should be monitored closely. Obtunded patients with reduced gag reflex should be intubated. Respiratory support including oxygen and ventilation should be provided as needed. If hypotension occurs, it should be treated with standard measures including intravenous fluids, Trendelenburg positioning, and dopamine by intravenous infusion. There is no antidote for the short-acting barbiturates. Forced alkaline diuresis is of no value for the short-acting barbiturates. Major complications associated with barbiturate intoxication include anoxic brain injury, aspiration pneumonia, rhabdomyolysis, and compartment syndrome. The occurrence of withdrawal signs and symptoms indicates the need to reinstitute barbiturate or substitute alternative benzodiazepine therapy and gradually reduce the dose until discontinued.

See also: Anxiolytics; Neurotoxicity; Poisoning Emergencies in Humans.

Further Reading

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Barium

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst, Shirley B Radding, and Kathyrn A Wurzel, volume 1, pp. 99–100, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-39-3
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Ba^{2+}

Uses

Barium is found in various alloys, paints, soap, paper, photographic chemicals, explosives, and rubber, and is used in the manufacture of ceramics and glass. Some of its compounds are used as mordents in fabric dying and in the preparation of phosphors. One major use is in a slurry of ground barite ($ZnS + BaSO_4$) for gas and oil drilling. Barium fluorosilicate has been used as an insecticide and some barium compounds are used as rodenticides. Medicinally, barium sulfate, being very sparingly soluble, is used as a radiopaque contrast material for X-ray diagnostic purposes, and other medical imaging uses.

Background Information

Sir Humphrey Davy discovered barium in 1808.

Exposure Routes and Pathways

Exposure pathways for barium primarily consist of ingestion (e.g., food and water) and inhalation. Barium is relatively abundant in nature; hence, most food contains small amounts of barium. Brazil nuts have very high barium concentrations (from 3 to 4000 ppm). It is also found in drinking water from natural deposits in certain regions. Barium is also detected in the air of most cities.

Toxicokinetics

Soluble barium compounds are absorbed by the lungs and gastrointestinal tract and small amounts are accumulated in the skeleton. The highest concentration of barium in the body is present in the lungs. Although some barium is excreted in the urine, it is reabsorbed by the renal tubules. It is primarily excreted in feces.

Mechanism of Toxicity

Ingestion of toxic doses of barium affects the muscles, especially the heart. Barium has a digitalis-type effect on the heart. Ventricular fibrillation and slowed pulse rate are noted. This may be related to barium's tendency to displace potassium; the resulting potassium deficiency causes muscle weakness.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} for rats is $630\,mg\,kg^{-1}$ for barium carbonate, $118\,mg\,kg^{-1}$ for barium chloride, and $921\,mg\,kg^{-1}$ for barium acetate.

Human

The toxicity of barium is related to the solubility of the compound. Barium sulfate, being very sparingly soluble, is relatively nontoxic. Soluble barium salts are toxic by ingestion (e.g., acetate, chloride, nitrate, sulfide, as well as carbonate and hydroxide compounds). Ingestion results in nausea, vomiting, stomach pains, and diarrhea. Severe gastrointestinal irritation is followed by muscle twitching and then a flaccid muscular paralysis. Barium can activate catecholamines, resulting in muscle twitching and other nervous system effects. Ingestion of barium compounds can lead to gastroenteritis, hypokalemia, hypertension, cardiac arrhythmias, and skeletal muscle paralysis. Potassium infusion is used clinically to reverse many of the toxic effects, but cannot reverse the hypertensive response. Barium released during welding can decrease plasma potassium levels.

Soluble compounds also irritate skin, eyes, and mucous membranes and can be absorbed following inhalation. Barium carbonate dust is a bronchial irritant. Barium oxide dust is a dermal and nasal irritant.

The estimated lethal dosage of the rodenticide barium carbonate in humans is $\sim 70\,mg\,kg^{-1}$. The LD_{50} for barium chloride is estimated at $\sim 14\,mg\,kg^{-1}$, and the LD_{Lo} is $\sim 0.8\,g$. Convulsions and death from cardiac and respiratory failures can occur. Survival for more than 24 h is usually followed by complete recovery. Direct aspiration of a large amount of barium into the airway resulted in tachycardia, rapid breathing, fever, and low oxygen saturation. A family eating fish accidentally battered with barium carbonate developed nausea, vomiting, diarrhea, and abdominal pain within minutes and the parents also

developed ventricular tachycardia, flaccid paralysis of the extremities, dyspnea (mother), and respiratory failure (father). Patients were treated symptomatically and all fully recovered.

Chronic Toxicity (or Exposure)

Animal

In guinea pigs, barium caused various changes in the blood and pathological changes in bone marrow, spleen, and liver. Cardiovascular effects are evident in rats after long-term exposures. Ultrastructural changes in the kidney glomeruli were noted in rats consuming barium ($1\,g\,l^{-1}$) in the drinking water for 36 weeks. Increased kidney weights were noted in female rats consuming barium (2500 ppm) in the drinking water for 15 months.

Human

Inhalation of insoluble sulfate and oxide, as dusts, produces a pneumoconiosis called baritosis, which is a relatively benign condition that is usually reversible with cessation of exposure. Cardiovascular effects are also of concern after long-term exposure in humans.

Clinical Management

Addition of sodium sulfate as a lavage solution may precipitate the very insoluble barium sulfate. As potassium deficiency occurs in acute poisoning, serum potassium and cardiac rhythm must be monitored closely. Administration of intravenous potassium appears beneficial. As renal failure is also a concern, urinary output also must be monitored closely.

Environmental Fate

Barium is a highly reactive metal that occurs naturally only in a combined state. The element is released to environmental media by both natural processes and anthropogenic sources.

Barium is released primarily to the atmosphere as a result of industrial emissions during the mining, refining, and production of barium and barium chemicals, fossil fuel combustion, and entrainment of soil and rock dust into the air. In addition, coal ash, containing widely variable amounts of barium, is also a source of airborne barium particulates. Most barium released to the environment from industrial sources is in forms that do not become widely dispersed. In the atmosphere, barium is likely to be present in particulate form. Although chemical reactions may cause changes in speciation of barium in air, the main mechanisms for the removal of barium compounds from the atmosphere are likely to be wet and dry depositions.

In aquatic media, barium is likely to precipitate out of solution as an insoluble salt (i.e., as $BaSO_4$ or $BaCO_3$). Waterborne barium may also adsorb to suspended particulate matter. Precipitation of barium sulfate salts is accelerated when rivers enter the ocean because of the high sulfate content in the ocean. Sedimentation of suspended solids removes a large portion of the barium content from surface water. Barium in sediments is found largely in the form of barium sulfate (barite). Coarse silt sediment in a turbulent environment will often grind and cleave the barium sulfate from the sediment particles leaving a buildup of dense barites. Estimated soil–water distribution coefficients (K_d) (i.e., the ratio of the quantity of barium absorbed per gram of sorbent to the concentration of barium remaining in solution at equilibrium) range from 200 to 2800 for sediments and sandy loam soils.

As pH levels increase above 9.3 and in the presence of carbonate, barium carbonate becomes the dominant species. Barium carbonate also exhibits fast precipitation kinetics and very low solubility and in alkaline environments limits the soluble barium concentration. Barium forms salts of low solubility with arsenate, chromate, fluoride, oxalate, and phosphate ions. The chloride, hydroxide, and nitrate of barium are water soluble and are frequently detected in aqueous environments.

Ecotoxicology

The uptake of barium by fish and marine organisms is an important elimination mechanism. Barium levels in seawater range from 2 to $63\,mg\,l^{-1}$ with a mean concentration of $\sim 13\,mg\,l^{-1}$. Barium was found to bioconcentrate in marine plants by a factor of 1000 times the level present in the water. Bioconcentration factors in marine animals, plankton, and in brown algae of 100, 120, and 260, respectively, have been reported. Relatively little information is available on the effects of barium compounds in aquatic organisms. Barium carbonate was practically nontoxic to fish (96 h LC_{50} in *Gambusia* was $>10\,g\,kg^{-1}$).

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value – time-weighted average is $0.5\,mg\,ml^{-1}$ for soluble barium compounds and $10\,mg\,ml^{-1}$ for barium sulfate. The permissible exposure limit is $0.5\,mg\,m^{-3}$ for barium in soluble compounds. The reference dose for barium is $0.07\,mg\,kg^{-1}\,day^{-1}$ and the tolerable daily intake (The Netherlands) is $0.02\,mg\,kg^{-1}\,day^{-1}$.

See also: Metals.

Further Reading

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.

Relevant Websites

http://risk.lsd.ornl.gov – Risk Assessment Information System.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Barium.

Bases *See* Alkalies.

Batrachotoxin

John P Dumbacher

- Representative Chemicals: Batrachotoxin; Homobatrachotoxin; Batrachotoxinin A; and several other batrachotoxinin A congeners
- Chemical Abstracts Service Registry Number: CAS 23509-16-2 (Batrachotoxin)
- Synonyms: *Phyllobates* toxin; *Pitohui* toxin; *Ifrita* toxin; poison dart frog toxin
- Chemical/Pharmaceutical/Other Class: Steroidal alkaloid neurotoxin
- Chemical Formulas:
  - Batrachotoxin: $C_{31}H_{42}N_2O_6$
  - Homobatrachotoxin: $C_{32}H_{44}N_2O_6$
  - Batrachotoxinin-A: $C_{24}H_{35}NO_5$

Background Information

Batrachotoxins are a class of steroidal alkaloid neurotoxins found in Colombian poison dart frogs of the genus *Phyllobates* (family Dendrobatidae). The frogs have special skin glands that store and secrete the toxins, and these glands are most densely packed on the back behind the head. Evidence suggests that the frogs acquire the toxins from a dietary source; however, no potential source of these frog poisons has been identified. Interestingly, of all of the so-called poison dart frogs, only three species of *Phyllobates* were actually used by Native Americans for poisoning dart tips, and the major toxic element responsible for poisoning are the batrachotoxins. More recently, identical toxins were found in New Guinean birds in the genus *Pitohui* (family Pachycephalidae) and *Ifrita* (family Cinclosomatidae). The toxins are most concentrated in the skins and feathers of birds.

Several naturally occurring batrachotoxins have been identified from frog and bird extracts. The most common are batrachotoxin and homobatrachotoxin, which contain a pyrrole moiety. These occur in frogs in roughly equal proportions, and have an LD_{50} in mice of $\sim$2–3 $\mu g\,kg^{-1}$ (subcutaneous injection). Toxicity via other routes has not been well studied. The pyrrole can be manipulated in nature and in the lab to give the non-pyrrole form, called batrachotoxinin-A, which is $\sim$1/500th as toxic as batrachotoxin or homobatrachotoxin. Several other congeners have been identified in nature, but the pharmacology of many of these remains unstudied.

Exposure Routes and Pathways

From *Phyllobates* frogs, exposure occurs through ingesting skin and flesh of the frogs. Toxin quantities can be high enough to make even handling these frogs dangerous, so presumably some absorption can occur through skin. Exposure to the toxins may occur by subcutaneous injection, such as a puncture from a poisoned dart tip. From birds, exposure can occur by eating flesh, however even handling the birds can cause 'allergic' reactions such as itchy eyes, runny nose, sneezing, and tingling around buccal membranes. These reactions are believed to be caused by powder or tiny feather fragments released from toxic feathers. Batrachotoxins are lipid soluble and soluble in a variety of organic solvents such as methanol, chloroform, and ethanol.

Toxicokinetics

Batrachotoxin can be absorbed through skin as well as from the gastrointestinal tract. Effects can occur within 10 min and can last for several hours to more than a day.

Mechanism of Toxicity

Batrachotoxins bind specifically to voltage-gated sodium channels in nerve and muscle membranes.

Once activated, bound batrachotoxins stabilize the channel in its open conformation. This allows sodium ions to flow freely across the membrane, and depolarize it, causing local tingling, irritation, and numbness, and in higher concentrations convulsions, paralysis, and cardiac or pulmonary failure. Because a relatively small proportion of activated channels can depolarize the membrane, batrachotoxins are highly toxic. Batrachotoxins bind strongly to sodium channel proteins, so binding is often referred to as 'irreversible', although light exposure (resulting in local tingling or numbness) generally subsides within a few minutes to 24 h.

Acute and Short-Term Toxicity (or Exposure)

Human

Very little is known about toxicity of batrachotoxins in humans. If it is assumed that human and mouse toxicity are equivalent (at $\sim 2.5\,\mu g\,kg^{-1}$ subcutaneously), then a median lethal dose for a 68 kg human would be $\sim 170\,\mu g$ of batrachotoxin. Other studies show that mice are less susceptible to neurotoxins than humans, so another estimate can be based upon toxicity relationships of batrachotoxin to aconitine, digitoxin, and strychnine and their toxicity in humans. Using these relationships, it is expected that a dose as small as 2–10 μg of purified batrachotoxin injected subcutaneously may be lethal to humans. Likewise, ingested amounts of as little as 120–500 μg are expected to be lethal. These are certainly rough estimates, and few, if any, human poisonings have been reported in medical literature. However, purified toxins as well as frog skin secretions should be handled with extreme care.

Human Use of Frog Secretions Containing Batrachotoxins

Very small amounts of frog secretions from *Phyllobates terribilis*, *P. bicolor*, and *P. aurotanea* can be used to poison dart tips, which are reportedly effective at immobilizing a variety of animals including jaguar, bear, deer, and humans. A single *P. bicolor* or *P. terribilis* can effectively poison 20–30 darts.

Human Knowledge of *Pitohui* and *Ifrita* Birds Containing Batrachotoxins

In New Guinea, traditional hunters are aware that *Pitohui* and *Ifrita* birds carry neurotoxins. Local names for these birds often reflect the fact that they are bitter or contain burning chemicals. Toxins in these birds are more diffuse than in the frogs, but even a single feather, if tasted, can cause an acute burning sensation that may last for several minutes to hours. Handling the birds can cause allergy-like reactions such as itchy watery eyes, running nose, and sneezing. To the author's knowledge, there have been no human deaths or serious poisonings due to bird ingestion, as it is generally recognized as inedible, and an unpleasant burning sensation sets in before much of the toxin is eaten. No anthropologists have reported local New Guineans using the toxins to immobilize prey.

In Vitro Toxicity Data

Batrachotoxin is an important research tool because of its action of holding voltage-gated sodium channels open as well as its specific effects at other ligand-binding sites. It was previously used commonly in channel and ligand research. There are no commercially available stocks of batrachotoxins, however, and work in Colombia is currently difficult or impossible, so these chemicals are used less frequently in research.

Clinical Management

No antidote is available.

See also: Animals, Poisonous and Venomous.

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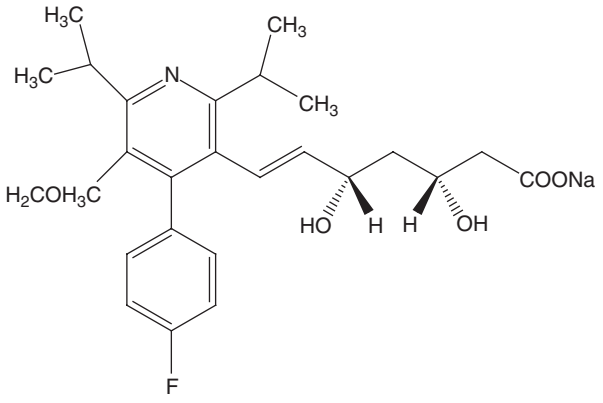
Baycol

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 143201-11-0
- Synonym: Cerivastatin sodium
- Chemical/Pharmaceutical/Other Class: Statins; HMG-CoA inhibitors
- Chemical Formula: $C_{26}H_{33}FNO_5Na$
- Chemical Structure:



Uses

Baycol was indicated as an adjunct to diet to reduce elevated total-cholesterol, low-density lipoprotein cholesterol (LDL-C), apo B, and triglycerides (TG) and to increase high-density lipoprotein cholesterol (HDL-C) levels in patients with primary hypercholesterolemia and mixed dyslipidemia (Fredrickson types IIa and IIb) when the response to dietary restriction of saturated fat and cholesterol and other nonpharmacological measures alone had been inadequate. Therapy with lipid altering drugs should bea component of multiple risk factor intervention in those patients at significantly high risk for atherosclerotic vascular disease due to hypercholesterolemia.

Background Information

Baycol was removed from the market in 2001 because of an unacceptable level of risk of adverse effects.

Toxicokinetics

Absorption: The mean absolute bioavailability of cerivastatin following a 0.2 mg tablet oral dose is 60% (range 39–101%). In general, the coefficient of variation (based on the intersubject variability) for both systemic exposure (area under the curve, AUC) and C_{max} is in the 20–40% range. The bioavailability of cerivastatin sodium tablets is equivalent to that of a solution of cerivastatin sodium. No unchanged cerivastatin is excreted in feces. Cerivastatin exhibits linear kinetics over the dose range of 0.2–0.8 mg daily. In male and female patients at steady state, the mean maximum concentrations (C_{max}) following evening cerivastatin tablet doses of 0.2, 0.3, 0.4, and 0.8 mg are 2.8, 5.1, 6.2, and 12.7 $\mu g l^{-1}$, respectively. AUC values are also dose-proportional over this dose range and the mean time to maximum concentration (t_{max}) is $\sim$2 h for all dose strengths. Following oral administration, the terminal elimination half-life ($t_{1/2}$) for cerivastatin is 2–4 h. Steady-state plasma concentrations show no evidence of cerivastatin accumulation following administration of up to 0.8 mg daily.

Results from an overnight pharmacokinetic evaluation following single-dose administration of cerivastatin with the evening meal or 4 h after the evening meal showed that administration of cerivastatin with the evening meal did not significantly alter either AUC or C_{max} compared to dosing the drug 4 h after the evening meal. In patients given 0.2 mg cerivastatin sodium once daily for 4 weeks, either at mealtime or at bedtime, there were no differences in the lipid-lowering effects of cerivastatin. Both regimens of 0.2 mg once daily were slightly more efficacious than 0.1 mg twice daily.

The volume of distribution (V_d) is calculated to be 0.31 kg^{-1}. More than 99% of the circulating drug is

bound to plasma proteins (80% to albumin). Binding is reversible and independent of drug concentration up to $100\,mg\,l^{-1}$.

Mechanism of Toxicity

Cerivastatin is a competitive inhibitor of HIVIG-CoA reductase, which is responsible for the conver sion of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) to mevalonate, a precursor of sterols, including cholesterol. The inhibition of cholesterol biosynthesis by cerivastatin reduces the level of cholesterol in hepatic cells, which stimulates the synthesis of LDL receptors.

Clinical Pharmacology

Cholesterol and triglycerides circulate as part of lipoprotein complexes throughout the bloodstream. These complexes can be separated via ultracentrifugation into HDL, intermediate-density lipoprotein (IDL), LDL, and very-low-density lipoprotein (VLDL) fractions. In the liver, cholesterol and TG are synthesized, incorporated into VLDL, and released into the plasma for delivery to peripheral tissues.

Chronic Toxicity (or Exposure)

Animal

Chronic administration of cerivastatin to rodent and nonrodent species demonstrated the principal toxicologic targets and effects observed with other HMG-CoA reductase inhibitors: hemorrhage and edema in multiple organs and tissues including the central nervous system (CNS) (dogs); cataracts (dogs); degeneration of muscle fibers (dogs, rats, and mice); hyperkeratosis in the nonglandular stomach (rats and mice, this organ has no human equivalent); liver lesions (dogs, rats, and mice). CNS lesions in the dog were found at a dose of $0.1\,mg\,kg^{-1}\,day^{-1}$. This dose resulted in plasma levels of cerivastatin (C_{max} measured as free drug) that were $\sim$17 times higher than the mean values in humans taking $0.8\,mg\,day^{-1}$. No CNS lesions were observed after chronic treatment with cerivastatin for up to 2 years in the mouse (at up to six times human C_{max} free drug levels) and rat (in the range of human C_{max} free drug levels).

A 2 year carcinogenicity study was conducted in rats with dietary administration resulting in average daily doses of cerivastatin of 0.007, 0.034, or $0.158\,mg\,kg^{-1}$. The high dosage level corresponded to plasma free drug levels (AUC) of approximately two times those in humans following a 0.8 mg oral dose. Tumor incidences of treated rats were comparable to controls in all treatment groups. In a 2 year carcinogenicity study conducted in mice with dietary administration resulting in average daily doses of cerivastatin of 0.4, 1.8, 9.1, or $55\,mg\,kg^{-1}$, hepatocellular adenomas were significantly increased in male and female mice at $\geqslant 9.1\,mg\,kg^{-1}$ (AUC free values about three times the human value at $0.8\,mg\,day^{-1}$). Hepatocellular carcinomas were significantly increased in male mice at $1.8\,mg\,day^{-1}$ (AUC free values in the range of human exposure at $0.8\,mg\,day^{-1}$).

In a combined male and female rat fertility study, cerivastatin had no adverse effects on fertility or reproductive performance at doses up to $0.1\,mg\,kg^{-1}\,day^{-1}$ (in the range of human C_{max} free drug levels). At a dose of $0.3\,mg\,kg^{-1}\,day^{-1}$ (about three times the human C_{max} free drug levels), the length of gestation was marginally prolonged, stillbirths were increased, and the survival rate up to day 4 postpartum was decreased. In the fetuses (F1), a marginal reduction in fetal weight and delay in bone development was observed. In the mating of the F1 generation, there were a reduced number of female rats that littered.

In the testicles of dogs treated chronically with cerivastatin at a dose of $0.008\,mg\,kg^{-1}\,day^{-1}$ (in the range of human C_{max} free drug levels), atrophy, vacuolization of the germinal epithelium, spermatidic giant cells, and focal oligospermia were observed. In another 1 year study in dogs treated with $0.1\,mg\,kg^{-1}\,day^{-1}$ ($\sim$17-fold the human exposure at doses of 0.8 mg based on C_{max} free), ejaculate volume was small and libido was decreased. Semen analysis revealed an increased number of morphologically altered spermatozoa indicating disturbances of epididymal sperm maturation that was reversible when drug administration was discontinued.

Cerivastatin caused a significant increase in incomplete ossification of the lumbar center of the vertebrae in rats at an oral dose of $0.72\,mg\,kg^{-1}$. Cerivastatin did not cause any anomalies or malformations in rabbits at oral doses up to $0.7\,mg\,kg^{-1}$. These doses resulted in plasma levels about six times the human exposure (C_{max} free) for rats and three times the human exposure for rabbits (C_{max} free) at a human dose of 0.8 mg. Cerivastatin crossed the placenta and was found in fetal liver, gastrointestinal tract, and kidneys when pregnant rats were given a single oral dose of $2\,mg\,kg^{-1}$.

Human

Because of possible adverse effects on liver function, caution was advised when Baycol (cerivastatin sodium

tablets) was administered to patients with a history of liver disease or heavy alcohol ingestion.

In Vitro Toxicity Data

No evidence of genotoxicity was observed *in vitro* with or without metabolic activation in the following assays: microbial mutagen tests using mutant strains of *Salmonella typhimurium* or *Escherichia coli*, Chinese hamster ovary forward mutation assay, unscheduled DNA synthesis in rat primary hepatocytes, chromosome aberrations in Chinese hamster ovary cells, and spindle inhibition in human lymphocytes. In addition, there was no evidence of genotoxicity *in vivo* in a mouse micronucleus test; there was equivocal evidence of mutagenicity in a mouse domi nant lethal test.

Market Removal for Safety

Baycol was removed from the market place in 2001 due to concerns about its safety. The primary adverse effects were:

Liver enzymes: HMG-CoA reductase inhibitors have been associated with biochemical abnormalities of liver function. These abnormalities usually occurred within the first six months of treatment, usually resolved after discontinuation of the drug, and were not associated with cholestasis.

Skeletal muscle: Cases of rhabdomyolysis (muscle fiber breakdown), leading to acute renal failure secondary to myoglobinuria, have been reported with cerivastatin and other drugs in this class. Myopathy, defined as muscle aching or muscle weakness, associated with increases in plasma creatine kinase values to greater than 10 times the upper limit of normal, was seen in 0.4% of patients in US cerivastatin clini cal trials.

Endocrine function: HMG-CoA reductase inhibitors interfere with cholesterol synthesis and lower cholesterol levels and, as such, might theoretically blunt adrenal or gonadal steroid hormone production.

Safety in pregnant women has not been established.

See also: Food and Drug Administration, US; Kidney.

Further Reading

Dipiro JT (2005) *Pharmacotherapy*, 6th edn. New York: McGraw-Hill.

Walsh PM (2003) *Physicians' Desk Reference*, 58th edn. Montvale, NJ: Thomson PDR.

Relevant Website

http://www.fda.gov – Baycol Information (from the US Food and Drug Administration).

BCNU (Bischloroethyl Nitrosourea)

Madhusudan G Soni

- Chemical Abstracts Service Registry Number: CAS 154-93-8
- Synonyms: Carmustine; *N*,*N*-bis(2-Chloroethyl)-*N*-nitrosourea; BiCNU; Carmubris; Nitrumon
- Chemical/Pharmaceutical/Other Class: Alkylating agent

Uses

Bischloroethyl nitrosourea (BCNU) has been used in human medicine as an antineoplastic agent (alone or in combination with other agents) in the treatment of Hodgkin's lymphoma, multiple myeloma, and in primary or metastatic brain tumors.

Exposure Routes and Pathways

Intravenous injection is the most common route of exposure. Doses range from 100 to 250 mg m^{-2} body surface for courses of 2 or 3 days.

Toxicokinetics

In animal experiments, BCNU is rapidly absorbed, following different routes of ingestion. A few minutes after administration, no unchanged BCNU can be detected in plasma. BCNU undergoes spontaneous decomposition under physiological conditions to release both alkylating and carbamoylating entities. In addition to chemical decomposition, BCNU may be denitrosated enzymatically via hepatic microsomal enzyme oxidation system to its corresponding urea. BCNU is rapidly distributed to most tissues including brain and cerebrospinal fluid. The volume

of distribution is $\sim 0.18\,l\,kg^{-1}$. Approximately 80% of the drug appears in the urine within 24 h as degradation products. Approximately 10% of the ingested BCNU is removed by respiratory excretion and 1% in feces. BCNU is reported to have a biological half-life of less than 20 min.

Mechanism of Toxicity

It is generally assumed that BCNU exerts its cyto toxicity through the liberation of alkylating and carbamoylating moieties. An alkylating entity, particularly chloroethyl carbonium ion, is strongly electrophilic and can alkylate a variety of biomolecules, including the purine and pyrimidine bases of DNA. The interstrand cross-linking is generally associated with the cytotoxicity of BCNU. The carbomylation of lysine residues of protein can inactivate certain DNA repair enzymes thus interfering with repair processes.

Acute and Short-Term Toxicity (or Exposure)

Animal

In dogs, high doses of BCNU resulted in severe bone marrow hypoplasia with delayed, reversible thrombocytopenia. The other major toxicities observed were cardiopulmonary (pulmonary edema, myocardial infarction, and pericardial hemorrhage), intestinal mucosal damage with hemorrhage, renal toxicity, and delayed hepatotoxicity. Similar toxicity was seen in monkeys except that cardiopulmonary toxicity did not occur. In rats, initially well-tolerated doses may cause death later. There is sufficient evidence for the carcinogenicity of BCNU in rats. BCNU is embryo- and fetolethal in rats and rabbits at doses nontoxic to the mother and can induce a variety of teratogenic effects in rats.

Human

Various cytotoxic effects of BCNU in humans are reported. The drug is not a vesicant, but local burning pain has been reported after intravenous administration. Nausea and vomiting occur ~ 2 h after injection. Flushing of the skin and conjunctiva, central nervous system toxicity, esophagitis, diarrhea, interstitial pulmonary fibrosis, and renal and hepatic toxicities have been reported. Hepatotoxicity and pulmonary toxicity may be dose limiting. Although bone marrow suppression is observed, this drug characteristically causes an unusually delayed onset of leukopenia and thrombocytopenia. The nadir of the leukocyte and platelet counts may not reach normal levels until 6 weeks after treatment.

Clinical signs associated with BCNU-induced pulmonary toxicity in humans are dyspnea, tachypnea, and a dry hacking cough. The incidence of these symptoms is between 20% and 30% and mortality varies from 24% to 80%. The onset of symptoms is usually within 3 years of treatment. There is a linear relationship between total dose received and pulmonary toxicity at doses $> 1000\,mg\,m^{-2}$, with 50% of patients developing pulmonary toxicity at total cumulative doses of $1500\,mg\,m^{-2}$.

Chronic Toxicity (or Exposure)

Animal

Administration of BCNU three times a week for six months, followed by 12 months observation, to Swiss mice at intraperitoneal doses of 2.5 and $5.0\,mg\,kg^{-1}$ and to SD rats at dose of $1.5\,mg\,kg^{-1}$ resulted in increases in tumor incidence in all treated animals, predominantly subcutaneous and lung neoplasms.

Human

In a long-term study of patients receiving BCNU in childhood and early adolescence (1–16 years), delayed onset pulmonary fibrosis occurring up to 17 years after treatment has been reported. Pulmonary toxicity characterized by pulmonary infiltrates and/or fibrosis has been reported to occur from 9 days to 43 months after treatment with BCNU. Most of these patients were receiving prolonged therapy with total doses of BCNU greater than $1400\,mg\,m^{-2}$. However, there have been reports of pulmonary fibrosis in patients receiving lower total doses. The occurrence of acute leukemia and bone marrow dysplasias have been reported in patients following long-term BCNU therapy. Renal abnormalities consisting of progressive azotemia, decrease in kidney size, and renal failure have been reported in patients who received large cumulative doses after prolonged therapy with BCNU.

In Vitro Toxicity Data

BCNU was mutagenic *in vitro* (Ames assay, human lymphoblast HGPRT assay) and clastogenic *in vitro* (V79 hamster cell micronucleus assay).

Clinical Management

Most of the adverse reactions of BCNU are reversible if detected early. When such effects or reactions do occur, the drug should be reduced in dosage or discontinued and appropriate corrective measures should be taken according to the clinical judgment

of the physician. Blood counts should be monitored weekly for at least 6 weeks after the dose. Baseline pulmonary function studies, hepatic functional tests, and periodic renal functional tests should be monitored. No proven antidotes have been established for BCNU overdosage.

Environmental Fate

There is no information available on the environmental fate of BCNU. However, one can predict that as a very reactive agent, BCNU spontaneously decomposes.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Toxicity Testing, Mutagenicity.

Further Reading

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Carter SK, Schabel FM, Broder LE, and Johnston TP (1972) 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and other nitrosoureas in cancer treatment: A review. *Advances in Cancer Research* 16: 273–332.

DeVita VT, Carbone PP, Owens AH, *et al.* (1965) Clinical trials with 1,3-bis(2-chloroethyl)-1-nitrosourea, NSC-409962. *Cancer Research* 25: 1876–1881.

Bee *See* Hymenoptera.

Behavioral Toxicity Testing *See* Toxicity Testing, Behavioral.

Behavioral Toxicology

Deborah A Cory-Slechta

Introduction

Behavioral toxicology is that scientific discipline which studies the effects of therapeutic drugs and other chemicals on behavior, the ultimate output of the nervous system, and also seeks to determine how such effects are caused. The impetus for such studies has come from multiple sources. Both human and experimental animal studies have been carried out to assess the behavioral consequences arising from exposures to chemicals used in the workplace as well as those dispersed in the environment. These efforts have been important in determining safe exposure and risk levels, as well as in furthering our understanding of these chemicals. A second force behind many such studies has been the need to screen newly synthesized chemicals for any potential adverse behavioral effects before their introduction into use, efforts which are obviously carried out only in experimental laboratory contexts.

Human behavior is, of course, extremely diverse and complex, composed of numerous different functions, any or all of which might be perturbed by exposure to a toxicant. Thus, understanding how a chemical affects human behavior may require a determination of its effects across these different behavioral functions. Furthermore, some human behaviors require an integration of several different behavioral functions. If we think about learning in a classroom, for example, in addition to cognitive functions, sensory functions are needed to process the information presented, and motor functions are required for executing the correct response. Thus, in the event that a chemical is suspected to produce effects on cognitive functions, the possibility that such effects, instead, result indirectly from changes in sensory or motor functions must always be considered.

The entire range of behavioral functions and the tests designed to evaluate them cannot be presented here. This entry first presents the types of methods that comprise the test batteries used in screening newly developed chemicals for behavioral toxicity. While screening batteries are extremely useful in providing a preliminary assessment of adverse behavioral effects, they are less useful for elaborating the actual nature of the behavioral deficits or for yielding an understanding of their underlying behavioral and neurobiological mechanisms. For such purposes, more specific tests of various behavioral functions are utilized. Such higher-order tests, in particular those related to sensory, motor, and cognitive functions, are subsequently presented in this entry and are followed by some discussion of the testing methods utilized in experimental animals to determine adverse behavioral effects of chemicals during the course of development as well as some of the test methods unique to human populations.

Screening Batteries

Because a newly developed chemical may have effects on any of the numerous behavioral functions that comprise a behavioral repertoire, screening batteries must necessarily assess a wide variety of functions with sufficient sensitivity to suggest potential behavioral toxicity even in a single behavioral domain. These screening batteries are typically executed in studies using rats and mice and generally consist of two components: a functional observational battery (FOB) and a measure of motor activity (see below). FOBs include an array of measures, generally of unlearned or instinctive behaviors, designed to detect any indications of gross changes in reflexes and in gross motor or sensory function. Most FOBs are relatively easy to implement since there is typically no behavioral training or sophisticated equipment required for any of the behavioral measures utilized as they are carried out and scored by an experienced observer. An FOB may include measures of general integrity, such as any signs of convulsions, palpebral closure, lacrimation, piloerection, salivation, and vocalizations. In addition, assessments of sensory capability, based on measures such as response to a finger snap or a tail-pinch, righting reflex, and assessments of motor function, as evaluated by the posture or gait of the animal, catalepsy, hindlimb foot splay, forelimb and hindlimb grip strength, and the time to begin ambulating, may be included. Finally, any signs of arousal or stress can be measured, such as ease of removal and handling, the animal's response to touch or approach, and urination and defecation. In addition, certain physiological responses, including body temperature and body weight, are measured. These evaluations are sometimes carried out in two different environments: a familiar one, such as the animal's home cage, and an unfamiliar flat surface of some type. This series of measures can be made relatively rapidly on each animal, consistent with the goal of screening of new compounds across a wide range of doses. In the event that behavioral activity of the chemical is indicated in such a screening test, more advanced and specific behavioral procedures would be required to delineate the precise nature of the behavioral impairment.

One question that has arisen with respect to the use of FOBs is whether changes in only one or two of the numerous measures made are really indicative of neurotoxicity. For example, how is a change in two seemingly unrelated measures interpreted (e.g., vocalizations and hindlimb grip strength). One answer that has been suggested is that neurotoxicity would be indicated by similar changes occurring within a single behavioral domain. Thus, changes in both forelimb and hindlimb grip strength would be indicative of altered motor function. Some have contended that if the toxicant under test produces body weight changes, then any changes also observed in the FOB may simply be due to 'sickness syndrome' or general malaise of the animal, not neurotoxicity. This is not necessarily a valid conclusion, however, since body weight changes may occur totally independently of any observed FOB effects. In fact, FOB changes are often reported in the absence of any body weight changes.

Motor Function

Motor function is a critical component of human behavior because it embodies the ultimate execution of a behavioral response. The feats of highly skilled athletes provide one example of incredibly refined motor performance, but even everyday functions such as walking or driving to work depend on adequate motor capabilities. Motor behavior is not a unitary behavioral function, but rather one with many different components. Various motor responses entail such aspects as strength, coordination and endurance, precision and duration, frequency of occurrence, and for ambulation, gait and balance as well. Measurement of these different aspects of motor function obviously requires different procedures. As is the case for measurement of virtually all behavioral functions, the paradigms for assessing motor capabilities range from simple assessments to more complex technologies. The former provide easily implemented but generally less specific and selective measures of function and the more advanced procedures provide specific measures of motor function as distinct from changes in sensory or motivational processes but may require some training of the experimental subject.

Motor Activity Levels

Motor activity measures the frequency of occurrence of integrated movements and/or ambulation of the organism over some designated period of time, a behavior that generally occurs at some baseline level in mammalian species and which may be altered by exposure to a toxicant. A measure of motor activity is typically one component of a screening battery, and most studies of motor activity are carried out using rodents. Generally, the animals are placed on a horizontal surface, which could be square, rectangular, or even a maze such as a T-shaped apparatus, and the number of defined movements over a specified time period are recorded. In nonautomated versions, movements are typically recorded by an

observer who, it is hoped, has no information with respect to the treatment condition (toxicant-exposed or control) of the subject which might bias the recording of data. In most automated versions as are typically used now, the movements of the animal either interrupt a light beam or trip a switch which then records a count.

Measurements of motor activity have been used to evaluate the potential central nervous system (CNS) effects of a wide variety of drugs and toxicants. One of the advantages of such measures of motor function is that no training is required of the subject. In addition, measures of motor activity can be made repeatedly across time so that the time course, including the onset and reversibility of toxicant effects, can be determined. In these types of repeated measurement experiments, moreover, an animal can serve as its own control, meaning that the experimenter looks for a change in the animal's normal pattern of motor activity after receiving the toxicant compared to the pattern observed before the treatment.

The experimenter must be cognizant of the fact that different devices for measuring motor activity may measure markedly different aspects of motor function. For example, in devices such as a figure-eight maze, an animal may rear up on its hindlimbs in front of a light beam. This response will break the beam of light and be counted as a response. In contrast, in devices like the open field shown in **Figure 1**, the investigator tallies the number of squares in a rectangular field entered by the animal with all four paws, and a rearing response might not be counted. Furthermore, beam height may differ in different devices and thus capture different aspects of behavior. Such differences can preclude the direct comparisons of various studies of the effects of a toxicant on motor activity and also underscore the importance of precise specifications of the response(s) being recorded in any given study with a particular device. Failure to do so may result in seemingly inconsistent results. Other influences must also be considered in the interpretation of changes in motor activity. For example, motor activity levels are known to be influenced by a variety of nonmotoric variables, such as time of day at which testing is carried out (rodents are nocturnal and show greater activity levels during dark hours), room lighting, and odors. As this list indicates, changes in sensory capabilities (perceived difference in the room odors or lighting) or circadian (nocturnal) rhythms could influence measures of motor activity independently of any direct toxicant-induced changes.

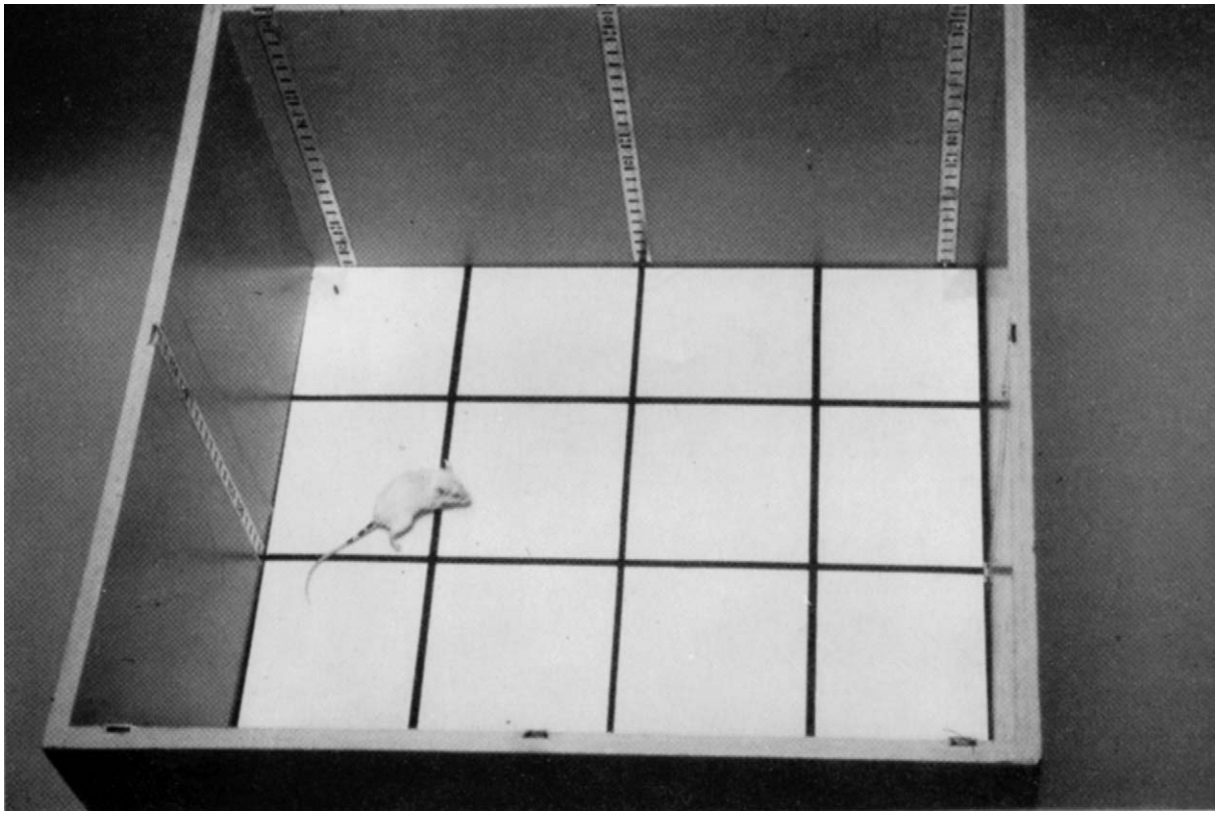

Figure 1 Open field apparatus in which the number of squares entered by the mouse or rat is counted over some fixed period of time. (Reproduced from Cory-Slechta DA (1989) Behavioral measures of neurotoxicity. *Neurotoxicology* 10: 271–296, with permission.)

Motor activity may be an insensitive measure of toxicant-induced changes when it relies on relatively gross measures such as total counts per unit time. For example, in the open field test mentioned previously and shown in **Figure 1**, the total number of squares entered into by the animal over a period of time is measured. However, the same total number may be achieved through very different patterns of behavior. For example, the organism might show an initial period of rapid movement followed by immobility or, alternatively, cycles of high activity followed by low activity or, finally, even a continuous but moderate rate of ambulation. All three could lead to the same total number of squares entered, but the disparate patterns suggest differences in behavior that are not being captured.

Strength, Coordination, and Endurance

Weakness and fatigue are common complaints resulting from exposures to a number of different chemicals. Both simple and more complex approaches to measuring these facets of motor function are available. A simple and commonly used procedure that has the advantage of not requiring any specific training of the animal is the rotarod device shown in **Figure 2**. A rat or mouse is placed on a rotating cylinder, the speed of which can be manipulated, and the time the animal remains on the rotating device before falling onto the plate below is recorded. Falling off more quickly may be an indication of changes in coordination and/or endurance. As with motor activity, time spent on the rotarod can be measured repeatedly, and a stable baseline performance can be generated across experimental sessions against which the impact of toxicants may be compared.

The difficulties with such an approach are also evident in **Figure 2**. Mice frequently attempt to scramble up the dividers; some attempt to run backwards. Others begin to jump off the device and will not remain on the device regardless of being repeatedly placed back on the rotarod. As these examples indicate, the rotarod device thus measures aspects of behavior in addition to coordination and endurance which must obviously be considered in interpreting such data. In other words, one cannot necessarily be certain that decreased time spent on a rotarod after toxicant treatment necessarily reflects changes in endurance and coordination or, for

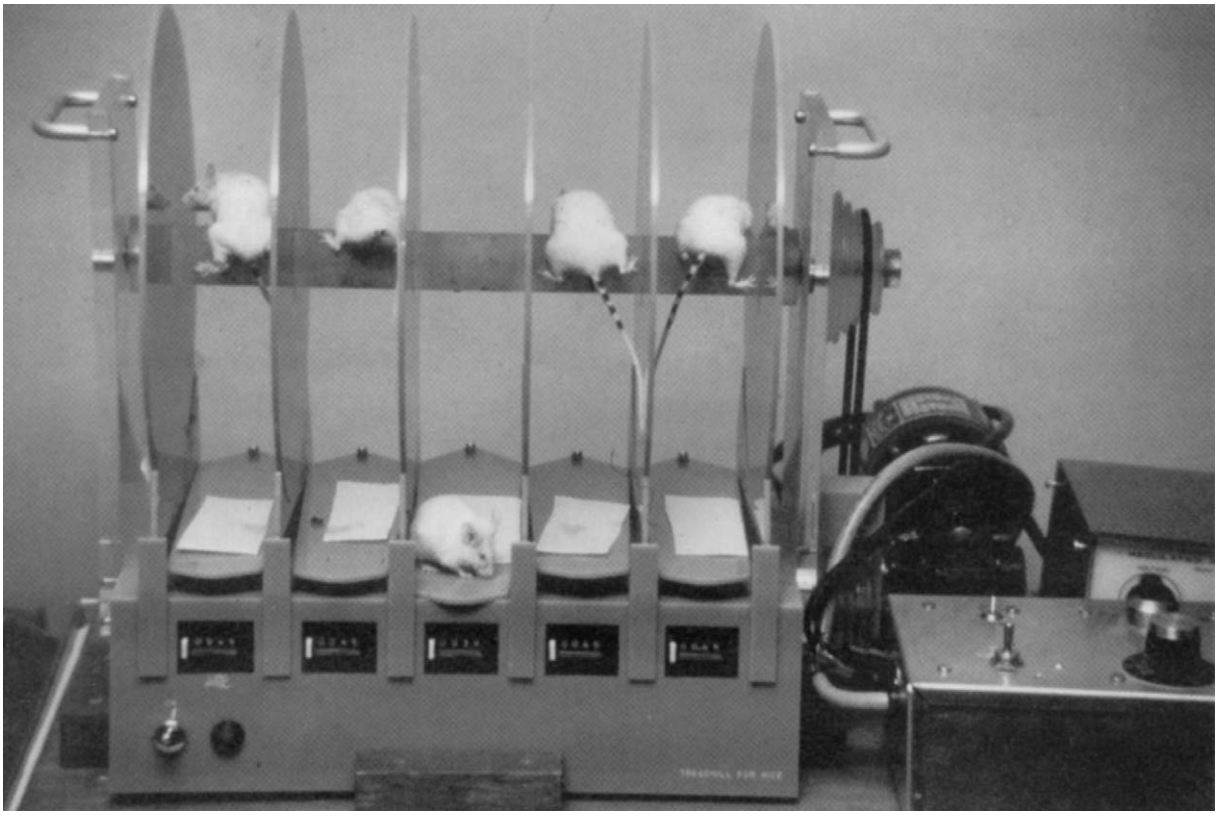

Figure 2 Illustration of the rotarod apparatus for mice. Each mouse is placed on the rotating cylinder; speed of revolution can be manipulated and time on rotarod typically constitutes the dependent variable of interest. (Reproduced from Cory-Slechta DA (1989) Behavioral measures of neurotoxicity. *Neurotoxicology* 10: 271–296, with permission.)

example, whether it could reflect, for example, increased distraction.

More advanced techniques that rely on learned behavior of animals (i.e., operant behavior) can provide controls for such nonmotoric behavioral factors and thus provide a more specific indication of changes in endurance and coordination. Rats can be trained to depress a lever with a specified amount of force in order to obtain a reward, for example, food delivery. The amount of force required to depress the lever can then be successively increased until the maximal force that can be exerted is reached. In addition, the force that the animal can sustain over time can also be measured as an indication of endurance. The ability to manipulate reward conditions facilitates the ability to differentiate motoric impairments from motivational deficits.

Gait and Balance

Walking, running, and many other motor responses depend on intact gait and balance, and such functions may be particularly vulnerable to chemicals that affect the peripheral nervous system. One simple procedure that has been devised to assess postural dysfunction is known as hindlimb splay. In this procedure, the hindpaws of a mouse or rat are dipped in ink and the animal is then dropped from a fixed height onto a piece of paper below as can be seen in **Figure 3**. An increase in the distance between the hindlimbs upon landing is indicative of damage to the peripheral nervous system with consequent effects on gait and ambulation. This approach is simple in that the rodent does not have to be specifically trained for the task, and this measurement can be made repeatedly across time without extensive equipment requirements so that time to onset and recovery of a toxicant's effects can be followed. However, hindlimb splay may not be a totally specific measure of altered motor function. Sensory disturbances, for example, might alter landing foot distance as well.

A more advanced type of approach, an automated hindlimb movement detection apparatus, is shown in **Figure 4**. In this scheme, a TV–microprocessor system is utilized to record the placement of a rat's hindpaws as it traverses from one rung to the next in a running wheel analogous to those offered in pet stores for rodents. Computer analysis of the recording provides a measurement of both quantitative and temporal characteristics of stepping, such as correct small steps and large steps, missteps, and the temporal parameters of these movements. Thus, an experimenter can measure with great precision how different parameters of gait differ before and after exposure to a toxicant. The animal need not be explicitly trained, and this approach provides a relatively specific measure of motor function *per se*. Procedures for measuring bodily sway in children have also been used in behavioral toxicology studies. In these procedures, the child stands on foam or on a hard surface under conditions of either eyes opened or closed, and the extent of the sway of his or her body is measured utilizing strain gauges.

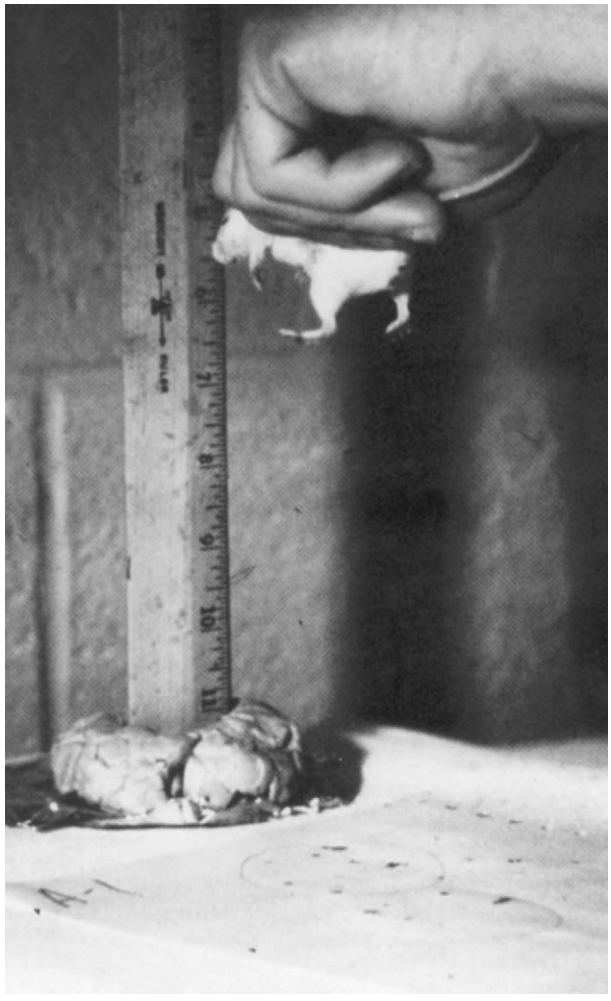

Figure 3 Illustration of the hind-limb splay procedure: the hind paws of the mouse are dipped in ink, the animal is then dropped from a fixed height onto a piece of paper and the distance between some parameter of the hindlimbs is measured. (Reproduced from Cory-Slechta DA (1989) Behavioral measures of neurotoxicity. *Neurotoxicology* 10: 271–296, with permission.)

Sensory Function

A wide range of sensory functions provide us with information about the environment. These functions include our abilities to hear, see, smell, and detect

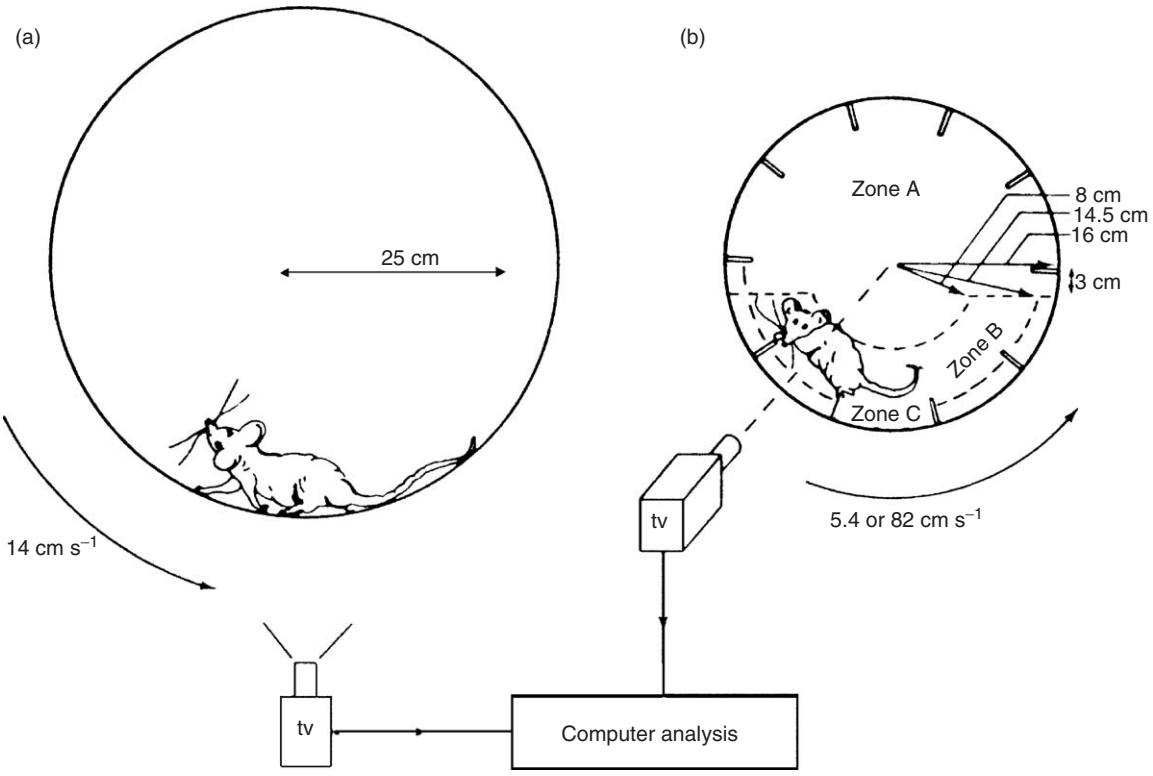

Figure 4 Automated hind-limb movement apparatus. (a) The camera can register the movements of the dyed soles of the paws of the rat from below. (b) The wheel with a transparent front facing the axially mounted color TV camera. (Reproduced from Tanger HJ, Vanwersch RAP, and Wolthius OL (1984) Automated quantitative analysis of coordinated locomotor behaviour in rats. *Journal of Neuroscience Methods* 10: 237–245, with permission from Elsevier.)

movement, vibration, and pain. Deficits in sensory function sometimes constitute some of the earliest or even the most pronounced manifestations of chemical exposures that affect the nervous system. As with the measurement of motor function, both simple and more advanced procedures are available to measure sensory function. Since almost all sensory procedures require the subject to make motor responses to indicate whether it has detected some sensory stimulus, changes in motor capabilities could conceivably be measured instead of sensory changes. Thus, while the simple procedures do not require any training of the subject, the experimenter must recognize that any changes measured may be due to changes in sensory function or motor function or both. The more complex techniques not only require training of the subject before it is possible to measure sensory function, but they also offer the possibility of differentiating the contributions of motor abnormalities from sensory changes. The more advanced procedures can also be used across species, including rats, nonhuman primates, and humans, thus alleviating some of the questions that arise with respect to the extrapolation of findings across species.

In most procedures used to evaluate sensory function, a sensory stimulus is presented to the subject, and a response by the subject, either learned or unlearned depending on the specific procedure, then indicates whether the subject has detected that stimulus. The stimulus may vary from one presentation to the next in its important dimensions such as frequency and intensity, yielding a complete profile of sensory capabilities for that specific sensory modality. For example, in measuring hearing, tones differing in their loudness and pitch are used so that hearing along the entire spectrum is measured.

A clinical neurological examination often includes components designed to measure sensory function, but relatively speaking, these tend to be less sensitive; thus, subtle changes in sensory function might not be detected. One of the simpler experimental procedures used to test sensory function is referred to as reflex modification and is based on unlearned reflexes, in particular the startle reflex. A stimulus such as a loud noise can elicit a startle response (i.e., a startle reflex). It is also known that a stimulus presented prior to the presentation of that loud noise (a prestimulus) can measurably decrease the magnitude of the startle response. Thus, a prestimulus is detectable if it decreases the magnitude of the subsequent startle response. The prestimulus can be varied in intensity and frequency dimensions during a testing session to

produce a complete profile of sensory changes in a particular modality. For example, across the trials of a test session, the intensity and frequency of an auditory stimulus can be modified, and a threshold (e.g., the intensity for a given tone frequency that inhibits startle on 50% of its presentations) can be determined for each tone frequency, generating a classical audiogram. The advantages of reflex modification include its utility across different stimulus modalities (e.g., visual, auditory, and proprioceptive), its utility across species, and the absence of any requirement for training subjects. This approach has already been successful in revealing auditory impairments resulting from exposures to neurotoxic compounds such as trimethyltin and PCBs.

One factor that must be considered in reflex modification procedures is that a less pronounced startle response could result from alterations in motor function *per se* rather than deficits in the subject's ability to detect the prestimulus. For this reason, it is imperative that some trials be interspersed throughout each test session in which no prestimulus is presented, only the startle stimulus. This allows the experimenter to determine whether the magnitude of the startle response remains constant after a chemical has been administered. If so, then any changes in the amplitude of the startle response during prestimulus trials necessarily reflect altered sensory function. Another caution regarding this procedure is that the startle reflex itself may diminish over time. Thus, the number of trials in an experimental session must be carefully controlled.

The more advanced methods for the measurement of chemical-induced changes in sensory function are termed operant psychophysical procedures. These methods have been used in almost identical forms across a range of sensory modalities and in numerous species, including rodents (rats and guinea-pigs), chinchillas, pigeons, nonhuman primates, and humans. **Figure 5** depicts an example of both a human and a nonhuman primate being tested for sensitivity to a vibratory stimulus presented to the hand using operant psychophysical methods. Here the subject is typically required to make a specified response within

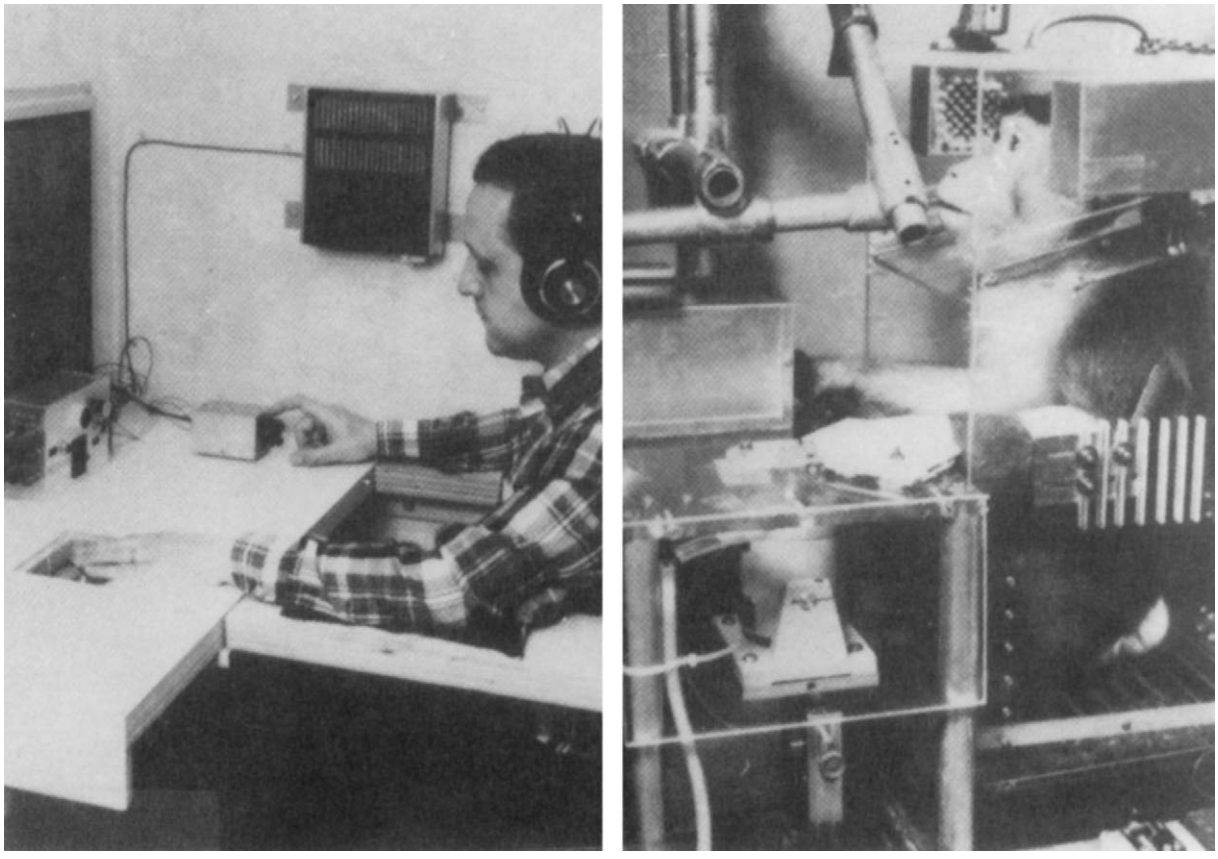

Figure 5 Photograph of a human and nonhuman primate working on the vibration sensitivity paradigm. In each case, the left hand of the subject is placed atop a device that delivers the vibratory stimulus, while the right hand holds down a telegraph key to be released when the subject detects the vibratory stimulus. A spout at the level of the monkey's mouth delivers a squirt of fruit juice for a correct response. (Reproduced from Maurissen JPJ (1979) Effects of toxicants on the somatosensory system. *Neurotoxicology and Teratology* 1(Suppl.): 22–31, with permission from Elsevier.)

some designated period of time to signify that a stimulus presentation was detected. Experimental training of the subject is required before any sensory capabilities can be precisely gauged. In **Figure 5**, the subjects were required to hold down a key when a tone sounded. If they detected a vibratory stimulus delivered to the fingertips during the tone delivery, they released the key and received a reward. To determine how much subjects were simply guessing as to whether a vibratory stimulus was presented, some trials involved no vibratory stimulus presentation. On those trials, the subjects were rewarded for releasing the key only after the tone ended, to indicate that they had detected no vibratory stimulus. As with measures of sensory function such as reflex modification, the various parameters of the sensory modality being evaluated are varied from trial to trial (e.g., intensity, magnitude, and frequency) allowing a determination of that specific sensory function along its significant dimensions. Since changes in sensory function may sometimes be quite selective (e.g., hearing loss for high frequency tones but not low frequency tones), the ability to map sensory changes along the entire spectrum of its significant dimensions is an important component of these methods.

There are several different variations of the methods by which stimuli are presented in the operant psychophysical procedures. In the method of constant stimuli, the subject is presented with several different values (e.g., intensities) of the stimulus in a random sequence or order across trials. The proportion of stimulus presentations detected at each intensity is then calculated, and the value yielding a 50% detection response is deemed the threshold. The method of limits presents a series of stimulus intensities which begin either well above or well below the presumed threshold value. The stimulus value is then either progressively decreased or increased, respectively, until a change in the subject's ability to report the stimulus presentation occurs. The intensity of the stimulus at which this change in detectability occurs is designated as the threshold. In the up-and-down, staircase, or titration method of stimulus presentation, the threshold is continuously tracked by raising or lowering the stimulus intensity depending on whether the subject correctly detected the stimulus. If the subject fails to detect the stimulus, presumably because it is below the threshold for detection, the intensity is raised on the next trial; if the stimulus was detected (i.e., was above threshold), the intensity is then lowered on the next trial. In this fashion, the stimulus intensity can be titrated around the threshold value of the subject.

One of the advantages of operant psychophysical procedures over methods such as reflex modification is that stimulus presentation and subsequent responses occur on a continuous or response-dependent basis. In other words, a response of the subject is recorded, and the next stimulus is presented. In the reflex modification procedure, stimuli are presented during trials which are experimenter initiated and which are separated by specified time interval. The continuous procedures permit the experimenter to measure the rate of responding over time and the time required to respond following stimulus presentations (latency). These measures provide the experimenter with information as to any possible motor dysfunction or motivational problems that the subject may experience as a result of chemical administration which could contribute to behavioral changes in operant psychophysical procedures. Motor dysfunction might increase the latency to respond following stimulus presentations, while an unmotivated subject might be expected to show periods of nonresponding. Armed with this information, the experimenter can proceed to determine which behavioral changes result from true sensory loss.

Cognitive Function

One of the major concerns aroused by exposures to chemicals that affect the nervous system is their potential to adversely impact cognitive functions such as learning and memory. Such a concern certainly has precedent. Lead exposure at high levels can leave children with permanent mental retardation. Recently, it has been demonstrated that even very low levels of lead exposure (i.e., environmental exposures) can produce subtle changes in cognitive processes. Pesticides are known to exert pronounced effects on cholinergic neurotransmitter systems, the very system that has been repeatedly implicated as a causative factor in Alzheimer's disease.

Learning

Learning might be defined simply as an enduring change in behavior that results from experience with changes in environmental events. As a topic of long historical interest in psychology and neuroscience, there are numerous different methods that have been applied to the study of learning ranging from the relatively simple to the more complex and advanced paradigms. Methods for assessing learning involve the processing of sensory stimuli, the execution of motor responses, and a motivated subject. Difficulties in distinguishing the contributions of sensory, motor, and motivational deficits from learning deficits are frequently encountered when using relatively simple learning paradigms. Some of the more complex

procedures are designed to specifically differentiate such functions from learning and thus allow the experimenter to determine whether the chemical has specific effects on learning *per se* as distinct from changes in sensory or motor function, motivational levels, or other nonspecific behavioral alterations.

Many of the earliest studies of learning utilized rats as experimental subjects and required them to run mazes of various shapes and sorts, generally from a start box to a goal box where some type of food reward (reinforcement) was available. For example, in a T-maze, named because of its shape, the subject is reinforced for running from the start box at the base of the T to that arm of the T which has been designated as the correct arm and contains the goal box where food is located. The designation of which arm (stimulus) is correct may be based on side (the right side is correct), color (the arm painted black), or some other stimulus feature. Choosing the wrong arm at the choice point means no reinforcement. After entering an arm and either being reinforced or not, the animal is removed, and after a period of time (the intertrial interval) the animal is placed back in the goal box and another trial initiated. Learning under such conditions is typically measured as the number of trials required to reach a specified accuracy level or until behavior reaches a stable level of accuracy, at which point it is stated that the subject has learned to 'discriminate' between the correct and incorrect arm. The experimenter may compare two groups of rats in such an experiment: one treated with a chemical and one not treated, with the latter serving as a control group indicating 'normal' performance under the particular experimental conditions.

More complex versions of mazes soon emerged in response to the need for more difficult tasks because the T-maze was a relatively simple problem for a rodent to solve and thus not always adequately sensitive to effects of drugs or chemicals. Moreover, once the animal learned which was the correct arm, learning is no longer being measured, only the performance of an already learned response. Two different approaches were offered to circumvent these limitations. One was the construction of more complicated mazes, such as the Hebbs–William maze, which is actually a series of mazes. The correct route to the goal box in this device can be modified as needed by moving the various arms and boundaries into new configurations and thus requiring the subject to learn a new problem, allowing a repeated assessment of learning.

A second approach is embodied in reversal learning. Using this approach, the correct and incorrect arms (stimuli) in the maze are reversed after the subject initially learns which is correct. For example, after the rat learns to run to the right arm of the maze with 90% accuracy, the discrimination is reversed, such that the left arm of the maze is now the rewarded arm. After criterion accuracy is achieved following this reversal, the designation of correct and incorrect stimuli may be reversed again, allowing the repeated measurement of learning over time. Eventually, however, this scheme is also learned by the subject, a phenomenon known as 'learning to learn', such that it comes to learn each successive reversal problem with maximal efficiency (i.e., after only one or two trials).

All maze procedures have limitations that must be considered when interpreting data obtained with these methods. One related particularly to their use with rodents is that subjects leave an odor trail in the maze that can influence the behavior of rodents subsequently tested in the maze. While the experimenter can clean the maze between subjects, it must also be noted that the rodent's sense of smell is much more sensitive than humans, making it difficult to be certain that indeed no odors are still present. Another potential problem is that these procedures obviously require interactions between the experimenter and the subject during the course of testing because the rat must be constantly retrieved from the arms and replaced in the start box. This raises the distinct possibility of both subject and experimenter bias, unless the experiment can be carried out by an individual with no knowledge of any treatment (e.g., exposure to drug or chemical) of any subjects.

Another limitation of simple maze methods for assessing learning is that they do not selectively measure learning. While a decrease in the speed of reaching a 90% accuracy level may be observed in response to a chemical treatment, it may not necessarily be due to alterations in cognitive processing, since changes in either motor performance or sensory capabilities may impact performance in the maze, altering learning independently of any real cognitive changes. Impaired motor function may increase the time taken to reach the goal box; delayed reward is known to impair learning. Further sensory deficits may cause the subject to be unable to utilize the environmental stimuli that normally guide its path to the goal box. Motivational changes (e.g., if the reward becomes less appealing) may clearly retard the rate at which learning occurs. Changes in motor, sensory, and motivational functions as potential contributors to the observed effect may then have to be ruled out in separate additional experiments.

The water maze is a method increasingly being used in behavioral studies for evaluating learning. Used typically with rodents, the subject is placed in a large tub of water made opaque by the addition of a

substance such as nonfat milk powder. Since most laboratory rodents do not prefer water, the reward is escaping from the water by locating a platform submerged just below the surface of the water which is not visible to the subject. Learning is measured as a decrease in the time to locate the hidden platform across successive trials; a learning deficit is suggested by a slower decrease in that time requirement or a greater number of trials to reliably locate the platform. The procedure is relatively simple and imposes no food restriction on the subject. However, despite its ostensible simplicity, it suffers from many of the same limitations as nonwater-based mazes. First, the procedure is not fully automated and thus requires subject–experimenter interaction which can introduce bias into the results. Furthermore, since it is a relatively simple problem, the maze may be learned rapidly and, thus, it is of little utility for experiments aimed at understanding the time course of a chemical's effects on learning. This problem can be alleviated to some extent by moving the platform to a new location each time the subject has mastered the previous location. Although one might suspect that odor trails would not be a factor in a water maze, it has indeed been shown that odors are present and can be utilized by other subjects later placed in the maze. In addition, water temperature plays an important role in this task since age-related deficits in learning in the maze can be alleviated by warming up old rats between trials.

Finally, a rat that requires a greater number of trials or exhibits a slower decrease in the time to locate the platform following chemical treatment is not necessarily exhibiting a learning deficit. Changes in motor capabilities may affect swimming performance and thus lengthen the time it takes the subject to swim to the submerged platform, even if it knows the location of the platform. It is known that subjects rely on environmental cues to find the platform; thus, changes in sensory capabilities could mean an inability of the subject to detect the necessary environmental cues, a deficit which would also increase the length of time the subject required to reach the platform. These alternative explanations of any deficits in swimming time must be ruled out by additional experiments before one can reasonably conclude that a cognitive impairment is present. One way to achieve this is to have the same subjects, in other circumstances, simply be required to swim to a platform, the location of which never changed. This response would also require intact motor and sensory capabilities in performing the same response but no learning since the platform location remains constant. The observation of treatment effects when the platform is moved around, in the absence of any treatment effects when the platform remains fixed, would provide support for an interpretation that the chemical induced learning deficits.

Another maze procedure frequently utilized to evaluate learning (and memory) with rodents is the radial-arm maze. The device itself consists of a central circular area from which eight arms radiate outward like the spokes of a wheel. At the end of each of the eight arms is some type of reinforcer, usually a food pellet. In essence, the subject has access to eight reinforcement deliveries, one in each of the eight arms, and the accuracy and speed (efficiency) with which the subject learns to retrieve all eight reinforcer deliveries is measured. Obviously, under these conditions, the most efficient performance is to obtain all eight reinforcements without revisiting an arm from which the food has already been obtained. The measure of learning is the number of trials required for the subject to reach some specified level of efficiency in the maze. One way of further increasing task difficulty is to provide reinforcement only in a specified number of the arms (e.g., four of the eight) and to change which of the arms provides reinforcement over time or trials. The radial-arm maze obviously presents a more difficult problem to the subject than the T-maze or other simple mazes, but the possibility of interference from motor or sensory deficits produced by chemical treatment still remains. Thus, an increase in the number of trials to reach efficient performance is not necessarily indicative of a learning deficit with this method. By measuring the time of entry into each arm, investigators can begin to get some indication of whether changes in overall activity levels are affecting performance.

In addition to mazes, learning can be measured in Skinner boxes, also known as operant chambers, and these types of approaches have been used across a variety of species, notably rodents, pigeons, nonhuman primates, and humans. In such chambers are some type of response device, speakers, and/or lights for presentation of auditory or visual stimuli, respectively, as well as some type of reinforcement delivery device. An operant chamber configured for a rat is shown in **Figure 6**. Discrimination paradigms are among the simplest measures of learning in operant chambers. In such procedures, a response is reinforced in the presence of one stimulus, the 'correct' stimulus, but not in the presence of another stimulus. Accuracy is defined as the percentage of the total responses that occur in association with the correct stimulus, and learning can be assessed as the number of experimental sessions required by the subject to achieve a criterion level of accuracy.

One distinct advantage of discrimination paradigms in the operant chamber is that behavior can

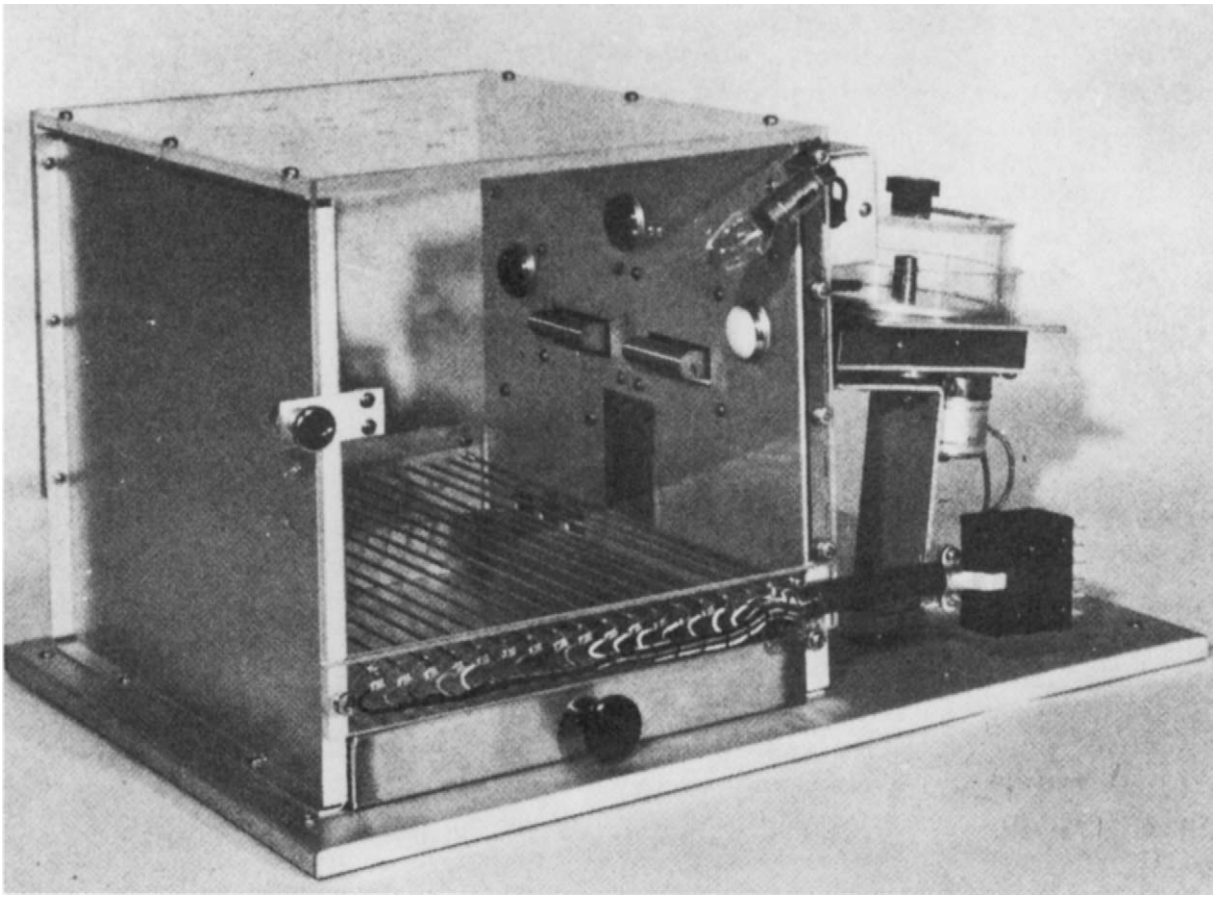

Figure 6 An operant chamber or Skinner box for a rodent. The front wall (right) shows two response levers, below which is situated a food pellet trough. Food pellets are dispensed from a feeder located behind the front wall into the pellet trough. Above and to the left of the levers are two keys which can be illuminated with various colors and used for external or environmental stimuli. Above and to the right of the rightmost lever is a speaker through which auditory stimuli can be projected and used as external (environmental) stimuli. (Reproduced from Reynolds GS (1968) *A Primer of Operant Conditioning.* Glenview. IL: Scott Foresman.)

occur at any time (i.e., the frequency with which it occurs is not constrained by trials as necessitated by the requirement of moving the animal from the goal box back to the start box in most maze-based methods). When responding can occur at any time, the rate or frequency of responding over time can be measured and used to gauge the possibility that motor deficits or motivational insufficiencies may contribute to any observed changes in learning accuracy. A decrease or slow-down in rate of responding would suggest such possibilities.

Another advantage of operant chamber-based procedures is the enormous flexibility they provide for behavioral assessment and the ease with which they can be carried out in these devices. For example, conditional discrimination problems, which are more difficult discrimination tasks, can be easily implemented in operant chambers. Matching to sample is one such method. In this task, the subject first makes a designated response to indicate that it is attending to a sample stimulus that is presented for a short period of time. Subsequently, a sample stimulus is presented for a short period of time. This is followed, after an interval of time, by the presentation of two or more stimuli, and reinforcement is contingent upon a response to the stimulus that matches the sample stimulus. The accuracy and speed with which the subject learns to match the sample stimulus is, of course, the measure of learning. Such tasks can be used with different species by simply increasing or decreasing the number of choice stimuli or the similarity of the stimuli appropriately. Because the procedure includes an initiating response on the part of the subject, a measure of rate of responding is possible, again providing information on potential motoric or motivational contributions to any deficits observed in matching accuracy.

Even the more complex matching to sample discrimination problems are eventually mastered by the subject, in which case discrimination reversals may be implemented in which the stimuli associated with

reinforcement and nonreinforcement are repeatedly switched. Eventually, however, the subjects will learn the reversal concept as well, such that they come to solve each reversal problem with maximal efficiency; that is, on the basis of only one or two responses (e.g., which stimulus is correct today?).

One of the most advanced methods for the assessment of learning is the repeated learning paradigm, also called repeated acquisition, sequence acquisition, or response sequence learning. It specifically addresses the limitations discussed previously. This method actually originated for the measurement of learning in human subjects and has since been adapted for a variety of species. In repeated learning, the subject must make a sequence of responses for reinforcement, and the correct sequence changes with each successive experimental test session. Because the procedure thus requires subjects to learn a new sequence of responses each day, learning can be measured repeatedly across time. A high rate of errors is typically evident during the early part of each test session, as the subject begins to learn the correct sequence for the specific session. The error rate gradually declines as the session progresses, and reinforcers for completing the correct sequence of responses occur at an increasing rate. The ability to measure learning repeatedly across time with this task provides the basis for the measurement of the time course of a chemical's effects (i.e., the time to onset of any learning disabilities and their potential reversibility). This is a particular advantage in situations where chemical exposure occurs in a chronic fashion.

The control for changes in sensory, motor, motivational, or other nonspecific behavioral changes as potential contributors to apparent chemical-induced learning impairments comes when the repeated acquisition task is run in conjunction with a 'performance' task in what is known as a multiple schedule format. The performance component also requires the subject to emit a sequence of responses for reward, but in this case, the sequence of responses stays constant across time. Thus, the subject simply performs an already learned response sequence. In the multiple schedule format, the repeated learning and performance components are presented alternately during the course of the experimental session, with a transition between them occurring either on the basis of time or on the number of reinforcers the subject has earned (e.g., after 15 min or 30 food deliveries switch from repeated learning to performance). Thus, during some portions of the test session, the subject is responding on the repeated acquisition task, while at other times during the session, the performance baseline is operative. Typically, different environmental stimuli, such as different colored lights, are used to indicate to the subject whether the performance or the repeated learning component is in effect.

Both the repeated learning and the performance tasks require intact motor and sensory capabilities, as well as appropriately motivated subjects. Learning *per se* is only required during the repeated acquisition task; the performance task simply requires completion of an already learned response sequence. Thus, if a toxicant or treatment has selective effects on learning *per se*, impairments in accuracy should only be evident during the repeated learning components of the session. If these changes arise, however, as a result of nonspecific behavioral changes (i.e., from sensory, motor, or motivational impairments), then accuracy impairments would be expected in both the repeated learning and performance components of the session since both require these behavioral capabilities. The elegance of this technique derives not only from its ability to distinguish learning effects from other types of behavioral changes but also from its ability to do so in the same subject during the same test session.

Behavior of a normal rat under these conditions is depicted in the top of **Figure 7**. In this diagram, the top tracing shows correct responses, which cumulate vertically; time is represented horizontally. P indicates the performance components of the session, whereas A indicates the repeated learning components. This 1 h behavioral test session began with a performance component and was followed by the repeated learning component, once again by the performance component, and finally by the repeated learning component. Illumination of lights in the operant chamber signaled to the subject that the performance component was operative, while turning out the lights signaled the repeated learning component was in effect. Each short pip mark in the top tracing indicates where the rat earned a food delivery for correctly completing the sequence of three responses required by the schedule. The bottom tracing shows the concurrent errors that occurred.

As **Figure 7** shows, this well-trained rat exhibited a relatively high level of accuracy during the first performance component, earning a steady rate of food rewards and making few errors. The switch to the repeated learning component is accompanied by an increase in errors and a decline in the number of food rewards earned, as the subject begins to learn the correct sequence of three responses for this specific session. Behavior during the second performance component is again composed of a steady rate of food rewards and the occurrence of relatively few errors. The second presentation of the repeated learning component is marked by both a gradual increase in the rate at which food rewards were earned and a

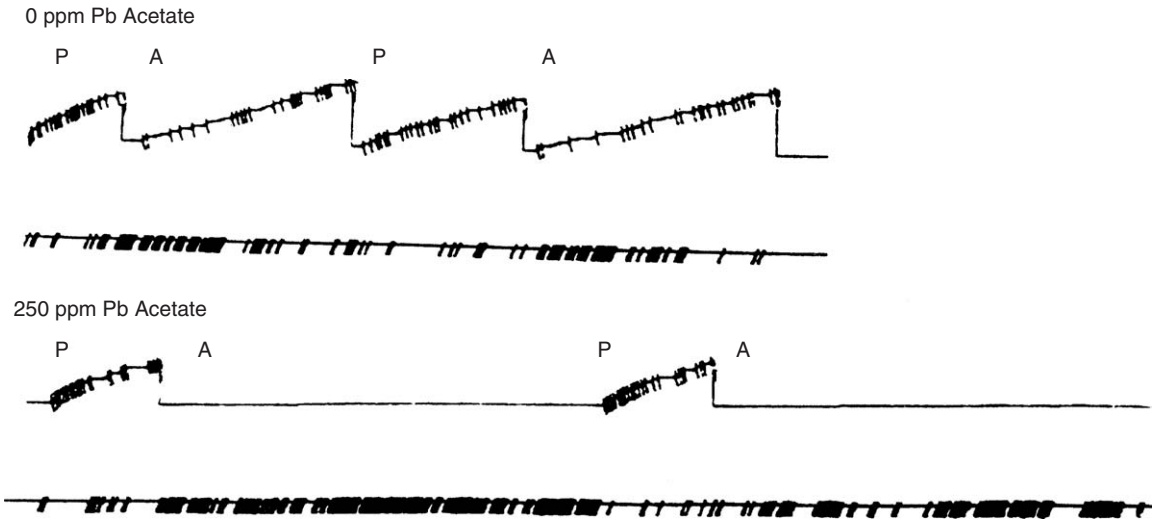

Figure 7 Behavior of a control rat (top) and a rat exposed to 250 ppm lead acetate in drinking water from weaning (bottom) on a multiple repeated acquisition (A; repeated learning) and performance (P) schedule of reinforcement. The top line of each record shows correct responses cumulating vertically with pips indicating food delivery for the completion of the correct sequence of responses; the bottom line shows errors. Time is represented horizontally. (Reproduced from Cohn and Cory-Slechta, unpublished data, with permission.)

decrease in the number of errors relative to levels occurring in the first presentation of the repeated learning component, consistent with a gradual learning of the correct sequence for this session.

The bottom set of tracings shows behavior under the same conditions for a rat that has been exposed from weaning to a relatively low level of lead in drinking water. It shows, in a rather dramatic fashion, a selective effect of lead on learning processes *per se*, as distinct from nonspecific behavioral changes. Specifically, behavior during both presentations of the performance component is unimpaired in that a substantial rate of food deliveries and a minimal rate of errors is evidenced. In contrast, there is no evidence of learning during either presentation of the repeated learning component of the schedule, in that virtually no food deliveries were obtained and a very high rate of errors was sustained. In fact, the rat continued to make errors throughout the entire time in the repeated learning components. Thus, in this case, the effects of lead on accuracy were restricted to the repeated learning component of the schedule. These impairments could not have resulted from deficits in motor or sensory function, or in appropriate motivation, since behavior in the performance components, which also required such functions, was perfectly normal.

Although an effect of a toxicant on behavior during the repeated learning but not during the performance component of a multiple schedule is strong evidence of a chemical's selective effects on cognitive functions, there are other factors that should be taken into consideration. Some investigators subscribe to the idea that a selective effect of a chemical on learning means that its effects should be evident across a variety of learning tasks. While this notion has some validity, it should not be considered a necessary condition since, as has already been described, all learning paradigms are not equal. The extent to which different learning tasks selectively measure learning *per se*, as distinct from sensory, motor, or motivational influences, clearly differs, as does the possible 'contamination' of the learning measure by changes in other behavioral properties. This is not to diminish the importance of these other types of behavioral effects, be they sensory or motor, for example, since such processes are clearly essential for integrated behavioral function, including cognitive functioning. Another important consideration is that the ability to detect effects of a chemical upon learning may depend to a large extent on the degree of task difficulty. It is well established that learning tasks that are relatively easy (i.e., those resulting in relatively high levels of accuracy) will be less sensitive to disruption either by drugs or by toxicants than are tasks of greater difficulty.

Memory

Memory, or remembering, is behavioral recall (i.e., the preservation of learned behavior over time). A distinction is often made between what is referred to as short-term or working memory, occurring over relatively short delay periods, and long-term or reference memory, considered more permanent memory. Obviously, the temporal parameters associated

with what is designated as short- and long-term memory are species dependent.

The measurement of memory is typically based on the persistence of a previously learned response following some time delay; differences in recall accuracy are compared before and after delay intervals. Typically, the longer the delay, the greater the decrease in accuracy. An impairment of memory by a chemical accelerates the rate at which accuracy decreases with increasing delay values.

Both simple and more advanced techniques are available to evaluate memory. Again, however, many of the ostensibly simple tasks cannot differentiate memory deficits *per se* from deficits produced by changes in other behavioral functions, be they motor or sensory functions, or in level of motivation. For example, an inability to execute the response as efficiently (motor impairment) may in essence mean that the delay interval for the subject is functionally longer, thus indirectly impairing accuracy. Alternatively, a treatment which somehow increased the speed of responding could cause the subject to respond before adequately evaluating stimulus options, and thus decrease accuracy independently of a real change in remembering. Here, again, the more advanced methods include the capabilities for differentiating real effects of a chemical upon remembering from those caused by other behavioral consequences of the exposure.

One widely used simple measure of memory is passive avoidance. In this task, the subject, most often a rodent, is placed in a chamber that has two quite distinct compartments. The subject receives a shock in the compartment it prefers (spends most time in), engendering an association between the shock and the distinctive characteristics of that compartment. At some later time (i.e., after some delay interval), the subject is placed back into this two-compartment chamber, and memory is evaluated on the basis of the time (referred to as latency) that elapses before the subject steps back into the side of the chamber in which it previously received shock. The contention is that the longer the subject waits to enter that compartment, the better it remembers the shock it received there.

While changes in latency on this task are produced by a variety of drugs and chemical treatments, the interpretation of these changes can be problematic. If, for example, the chemical causes hyperactivity, the subject might reenter the shocked compartment sooner even if it does remember its association with shock. If the treatment disrupts sensory capabilities, altering perceived distinctions between the compartments, this too may result in a more rapid reentry into the compartment in which the subject had previously been shocked. If the administration of a chemical causes a sedative effect in the subject, rendering it less mobile, the time to reenter the shocked compartment may be increased relative to that seen in nontreated controls, but this would not be considered facilitation of memory. Again, such possible alternative interpretations must necessarily be worked out in additional experiments or with additional manipulations. Depending upon the experimental design, chemical treatments could alter shock sensitivity and thereby modify performance.

The more advanced procedures for memory evaluation not only require more extensive training of the subject, but they also control for some of the possible confounds mentioned previously. There are two general types of more advanced procedures for the assessment of memory. One uses the previous responding of the subject as the event to be remembered, such as in the delayed alternation paradigm. In this procedure, the subject has access to at least two response manipulanda and is required to alternate its responses on the two for reward after some delay interval ends. That is, a response on manipulanda A initiates a delay interval, after which a response on B produces reward. This event initiates another delay interval, after which a return to manipulanda A produces reward. Typically a series of delay intervals are tested in each session, with the length of the delay interval varied randomly across the trials of a session and the specific lengths of the delays appropriate to species. Responses during the delay start the delay over again, thus increasing the time to reward. On this task, then, the subject has to remember which response manipulanda it responded on before the delay interval started in order to respond correctly after the delay. Typical behavior observed under these conditions is a decrease in remembering (accuracy) as the length of the delay interval increases. A chemically induced impairment of memory would then be manifest as a more pronounced decrease in accuracy as delay length increases than is observed under nontreatment (control) conditions.

Critical to the interpretation of any memory-related deficits with the delayed alternation task is the inclusion of a zero-second or no-delay condition. The no-delay condition requires no memory, as there is no delay. Therefore, if a treatment is impairing accuracy under the no-delay condition as well as at the various delay intervals, it is likely that the effects are due to changes in behavioral processes other than remembering. The pattern of change consistent with a selective memory impairment of a toxicant, then, is one composed of no change in accuracy at the zero-second delay but a more pronounced decrease in

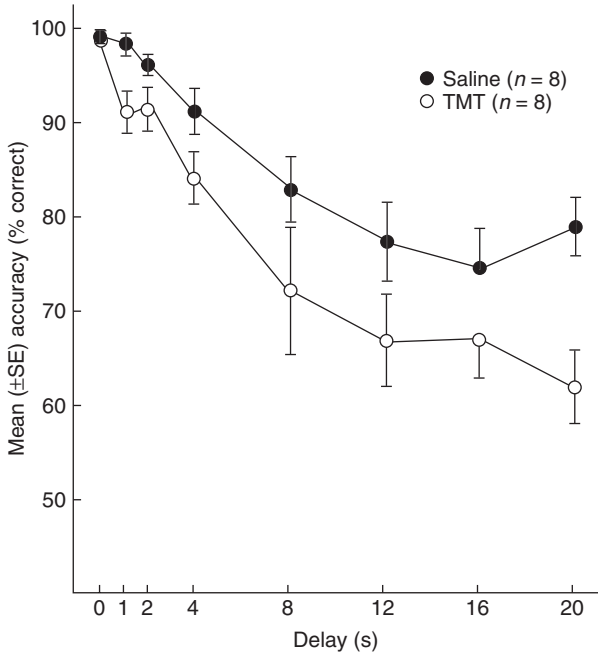

Figure 8 Effects of 7 mg kg^{-1} trimethyltin on delayed alternation performance. Lower accuracy values were evident in TMT-treated rats at all delay values, but no impairment was seen in the zero-second delay condition, consistent with a specific effect on memory function. (Reproduced from Bushnell PJ (1988) Effects of delay, intertrial interval, delay behavior and trimethyltin on spatial delayed response in rats. *Neurotoxicology and Teratology* 10: 237–244, with permission from Elsevier.)

accuracy with increasing delay values relative to nontreated control subjects.

An example of an apparently selective impairment of memory independently of changes in other behavioral processes is shown in **Figure 8**. As can be seen, the accuracy level of a group of nontreated normal rats (solid circles) declines as the delay value increases, as expected. Corresponding data for a group of rats treated with the organic metal, trimethyltin, are shown in the open circles. In this group, accuracy was unaffected at the zero-second delay but decreased more rapidly than did that of normal rats as delay value lengthened.

Other methods for measurement of memory function rely on explicit discrimination tasks. The matching to sample task described earlier is one example. In this paradigm, a sample stimulus is presented briefly to the subject. The subject must then pick the sample stimulus when subsequently presented with multiple stimulus options (i.e., the subject must match the sample). When delay intervals are imposed between the presentation of the sample stimulus and the subsequent presentation of multiple stimuli, the task becomes a memory task. In this case, the subject must remember the sample stimulus in order to perform correctly. As in the delayed alternation procedure, delay intervals of various lengths are used, including the no-delay or zero-delay condition, and a delay function similar to that shown in **Figure 8** is expected. Many of the caveats mentioned with respect to interpreting memory effects in the delayed alternation task likewise apply to the delayed matching to sample paradigm. Separation of a chemical effect arising directly from changes in memory processes rather than from changes in motor, sensory, or motivational functions depends on the inclusion of a no-delay condition. Furthermore, as with learning paradigms, the contention that if a chemical produces a true memory deficit it will be observed across different memory tasks must be tempered by the fact that not all memory paradigms produce an equally selective measure of memory.

Schedule-Controlled Operant Behavior

Learned voluntary behavior is a function of the consequences that follow it. If a response is followed by a reinforcing stimulus, the rate of that response subsequently increases; if followed by a punishing stimulus, or by the absence of a reinforcing stimulus, the rate of responding subsequently decreases. In addition to determining the subsequent frequency of that response, these consequence stimuli will also determine the intensity and temporal pattern with which that response will be emitted in the future.

In the real world, consequence stimuli do not necessarily follow every occurrence of the response. In fact, typically, consequences follow the response on an intermittent basis. Paychecks, for example, are typically distributed on a weekly, biweekly, or even monthly basis, not after each instance of work-related activity that occurs. The pianist plays the entire piece of music before the audience applauds. This strategy of intermittent reinforcement of responding actually provides greater behavioral efficiency and economy as well as greater response strength and persistence than does continuous reinforcement. A response that has been reinforced after every occurrence declines much more rapidly when reinforcement is withheld (extinction) than does one that has been reinforced on an intermittent schedule.

The term schedule of reinforcement refers to the nature of the rules governing the allocation of consequences for a particular response. Behavioral performance controlled by a schedule of reinforcement is referred to as schedule-controlled operant behavior. These schedules of reinforcement are critical because they govern the rate and pattern of responding in time which underlie other behavioral functions. For example, the rate of learning may well be influenced by the underlying schedule of reinforcement. If reinforcement of the correct response during

a learning task is too infrequent, the task may not be adequately learned or not learned at all. Likewise, remembering that response, as in a memory task, may depend on the extent to which it was sufficiently reinforced to begin with.

Consequence stimuli can occur on the basis of time elapsing or on the basis of the number of responses that have occurred or both. In the human environment, schedules of reinforcement exhibit a remarkable complexity. For the purposes of understanding how these various reinforcement schedules or payoff schemes control the frequency and the pattern of behavior in time, simpler versions were initially studied in a laboratory context. As the understanding of simple reinforcement schedules evolved, increasingly complex schedules that more closely mimicked the human environment were elaborated and examined in laboratory experiments.

One of the important aspects of schedule-controlled behavior that deserves note is the remarkable similarity of behavior patterns generated by these schedules across a wide variety of species, even when type of response and type of consequence stimuli differ – a phenomenon of obvious importance for the issue of cross-species extrapolation because it shows the similarity and contiguity of such behavioral process across species.

Simple Schedules of Reinforcement

There are four simple schedules of reinforcement: the fixed interval (FI) and the variable interval (VI), both of which are temporally based reinforcement schedules, and the fixed ratio (FR) and the variable ratio (VR) schedules, both of which are response-based schedules. The FI and the VI schedules both stipulate that a certain amount of time must elapse from the occurrence of a previously reinforced response before a response will again produce reinforcement. On the FI schedule, that time interval remains constant and the parameter value of the schedule indicates the length of that temporal interval (e.g., FI 1 min means that the first response occurring at least 1 min after the preceding reinforced response will result in reinforcement). On the VI schedule, the length of the interval varies from one interval to the next with the parameter value of the schedule indicating the average of the different interval lengths. For example, on a VI 1 min schedule, the average time between reinforcement opportunities is 1 min, but each interval may be either longer or shorter. Responses during the interval have no specific consequence attached to them on either the FI or VI schedules.

Because of the differences in the way in which they schedule reinforcement, the FI and VI control quite different rates of responding (responses per unit time) and patterns of responding, as can be seen in **Figure 9**. The FI schedule generates a characteristic 'scallop' pattern of responding, which engenders pausing, that is, little or no responding immediately after reinforcement delivery (indicated by the short pip marks), followed by a gradual increase in the rate of responding as the time of reinforcement availability again approaches. In the human environment, studying for an examination has features that are characteristic of FI performance: little or no responding early in the semester but a gradual increase as the time of the examination approaches. While one

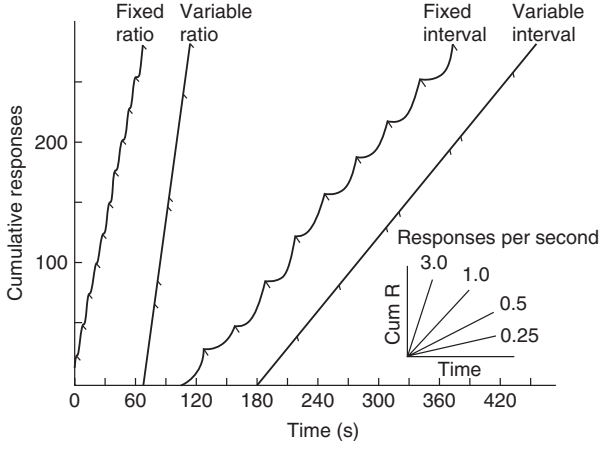

Figure 9 Schematic cumulative records of performance on the fixed ratio (FR), variable ratio (VR), fixed interval (FI), and variable interval (VI) schedules of reinforcement. Responses are cumulated vertically over time. Each downward deflection of the pen represents reinforcement delivery; horizontal lines indicate pausing. (Reproduced from Seiden LS and Dykstra LA (1977) *Psychopharmacology: A Biochemical and Behavioral Approach*. New York, NY: Van Nostrand Reinhold.)

might expect that the performance under such conditions would be characterized by a single response as soon as the interval ends, such a pattern would require the subject to have perfect timing capabilities. Responding at a very rapid rate as the end of the interval approaches ensures that reinforcement delivery will occur with minimal delay as soon as it is available.

The pattern of responding on the VI schedule differs from that on the FI (**Figure 9**) in that no pausing occurs after reinforcement delivery. Instead, the subject continues to respond at a steady and relatively uniform rate over time. The absence of pausing on the VI schedule is thought to reflect the lack of predictability of reinforcement. On the VI schedule, reinforcement may be available immediately after a previous reinforcement delivery since the interval length varies. Thus, pausing after reinforcement could result in a reduction in the rate or number of reinforcement deliveries. One example of VI-maintained behavior sometimes cited is that of getting a busy signal when calling someone on the telephone. The caller continues to redial and is eventually reinforced by a ringing sound on the other end. The persistent redialing reflects the variable length or interval of telephone conversations and, therefore, the unpredictability of when the line will no longer be busy.

In the other two simple reinforcement schedules, reinforcement availability is based on the number of occurrences of the designated response. On an FR schedule, the completion of the number of responses specified by the schedule parameter value is required for each reinforcement delivery. An FR 100 schedule, then, requires 100 occurrences of the designated response for reinforcement delivery. The classic examples of FR schedules are the piecework systems that operated in factories early in US history, where workers were paid for each piece or unit they produced. The FR schedule generates its own characteristic behavior pattern which consists of a pause or period of no responding after each reinforcement delivery, followed by an abrupt transition to a very rapid rate of responding – a pattern known as 'break and run' and shown in **Figure 9**.

A VR schedule also requires the occurrence of a designated number of responses for reinforcement delivery, but the response requirement varies from one reinforcement delivery to the next in an unpredictable fashion. The parameter value of the schedule indicates the average response requirement. Thus, on a VR 100 schedule, the average number of responses required for reinforcement is 100, but the actual number varies from one reinforcement delivery to the next. Perhaps the most obvious example of behavior maintained by a VR schedule is that of gambling. A slot machine may pay off on the average once every 100 plays, but the number of plays between payoffs varies in an unpredictably way; thus, one play that results in a payoff may follow immediately after a preceding payoff or may follow only after a large number of subsequent plays. The VR schedule maintains the highest rates of responding of the four simple schedules (**Figure 9**). In essence, it is characterized by a continuous high rate of responding without pausing after reinforcement deliveries.

Like the VI, the pattern of responding on the VR schedule reflects the lack of predictability of reinforcement availability. Since reinforcement availability may always be imminent, pausing would delay reinforcement. The high rates characteristic of VR and FR schedules are thought to be due to the ratio basis of reinforcement in that the faster the response requirement is completed, the faster reinforcement is available. Increases in rates of responding on interval-based schedules such as the FI and VI cannot accelerate the availability of reinforcement; one must still wait for the time interval to end.

Complex Schedules of Reinforcement

As mentioned previously, the complexity of reinforcement schedules encountered in the human environment is much greater than those embodied in the simple schedules studied in the laboratory. Combinations and variants of the simple schedules of reinforcement produce greater approximations of this complexity. One such example is a multiple schedule of reinforcement, in which component schedules alternate over the course of a behavioral test session. On a multiple FI–FR schedule, for example, the session could begin with an FI schedule in effect and would be indicated to the subject by some explicit stimulus (e.g., illumination of a red light). After some specified period of time elapsed or after the delivery of designated number of reinforcers on the FI schedule, the red light would change to a green light, and the schedule would switch to an FR. The FR schedule component would then remain in effect until a designated time had elapsed or a specified number of reinforcers had been delivered, and would be followed by a switch back to the FI component, and so on. After training on this schedule, patterns of behavior characteristic of each schedule component emerge; thus, during the FI component, a scalloped pattern of responding is maintained, whereas during the FR component break-and-run performance is exhibited. In addition, after experience on the schedule, the colored light stimuli associated with each schedule component come to exert strong control over

behavior, such that performance appropriate to the schedule occurs immediately upon switching the color of the light. That is, these stimuli serve as discriminative stimuli signaling the schedule in effect.

This arrangement allows the experimenter to measure two very different types of schedule-controlled performances in the same subject during the same test session, making it a highly efficient experimental paradigm. This permits a determination as to whether a chemical may have selective effects on certain schedules (e.g., change FI performance without affecting the FR). If the compound being evaluated affects the control of the stimulus lights over responding, it might be manifest as a delay in transition to schedule-appropriate behavior whenever the light colors switched.

A mixed schedule of reinforcement is identical to a multiple schedule of reinforcement, except that there are no external stimuli provided to the subject to indicate that the operative reinforcement schedule has switched. Thus, the only indication to the subject as to 'what pays off' is the feedback it receives from its own behavior. This minimizes the extent of stimulus control over behavior relative to that of a comparable multiple schedule of reinforcement.

A chained schedule of reinforcement, like a multiple schedule of reinforcement, also has different external stimuli associated with each component of the schedule, but it requires the completion of a sequence of components for reinforcement delivery. Thus, on a chained FI–FR schedule, a red light may signal that the FI component is in effect. Completion of the FI with the first response after the interval ends produces the external stimulus (e.g., green light) associated with the FR component. Completion of the response requirement during the FR component then produces reinforcement, and the chain subsequently begins over – a course which continues throughout the behavioral session. A tandem schedule is identical to a chained schedule, but like the mixed schedule, it provides no external stimuli to signal which component schedule is in effect.

The schedule which probably most resembles those operative in the human environment is known as a concurrent schedule of reinforcement. In the real world, we are routinely faced with a multitude of simultaneously operative schedule options with various schedule conditions and consequences, and we must make choices among them. The foraging (food seeking) environment of many species likewise provides such concurrent options with differential probabilities of reinforcement among which species must make choices. Concurrent schedules provide an experimental analog of this facet of the environment and require the subject to make choices among component reinforcement schedules and reinforcers. For example, in an operant chamber such as shown in **Figure 6**, different response manipulanda might be associated with different but simultaneously available reinforcement schedule options, perhaps associated with different reinforcing events as well. In some cases, once the subject chooses one option, the alternative schedule options are no longer available for some period of time. Others allow subjects to switch back and forth between schedule options. These types of schedules allow experimenters to ask questions about how much behavior the subject is willing to emit for specified reinforcers, preferences for reinforcers and response patterns, relative magnitude of reinforcement and allocation of behavior depending on effort and reinforcement availability.

Measurement of Schedule-Controlled Behavior

The universal measure of schedule-controlled behavior is the rate of responding, which is simply the total number of responses divided by total time. While this is a useful measure of behavior, it provides no indication of other aspects of schedule-controlled behavior, such as the extent of pausing or the patterns of behavior over time. For such purposes, a more fine-grained analysis or microanalysis of performance must be undertaken.

One such measure, applicable to both FR and FI schedules, is postreinforcement pause (PRP) time, which is simply measured as the time from reinforcement delivery until the first response occurs in the next interval (FI) or ratio (FR). For the FI schedule, one may be interested in the extent to which the scalloped pattern of performance occurs as an indication of the extent to which responding is controlled by the contingencies operative on the schedule. For this, one of two measures is utilized: the index of curvature or the quarter life. Index of curvature simply utilizes a mathematical formula to indicate how the observed scallop deviates from a straight line that would be generated by a constant rate of responding throughout the interval. Quarter life measures the time it takes for the first 25% of responses in the interval to occur.

Another measure of schedule-controlled behavior is that provided by the distribution of the times between successive responses or interresponse times (IRTs). These can be generated as a frequency distribution and have been shown to be important targets of chemical exposure. For example, lead exposure appears to affect primarily the very short IRTs on FI schedules. Many different drugs from a variety of different classes have been shown to increase the frequency of long IRTs and to decrease the frequency of

short IRTs on an FI schedule – a phenomenon known as 'rate-dependency' and which results in a more uniform and less scalloped pattern of responding. Rates of responding can also be calculated on schedules of reinforcement after the PRP or the IRTs longer than some designated time (pauses) have been subtracted out. This results in a 'truer' rate of responding and is known as running rate.

Behavioral Teratology

Behavioral teratology, or neurobehavioral teratology, is often referred to separately from behavioral toxicology. Behavioral teratology focuses on the behavioral impact of toxic exposures occurring prenatally or during early development. In some cases, these studies may only track the consequences of chemical exposures into early postnatal life, but in others effects may be studied well into the juvenile and even adult stages of the life cycle. Because the possibility has been raised that developmental exposures may accelerate the processes of aging, some studies are now beginning to follow subjects throughout the lifespan. Behavioral teratology studies typically include a series of tasks designed to evaluate multiple behavioral functions. Consequently, such experiments may include assessment of the development of various reflexes and developmental landmarks (e.g., eye opening), performance on a functional battery (FOB), motor activity, sensory capabilities, learning, and even schedule-controlled operant behavior. In addition, some such experiments may include evaluation of behaviors deemed 'species specific' (i.e., behaviors that are innate and unique to that species), such as the ontogeny of aggression, play, or vocalization in rodents.

In cases in which outcome is followed through maturity, many of the behavioral paradigms that have already been described are utilized. Assessment of behavioral changes early in life, however, may require modification of such procedures and even the development of specialized behavioral preparations. One example of such a specialized preparation which concurrently measures sensory and motor capabilities (though not independently) is that known as 'homing behavior', a behavior utilized by rodent pups to locate the nest should they wander. In this procedure, a rat pup is placed in the center of a rectangular apparatus, one side of which contains clean bedding material, whereas the other side contains bedding material from the home nest with its scent familiar to the pup. The time taken for the pup to orient to or to reach the home cage bedding is then measured. Such a test is deemed apical because it requires the integration of both motor and sensory capabilities.

There are certain issues uniquely related to behavioral teratology studies that require special consideration. One is that of toxicant effects on the dam (mother). Since the behavior of the dam may ultimately influence behavior and development of the offspring, great care must be taken to determine whether any observed effects of a chemical in the offspring are direct effects of the toxicant itself or whether they arise indirectly as a result of the effect of the compound on the dam's behavior. This is typically done by using a variety of fostering procedures. A cross-fostering procedure distributes the pups of treated dams to dams that are treatment free, in which case there should be no chemical-induced changes in maternal behavior.

There are also issues related to statistical analyses of the data that are unique to behavioral teratology. The offspring of a given litter are not considered as individual subjects since, as members of a litter, they have all experienced factors of the fetal environment which may be unique to their dam. This means that the total number of subjects in a treatment group is really equivalent to the total number of litters represented in that group, a factor which can change the degrees of freedom in the statistical analyses.

Human Testing

Behavioral toxicological studies in humans have focused primarily on adults occupationally exposed to chemicals and children exposed to toxicants environmentally. There is frequently a good deal of overlap in the specific behavioral functions evaluated in each case, although the tests utilized must be age appropriate. However, studies in children also often include measurements of developmental profiles and landmarks which are not relevant to studies of occupationally exposed adults. In adults, in contrast, assessment of exposure-related symptomatology is possible. Both types of studies also generally assess a broad variety of behavioral functions and may include tests of motor function, sensory capabilities, complex or cognitive behaviors, attentional processes, and vigilance, usually in the context of a standardized test or test battery. In the past, many such functions would be evaluated as part of a neurological or clinical examination. However, it has become increasingly clear that such examinations, meant to diagnose disease or brain damage, are neither sufficiently sensitive nor quantitative for purposes of detecting subtle effects of toxicants and ultimately for setting standards of exposure.

The test batteries commonly used in human studies have come primarily from the field of clinical neuropsychology, in which human testing has predominated. Behavioral measures such as are utilized

in experimental animal studies were, in the past, rarely included in human studies. This has changed, however, and will likely increase even more in the future given the advantages of utilizing the same tests across species. In part the overall emphasis on broad testing of behavioral functions in human studies has been driven by the lack of any information on the behavioral properties of many of these chemicals as well as by the need to establish dose–effect and dose–response relationships.

Many of the same issues raised with respect to experimental animal studies also apply to human testing and to the choices of particular tests to be utilized. There are numerous tests that can be utilized for measurement of behavioral functions in humans, and questions remain as to the correct choice. One consideration related to the various tests is deemed validity and refers to the degree to which the test actually measures the behavioral function that it was designed to measure. For example, does a test of memory really evaluate memory function? In addition, how specifically does the test measure that function? The related issue was raised in experimental animal studies in which the possibility that changes in motor, sensory, motivational processes, etc. might contaminate a measure of memory function, and appropriate controls were included in the more advanced procedures to evaluate those possibilities.

Another important issue relates to the reliability of the test. That is, how reproducible or consistent are the test results across multiple administrations? Inadequate reliability almost guarantees that a subtle toxicant effect will not be detected against a background of scores of broad individual variability that will be present in any normal population. An issue that has not received adequate attention is the sensitivity of these tests to detect toxicant effects, a factor that is of particular importance if the test results are used in the context of setting exposure standards. If a particular test indicates effects of lead, for example, at a blood lead concentration of $40\,\mu g\,dl^{-1}$, one may wonder whether this represents the bottom limits of sensitivity of the test or the actual blood lead value at which such effects occur. In other words, could the test have detected effects at even lower levels of exposure if it had been more sensitive? A deficiency in test sensitivity could mean that exposure standards will be set at levels that are too high and will not protect the exposed populations.

A related question of relevance, particularly to tests of achievement such as the so-called intelligence tests, is standardization. This refers to the population from which the normative scores for the test were collected. This issue is often raised in the interpretation of intelligence tests for populations that are culturally and socially distinct from the populations of white middle-class English-speaking children from which normative scores for such tests have typically been derived.

Developmental Assessments

As mentioned previously, several unique considerations affect the assessment of toxicant-induced behavioral changes in children. One such consideration is the rapid development that children undergo from birth through even the preschool and early school stages. Moreover, this development is marked by wide individual differences in the rate at which it occurs and, for some facets of behavior, gender-related differences as well. An additional difficulty is that many of the behavioral processes that are of particular interest, such as complex cognitive behavior, are more difficult to evaluate at a young age. While it seems clear that children certainly have both learning and memory capabilities even from birth, assessment of such changes has typically relied on tests which may require language or motor skills well beyond the capabilities represented by these early stages of development.

Because of this rapid change in the behavioral repertoire over the course of early development, the tests that are utilized in studies of children tend to differ at different ages. One test frequently utilized in the first few days after birth is the Brazelton Neonatal Behavioral Assessment Scale, which is composed of two subscales. The first taps a range of behavioral items such as habituation and responsiveness to environmental stimuli. The second primarily measures a variety of unconditioned reflexes. While the Brazelton scale is obviously limited in the extent to which it can tap cognitive functions, or define specific behavioral deficits, its utility in detecting drug-induced changes has been established.

A recently developed technique for infant assessment is embodied in the Fagan Test of Infant Intelligence, which assesses visual recognition memory. In this test, an infant faces a display with two screens. On one screen, a visual stimulus is presented for a specified period of time. Subsequently, that visual stimulus is projected on one screen and, at the same time, another visual stimulus is projected onto another screen. An observer records the amount of time the infant spends gazing at each screen. Normal infants look away from the visual stimulus which they have already seen and spend more time gazing at the novel stimulus, a trait which has been shown to correlate with higher scores later in development on the Stanford–Binet intelligence test.

A widely used test at a slightly later stage of development is the Bayley Scales of Infant Development, appropriate to children from 2 to 30 months of

age. The test is composed of three subscales: motor, mental, and behavioral. Each is arranged with respect to chronological development. One of the advantages of this test is the ability to carry out repeated testing over the normed age range.

As children reach preschool and school age, the number of test choices available increases. For example, the McCarthy Scales of Children's Abilities provides an analog of an intelligence test score by combining the scores from its five subscales into a general cognitive index score. Its applicability extends from children aged 2.5–8.5 years. Like the Bayley Scales, it too allows for repeated measurement over time, which is a particular advantage for longitudinal studies; utilization of the same test instrument over time, given appropriate reliability of the instrument, provides greater assurance of the continuity and of the onset or disappearance of an effect than does the use of different instruments at different ages.

Various intelligence tests are available for preschool age children, such as the Weschler Preschool and Primary Scale of Intelligence (WPPSI). The advantage of this particular instrument is that it represents an extension of the well-standardized and widely used Weschler Intelligence Scale for Children (revised; WISC-R). The WISC-R is an intelligence test for children of 6 years of age or older; the WPPSI extends this age range to include children of ages 4–6.5 years. In addition, both rely on the same two subscales, verbal and performance, to measure a variety of behavioral functions, thus providing a type of continuity from the preschool to the school-aged child for repeated assessment of behavioral function.

One of the major concerns with developmental and intelligence tests such as the WISC-R and others is to be able to rule out contributions from numerous sociodemographic and other variables known to covary with intelligence test score. Variables which may potentially modulate intelligence include birth weight, length of gestation, maternal age, birth order, parental education, parental IQ, socioeconomic status, and quality of the home environment. Appropriate statistical controls or subject matching must be undertaken to evaluate the contributions of these variables to outcome measures.

While these developmental and intelligence tests may clearly be important to the determination of the levels and conditions of exposures to a toxicant associated with adverse behavioral function, they are less useful, as noted previously, in providing a precise delineation of the behavioral functions actually affected by a chemical. Measures such as intelligence test scores are global measures in that they rely on the integration of all behavioral functions. Even performances on subscales of these tests are jointly dependent on integrative motor, sensory, and cognitive functions. Thus, even a preferential deficit on a verbal scale, which is clearly geared toward cognitive function, may not provide a precise understanding of the nature of the behavioral deficit.

To achieve a true understanding of the behavioral processes affected by a chemical will necessarily require direct measurement of those specific functions, much as is done in the experimental animal studies described previously. Some neuropsychologists have recognized this problem and have begun to employ measures of specific behavioral functions such as learning, memory, sustained attention, and abstract thinking in an attempt to determine the source of the deficits in global intelligence test scores produced by lead exposure. An alternative approach is to utilize many of the behavioral tasks already employed in experimental animal studies – tasks which are designed to evaluate specific functions and which have already been widely used across species, including humans, in other research contexts. The repeated learning paradigm actually originated in studies using human subjects and was later adapted for nonhuman primates and rodents. Procedures such as delayed matching to sample and operant psychophysical procedures have also been used across species with appropriate parametric modifications. These types of paradigms may play a more significant role in future developmental studies of children because they provide direct and specific measures of behavioral functions that are more difficult to differentiate in standardized tests.

Adult Assessments

Assessments of behavioral toxicity in adults frequently occur in the context of occupational exposures to chemicals. Like studies carried out in school-aged children, these evaluations have relied largely on standardized tests, including intelligence tests. They also tend to employ a broad variety of tests so that numerous behavioral functions can be tested, particularly when the effects of a toxicant are ill-defined. As such, the same considerations must be taken into account with respect to the choice of tests utilized. These include validity, reliability, and sensitivity, as well as standardization issues related to the population from which test norms were derived.

One distinction between many of the studies of behavioral toxicity in children and adults is that while the former have tended to be primarily longitudinal in nature, following the effects of toxicant exposures to children across the course of development, most of the occupational exposure studies are cross-sectional studies that encompass only a single time point of measurement of behavioral function.

This no doubt reflects the added difficulties of carrying out studies in the workplace, where it may be more difficult to obtain appropriate amounts of the subjects' time for behavioral evaluation and where resistance to such experiments may be encountered from either the employer or the employee.

One of the most common inclusions in these test batteries that have been utilized in studies of occupational behavioral toxicity is the Weschler Adult Intelligence Scale (WAIS), which is actually a battery of tests subsumed under verbal and performance subscales which, combined, provide a full-scale intelligence test score. The series of verbal tests includes information (general information questions), comprehension (interpretation test), arithmetic, similarities (between nouns), digit span (repeating sequences of digits), and vocabulary. The performance tests include digit symbol (associating digits with symbols), picture completion, block design (duplicating block patterns), picture arrangement, and object assembly. Because of the obvious overlap of behavioral functions in some of these subtests, and the consequent global and nonspecific nature of any change detected in full-scale intelligence test scores, some investigators have opted to use only selected tests from the battery to provide a shortened version of the WAIS for occupational behavioral toxicity studies.

Two different test batteries, the World Health Organization (WHO) Neurobehavioral Core Test Battery and the Neurobehavioral Evaluation System (NES), are currently the most widely used test batteries in occupational behavioral toxicology studies. Both include components of the WAIS described previously in addition to other psychometric tests of behavioral function. The WHO Neurobehavioral Core Test Battery is a pencil and paper-administered test battery, whereas the NES is a computerized test battery that has been translated into several languages and in fact presents a more extensive set of tests than does the WHO in that it includes tests of psychomotor performance, cognition, memory and learning, and perceptual ability and affect.

Memory dysfunction has been a frequent complaint in populations of workers exposed to various neurotoxicants and is tapped by several different tests used in a human testing context. One of the most widely used for this purpose is the digit span that constitutes one of the WAIS subtests. As indicated earlier, this test requires the subject to recall a series of digits, and the length of the list is successively increased contingent upon the subject correctly recalling the members of the list. In some cases, words or letters are utilized instead of digits. As is the case with experimental animal studies, more complex versions of these tests have been devised and implemented. In procedures such as continuous recognition memory or memory scanning, subjects may be shown a list of digits or letters and then shown, after a delay interval, a longer list of various digits or letters and asked to recall those that were on the original list. Analogies to such tests are embodied in procedures such as the Benton Visual Recognition Test, which requires a subject to reproduce a drawing or geometrical design.

Paired-associates learning is also frequently used in a memory context in occupational exposure test batteries as well as in studies with children. In these paradigms, a list of paired words is read to the subject, who must then recall the second member of the pair when the first is read after a delay. The task can be made relatively simple by using pairs which have some type of obvious relationship or made more difficult by having pairs with no apparent relationship. The test can be used in a memory context by including a delay between the experimenter's reading of the list and the subject recalling the second member of each pair. In addition, the task can be used in a repeated learning context, much as the repeated acquisition paradigm described earlier, by using new lists of paired-associates after the subject masters the initial list. This particular approach has a long history of use with human subjects and has been found to be sensitive to toxicants such as lead.

Measures of vigilance, attention, or distractibility are also frequently included in assessments of occupational behavioral toxicology. These range from very simple procedures, such as reaction time, to more complex tasks, such as simulated cockpit or tracking tasks. Even reaction time can be varied from a very simple to a highly complex procedure. In a simple reaction time task, the subject is typically presented with some type of screen on which a single visual stimulus will appear at intermittent and unpredictable intervals. The subject must respond on the single response manipulanda as soon as the stimulus appears. Complex reaction time presents the subject with multiple stimuli as well as multiple response options. For example, there may be four different stimuli, each of which is presented at random and unpredictable intervals. The appropriate response depends on which of the stimuli is presented, and the subject is asked to respond on the appropriate manipulanda as quickly as possible after detecting the stimulus. Thus, the more complex reaction time task involves not only attending to the screen to detect the stimulus presentation but also making a decision as to the correct manipulanda and then executing the response. Obviously, the number of options can be modified to fit the experimental situation.

One of the important parameters of the reaction time task is the rate at which the attention of the subject deteriorates, such that reaction time is slowed down or even that the subject misses stimulus presentations entirely. This rate of deterioration of performance will depend on many factors, one of which is the rate at which stimuli are presented to the subject and another being the length of the session during which reaction time is measured. While one might intuitively think that the slower the rate of presentation, the more rapid the rate of deterioration of performance, in fact sometimes the opposite is true. A very rapid rate of presentation of stimuli can render the subject exhausted and less alert or less motivated. With respect to session length, one typically expects to see a gradual decrement in performance as the session progresses, such that an adequately long session must be implemented to catch this function. Finally, a critical variable in reaction time studies is the prominence of the stimuli used. In fact, this parameter can be manipulated to change the sensitivity and difficulty of the task.

Reaction time tasks are by no means limited to the presentation of discrete visual stimuli. Other variants have included those in which the subject must respond to a stimulus that is different from a continuously presented array of stimuli. The so-called clock test is one example. In this procedure, the subject is instructed to respond when the hand of a clock ticks off 2 s at once rather than the typical 1 s tick; the 2 s tick is an infrequent and unpredictable occurrence. In other situations, a continuous presentation of letters or numbers may be presented and the subject instructed to respond to one particular letter or number whenever it appears.

Pursuit and tracking tests represent even more complex versions of vigilance assays. In these kinds of tasks, subjects must continuously monitor a stimulus which drifts off a home position on the dial. The situation can be made quite complex, as in flight simulators in which there may be multiple dials which must be continuously monitored and returned to the home position, with drift occurring at varying rates on each dial across time. The various vigilance tasks described previously have a long experimental history and have been shown to be sensitive to a wide variety of influences, including fatigue and various drugs and chemical exposures.

The kinds of vigilance procedures described obviously require reasonably intact motor function and are often interpreted with that in mind. However, these techniques also depend on sensory processes. In fact, assuming intact motor functions, vigilance tasks such as those described can be adapted to provide some indication of sensory function changes by modifying the saliency (intensity) of the sensory stimuli used in the paradigm. However, more direct approaches to the evaluation of sensory function following occupational or environmental exposures to toxicants are provided by the types of operant psychophysical procedures elaborated previously. In fact, psychophysical procedures were developed using human subjects and only later adapted for various species of experimental animals. The psychophysical procedures clearly provide more direct and straightforward assessments of sensory detection capabilities in the absence of confounding changes in a subject's motor capabilities or motivation to respond.

Assessments of motor function are often included in the neuropsychological test batteries utilized in occupational exposure studies. Typically, these tend to be relatively simple measures of motor capabilities, probably for two reasons. The first is that the inclusion of vigilance tasks such as those described previously depends on motor coordination in addition to sensory capabilities; therefore, toxicant-induced changes in such performances may already be indicative of motor impairment. This can then be pursued by inclusion of some additional and more direct assessments of motor function in the battery. The second reason relates to logistical reasons and practicalities. Test batteries such as the WHO Neurobehavioral Core Test Battery and the NES are typically taken to the site where measurements of subjects are to be made. Thus, portability is a major consideration, and more complex assessments of motor function would incur greater equipment needs. Since the purpose of these batteries is generally to screen for adverse effects, studies providing more precise delineations of affected functions can be pursued at a later time.

Simple tests of motor function utilized are generally those such as finger tapping in which subjects are asked to tap a key or a button at as rapid a rate as possible for a designated period of time. The subjects may be asked to carry out this task with the preferred hand as well as with the alternate hand. In some cases, toe tapping has been used in addition to finger tapping. Other batteries have relied on the tests of manual dexterity that are frequently used in screening prospective applicants for some types of factory work jobs. One of the most frequently used of such tests is the Santa Ana test, which requires the subject to remove pegs from a hole and to reinsert them into the hole after turning them 180°. The measurement of interest in this case is the number of pegs that are successfully rotated within the specified time interval. The Purdue Pegboard test is likewise used in this capacity. It requires the proper orientation and placement of

pins in a series of holes. Such tests have indeed successfully defined subjects occupationally exposed to chemicals from those nonexposed.

One final common inclusion in many studies of occupational behavioral toxicology and in some test batteries is assessments of symptoms experienced by those exposed to chemicals. While this might be perceived as an ostensibly simple procedure, it entails numerous potential confounds. These evaluations are typically administered via questionnaires. Items for the questionnaire must be carefully constructed with respect to not only the choices of items but also the wording of the text and the manner in which the response is recorded. Clearly, the motivation of the subject in answering the questions must be considered. One problem can arise when the list of symptoms includes only those that are associated with the toxicant of concern. It is necessary to include symptoms that are not associated with the particular toxicant under evaluation so that some assessment of the tendency of the subject to respond positively to all symptoms can be evaluated. Several such evaluations of subjective and mood states are available. The most widely used is the Profile of Mood States (POMS), which consists of 65 adjectives of various moods that the subject answers according to a 5-point rating scale. The POMS has been used extensively in the evaluation of the acute effects of CNS drugs and toxicants.

Acknowledgments

Supported by NIEHS Grants ES05903, ES05017, and ES01247.

See also: Multiple Chemical Sensitivities; Occupational Toxicology; Pesticides; Pollution, Air Indoor; Psychological Indices of Toxicity; Sensory Organs; Sick Building Syndrome.

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Belladonna Alkaloids

Madhusudan G Soni

- Representative Compounds: Atropine; Scopolamine; Homatropine
- Chemical/Pharmaceutical/Other Class: Naturally occurring antimuscarinic drugs

Uses

Belladonna alkaloids are used in clinical medicine for their ability to block the effects of parasympathetic nerve stimulation.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposure to belladonna alkaloids. They are available in oral and tincture forms.

Toxicokinetics

The belladonna alkaloids are absorbed rapidly from the gastrointestinal tract. They also enter the circulation when applied locally to the mucosal surfaces of the body. Absorption from intact skin is limited. Belladonna alkaloids are metabolized mainly in liver to glucuronide conjugates after metabolic hydroxylation of the aromatic ring. The volumes of distribution for important belladonna alkaloids, atropine, and scopolamine, are ~ 1.7 and $1.4\,l\,kg^{-1}$, respectively. Elimination of belladonna alkaloids is rapid. About half of the atropine is excreted unchanged in urine. Traces of atropine are found in various secretions including breast milk. The elimination half-life for intravenously injected atropine ranges from 1.9 to 4.3 h.

Mechanism of Toxicity

Toxic doses of belladonna alkaloids prominently lead to central excitement. The earliest symptoms of

atropine toxicity are due to blocking of acetylcholine receptor sites on cells of organs innervated by the craniosacral division of the visceral effector nervous system. Scopalamine differs from atropine intoxication in that the cardiac rate is rarely increased and cerebral excitement is of short duration. Although the mechanism of action of these compounds is well studied the mechanism of toxicity is not entirely understood.

Acute and Chronic Toxicity (or Exposure)

Animal

Cats, dogs, and birds are sensitive to belladonna alkaloid toxicity; horses and oxen less so; and pigs, goats, and sheep are comparatively resistant to the alkaloids. Parenteral administration of lethal doses of atropine to young rabbits produces two distinctly different types of deaths. About half of the animals died promptly in a convulsive state, perhaps comparable to the commonly encountered clinical syndrome of central excitement, but a smaller group suffered delayed deaths in ~ 2 weeks with endarteritis obliterans in the distal portion of the injected limb. Chronic belladonna alkaloid or atropine poisoning has evidently not been encountered as a clinical entity, but the parenteral administration of large doses of atropine ($16\,mg\,kg^{-1}$ daily) for periods of 1–3 weeks produces in young puppies a syndrome clinically similar to advanced fibrocystic disease of the pancreas.

Human

The deliberate or accidental ingestion of belladonna alkaloids is a major cause of toxicity in humans. The most dangerous and spectacular manifestation of poisoning arises from the intense excitation of the central nervous system (CNS). Infants and young children are especially susceptible to the toxic effects of atropinic drugs. In adults, delirium or toxic psychoses without undue peripheral manifestations have been reported after instillation of atropine eye drops. Transdermal preparation of scopolamine has been reported to cause toxic psychoses, especially in children and in the elderly. Serious intoxication may occur in children who ingest berries or seeds containing belladonna alkaloids. In case of full-blown poisoning, the syndrome may last 48 h or longer. Depression and circulatory collapse are evident only in cases of severe intoxication; the blood pressure declines, respiration becomes inadequate, and death due to respiratory failure may follow after a period of paralysis and coma.

Clinical Management

The diagnosis is suggested by the widespread paralysis of organs innervated by parasympathetic nerves. Intramuscular injection of physostigmine may be used for confirmation. If the typical salivation, sweating, and intestinal hyperactivity do not occur after physostigmine injection, intoxication with atropine or a related agent is almost certain. Measures to limit intestinal absorption should be initiated without delay if the poison has been taken orally. The most effective antagonist to the CNS manifestation is physostigmine salicylate in doses of 0.5–2.5 mg by any parenteral route. Because physostigmine is metabolized fast, repeated doses may be needed. If marked excitement is present and more specific treatment is not available, diazepam is the most suitable agent for sedation and for the control of convulsions. Large doses should be avoided. Artificial respiration may be necessary. Ice bags and alcohol sponges help to reduce fever, especially in children.

See also: Cholinesterase Inhibition; Neurotoxicity; Poisoning Emergencies in Humans.

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Relevant Website

http://www.emea.eu.int – European Medicines Agency.

Benchmark Dose

Qiyu Jay Zhao

Introduction

Until recently, safe doses for noncancer effects have been derived directly from toxicology study doses such as no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs). The NOAEL represents the highest experimental dose for which no adverse health effects are observed, while the LOAEL represents the lowest dose for which the adverse health effect is observed. Dividing the study NOAEL or LOAEL by a series of uncertainty factors yields a dose that is generally considered safe. A recent alternative to using the NOAEL or LOAEL in this calculation is the use of a benchmark dose (BMD) to serve as the starting point for deriving safe doses.

BMD Definition

As defined by US EPA: a BMD is "a statistical lower confidence limit for a dose that produces a predetermined change in response rate of an adverse effect…compared to background." The BMD modeling is conducted by fitting a flexible mathematical model to the observed data, and the dose corresponding to the level of predetermined change (i.e., benchmark response (BMR)), is determined from the model. A common model fitting approach is to use maximum likelihood methods, and the resulting central estimated dose for the BMR is the BMD (see **Figure 1**). To replace a NOAEL in the computation of the safe dose, a lower confidence limit (usually 95%) on the BMD is often used instead of the BMD to account for statistical uncertainties in the study. This lower bound value is commonly referred to as the BMDL.

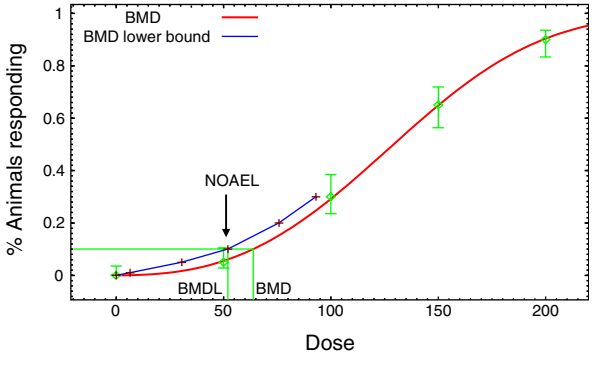

Figure 1 Example of BMD modeling results.

Characteristics of the BMD Approach

The BMD approach was developed to better define the point of departure in the computation of the safe dose in order to overcome the shortcomings of using NOAELs or LOAELs. The traditional NOAEL approach has several limitations: (1) it is limited to one of the doses in the study, making it dependent on study design; (2) it does not account for variability in the estimate of the dose–response; (3) it does not account for the slope of the dose–response curve; and (4) it cannot be applied when there is no NOAEL, except through the application of an uncertainty factor when a LOAEL is used.

In comparison with the NOAEL approach, the BMD approach provides four major advantages. First, the BMD is derived based on data from the entire dose–response curve for the critical effect, rather than only from the single dose (e.g., NOAEL). Therefore, the BMD reflects the slope of the dose–response curve. Second, the BMD approach treats sample size appropriately when the lower confidence limit on the BMD (i.e., BMDL) is used. For example, the smaller the sample, the larger the uncertainty associated with the BMD estimates and the lower the confidence limits (all else being equal). Therefore, data with lower statistical power will result in lower BMDLs (making their use health protective), and better experiments with more statistical power are 'rewarded' with higher BMDLs. Third, the BMDL is not constrained to be one of the experimental doses, and calculation of the BMDL allows for estimation of a NOAEL surrogate when only a LOAEL is available. In addition, the dose-independent BMDL also facilitates comparison of toxicity potencies across chemicals or endpoints. Fourth, the BMD approach can be useful when the dose spacing in a study is such that the LOAEL is much larger than the NOAEL. Thus, any good study can be used, even in the absence of a NOAEL, as long as sufficient and appropriate dose–response data are provided so that the dose corresponding to the BMR can be estimated.

Data Requirements and Parameter Selection

A number of dose–response models have been developed for BMD analyses. The form of the model used and the necessary inputs for the modeling depend on the type of data to be modeled. For quantal data (e.g., histopathology incidence data), the incidence of the effect of interest and the total size of the group are needed, and for continuous data (e.g., liver enzyme activity), the group size, mean, and a measure of variability (i.e., standard deviation or standard error) are required.

To estimate the BMD and BMDL, it is necessary to define a desired BMR. For quantal data, the BMR is defined as an incidence change from the estimated control. Usually, a 10% extra risk $\{[P(\text{dose}) - P(0)]/P(0)\}$ is used to define effective doses for comparing potencies across chemicals or endpoints. This response level is used because it is at or near the limit of sensitivity in most chronic bioassays. If a study has greater than usual sensitivity, then a lower BMR (5% or even 1%) can be used. For continuous data, BMR is defined as a percentage change from the estimated control mean, or as a change of a certain number of standard deviations from the control. If there is a minimal level of change in the endpoint (e.g., liver enzyme activity) that is generally considered to be biologically significant, then that amount of change can be used as the BMR. In the absence of endpoint-specific data to determine the appropriate level of response as adverse, a change in the mean equal to one control standard deviation can be used. This default approach is used because when values beyond the 98th–99th percentile of control animals are considered abnormal, a dose that causes a shift in the average of 1 standard deviation results in approximately an excess risk of 10% of the animals in the abnormal range. Whenever, a BMR is chosen based on biological considerations, US Environmental Protection Agency recommends to present the resulting BMDL with the BMDL estimated for the default BMR.

To make the BMD from continuous data comparable to the BMD from quantal data, the BMR for continuous data can also be expressed as incidence data. To do this, individual animal data are categorized based on a predetermined cutoff value (e.g., a $>10\%$ change in organ weight). This incidence data could then be modeled as a quantal endpoint, with the BMR expressed in terms of an incidence change from the control. This approach is not optimal, since some information would be lost in this data categorization. A better way to convert the continuous BMR to a quantal BMR is to use a 'hybrid' approach, which uses all of the information contained in the original observations. The hybrid approach fits continuous models to the continuous data. Based on the probability information in the continuous dose–response curve and a cutoff value for defining adverse response, a BMD for a specified quantal BMR (e.g., 10%) can be calculated. This result can be compared directly to other BMDs estimated from quantal data. A limitation for using 'hybrid' approach is that it requires definition of a background incidence of abnormality, or the specification of a level of response that can be considered the cutoff point between normal and abnormal responses. The selection of the cutoff point is often difficult.

BMD Model Evaluation

Although the BMD offers several advantages over the NOAEL, it can only be used in cases where available data are suitable for modeling. A good BMD model software should provide statistics for assessing model fit, including measures of global and local data fit. Guidance is available from US EPA to evaluate the statistical fit of various models.

Although statistical evaluation is critical, the use of scientific judgment remains essential when conducting dose–response modeling. An ideal data set should provide information on the shape of the dose–response curve, especially at the region close to the BMR. When this occurs BMD estimates from various models should provide similar results as long as these models provide a comparable data fit (i.e., BMD estimates are not model dependent). On the contrary, the BMD model is of limited utility if the dose spacing is such that there is no information on the shape of the dose–response curve, such as when there is 0% response in the control group, and very high (e.g., over 80%) response in the low-dose group. In some cases, however, applicable models might diverge with respect to BMD estimates. In such cases, it is necessary to analyze the data and determine whether there is a reason to prefer certain models, such as there is an underlying biological basis for choosing a dose–response shape in the region of the BMR or one of the models fits the data better in the 10% response region. For some data sets, 'plateauing' or nonmonotonicity of the response rates may occur in the high-dose region. If such plateauing drives the model fit, resulting in poor fit in the low-dose region, it may be appropriate to consider excluding the high dose(s) from the modeling. These examples are just a few of the many qualitative considerations needed to select an appropriate modeling result.

US EPA has developed a benchmark dose software package that provides various models for quantal, continuous, and nested data (developmental toxicity study results). The software can be downloaded from US EPA's website (see section Relevant Websites) free of charge, and it is frequently updated. This website also provides support documentation, including a software user's manual and a guidance document on the interpretation and use of BMD modeling.

See also: Risk Assessment, Human Health.

Further Reading

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Benomyl

Jamaluddin Shaikh

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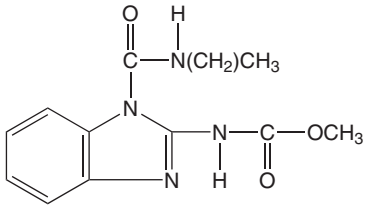
- Chemical Abstracts Service Registry Number: CAS 17804-35-2
- Synonyms: Agrocit; Fundazole; Benomyl 50W; Benlate; Benlate T; Methyl 1-(butylcarbamoyl)-2-benzimidazolylcarbamate
- Chemical/Pharmaceutical/Other Class: Benzimidazole fungicide
- Chemical Structure:



Uses

Benomyl is a protective and eradicant fungicide that is especially effective against a wide range of fungi affecting fruits, nuts, vegetables, turf, and field crops. The manufacturer (DuPont Crop Protection) ceased all manufacturing and sales of benomyl on December 31, 2001.

Exposure Routes and Pathways

Benomyl is formulated as a powder (Benlate and Benlate T). Dermal and oral routes are the most common exposure pathways.

Toxicokinetics

Benomyl is poorly absorbed as it is degraded in the gastrointestinal tract. Blood levels after oral administration are only one-tenth of those found after intraperitoneal injection in rats. Benomyl is hydroxylated and/or methylated, then conjugated, and promptly excreted in the urine. There is minimal or no tissue storage of benomyl or metabolites based on a 2 year feeding study in rats and dogs. In rats, the major metabolites of benomyl are converted to sulfate and/or glucuronide conjugates and excreted in urine (78%) and feces (8.7%). In mice, rabbits, and sheep, 44–71% of benomyl metabolites are found in urine and ~21–46% in feces.

Mechanism of Toxicity

Benomyl is a microtubule-disrupting agent in fungi. This agent may cause chromosomal aberrations (e.g., aneuploidy). There is very little evidence of benomyl toxicity in mammals, however. Benomyl itself does not have any direct effect on acetylcholinesterase. Under certain conditions, however, benomyl breaks down to produce carbendazim and butyl isocyanate, of which the isocyanate is an irreversible inhibitor of acetylcholinesterase with comparable potency to some active organophosphorus inhibitors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Benomyl has very low acute toxicity in laboratory animals. The oral LD_{50} value in rats is greater than $10\,g\,kg^{-1}$.

Human

Benomyl is a potential mild skin, eye, and respiratory tract irritant. Systemic poisoning is rare. Contact dermatitis has been reported in occupationally exposed workers. The skin lesions, which consisted of redness and edema, occurred on the back of hands, forearms, and in other places not covered by clothing. These

lesions generally clear within 3 weeks. Hyperpigmentation and photosensitization have also been reported.

Chronic Toxicity (or Exposure)

Animal

Rats given benomyl (150 mg kg^{-1} day^{-1} in the diet) for 2 years showed no signs of toxicity. Dogs receiving 150 mg kg^{-1} day^{-1} benomyl in their diet for 90 days showed no overt signs of toxicity but evidence of alterations in liver function. More severe liver changes and cirrhosis were noted after 2 years of dosing in dogs at this dosage. Liver tumors were noted in both male and female mice in long-term studies using from 40 to 400 mg kg^{-1} day^{-1} benomyl. In a 2 year study, however, rats given up to 2500 mg kg^{-1} day^{-1} of benomyl showed no significant signs of toxicity.

Human

Little is known regarding chronic effects of benomyl in humans.

In Vitro Toxicity Data

Benomyl induced aromatase activity in a human ovarian tumor cell line. Benomyl does not cause mutations or structural chromosomal aberrations in somatic or germ cells. Benomyl does not interact directly with DNA in mammalian or nonmammalian systems. Benomyl causes chromosomal aberrations (aneuploidy and/or polyploidy) both *in vitro* and *in vivo* due to disruption of microtubules. Benomyl is not clastogenic, however.

Clinical Management

Dermal decontamination should be accomplished by repeated washing with soap. Leather clothing can absorb benomyl; any contaminated leather clothing should therefore be discarded. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min. Emesis can be induced in cases of recent ingestion. In such cases, ipecac can be used to induce emesis. Emesis is not encouraged if the patient is comatose or convulsing. Activated charcoal slurry with or without saline cathartic and sorbitol may be used.

Environmental Fate

Benomyl binds strongly to soil and does not leach to any substantial degree. Its half-life after turf or soil application is 3–6 and 6–12 months, respectively. It did not accumulate from year to year with repeated applications, however. Benomyl completely degrades to carbendazim within several hours in nonalkaline water. The half-life of carbendazim is 2 months.

Ecotoxicology

Benomyl is moderately toxic to birds. The LC_{50} (5 day) in bobwhite quail and mallard ducks is $>10\,000$ ppm. The benomyl LD_{50} value in redwing blackbirds is 100 mg kg^{-1}. It is highly toxic to fish. The order of susceptibility to benomyl for various fish species is catfish < bluegill < rainbow trout < goldfish. A single application of benomyl reduces some soil dwelling organisms. Multiple applications at low concentration over a long time is very lethal to earthworm. The LC_{50} (7 day) in earthworms is 1.7 mg l^{-1} and the LC_{50} (14 day) is 0.4 mg l^{-1}. Benomyl is relatively nontoxic to bees.

Exposure Standards and Guidelines

The reference dose is 50 μg kg^{-1} day^{-1}, the acceptable daily intake is 20 μg kg^{-1} day^{-1}, and the permissible exposure limit is 5 mg m^{-3} (8 h).

See also: Chromosome Aberrations; Pesticides.

Further Reading

Mull RL and Hershberger LW (2001) Inhibitors of DNA biosynthesis-mitosis: Benimidizoles – the benzimidazole fungicides benomyl and carbendazim. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1673–1699. San Diego, CA: Academic Press.

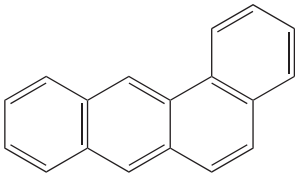
Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Benz[*a*]anthracene

Madhusudan G Soni

- Chemical Abstracts Service Registry Number: CAS 56-55-3
- Synonyms: 1,2-Benzanthracene; 2,3-Benzphenanthrene; 2,3-Benzophenanthrene; Tetraphene; Naphthanthracene; Benzanthrene; BA
- Chemical/Pharmaceutical/Other Class: Polycyclic aromatic hydrocarbon
- Chemical Structure:



Uses

There is no commercial production or known use of this compound.

Exposure Routes and Pathways

Human exposure to benz[*a*]anthracene occurs primarily through smoking of tobacco, inhalation of polluted air, and by ingestion of food and water contaminated by combustion effluents.

Toxicokinetics

Like benzo[*a*]pyrene, benz[*a*]anthracene may cross the gastrointestinal lining, pulmonary endothelium, or percutaneous barriers. Benz[*a*]anthracene is biotransformed to five dihydrodiols and a number of phenolic metabolites by P450 mixed-function oxidases. Detectable levels of benz[*a*]anthracene can be observed in most internal organs from minutes to hours after administration. Regardless of route of administration, once metabolized, hepatobiliary excretion and elimination through feces is the major route.

Mechanism of Toxicity

The arrangement of the aromatic rings in the benz[*a*] anthracene molecule gives it a "bay region" often correlated with carcinogenic properties. In general, the bay-region polycyclic aromatic hydrocarbons and some of their metabolites are known to react with cellular macromolecules, including DNA, which may account for both their toxicity and carcinogenicity. Benz[*a*]anthracene has been proposed to exert toxic effects through irreversible (covalent) binding of its electrophilic metabolites to nucleophilic sites within biological molecules. The species thought to be responsible for the genotoxic effects of benz[*a*]anthracene are diol epoxides. The genotoxic effects are due to the reactions of hard electrophiles derived from the diol epoxides with DNA. The ease of production of these hard electrophiles is related to the extent of delocalization of positive charge formed during the formation of benzyl carbonium ion intermediate.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal studies suggest that exposure to bay-region polycyclic aromatic hydrocarbons can damage the hematopoietic system leading to progressive anemia as well as agranulocytosis. Subcutaneous injection of 5 mg benz[*a*]anthracene daily to rats from the first day of pregnancy resulted in fetal death and resorption. Benz[*a*]anthracene has been shown to induce benzo[*a*]pyrene hydroxylase activity in the rat placenta.

Human

Direct evidence of acute toxicity resulting from oral exposure of humans to benz[*a*]anthracene was not found in the published literature.

Chonic Toxicity (or Exposure)

Animal

Benz[*a*]anthracene has been shown to be carcinogenic to experimental animals. Benz[*a*]anthracene given by several routes of administration has proven to be carcinogenic in mouse. Following repeated administration to young mice, it produced hepatomas and lung adenomas.

Human

No case reports or epidemiological studies on the significance of benz[*a*]anthracene exposure to humans are available. However, coal tar and other materials which are known to be carcinogenic in humans may contain benz[*a*]anthracene.

In Vitro Toxicity Data

Benz[*a*]anthracene was mutagenic to *Salmonella typhimurium* in the presence of an exogenous metabolic system. It was also mutagenic to mammalian cells *in vitro* in the presence of an exogenous metabolic system. In cultured mammalian cells, benz[*a*]anthracene induced unscheduled DNA synthesis and morphological transformation.

Exposure Standards and Guidelines

Benz[*a*]anthracene alone is not regulated; however, all polycyclic aromatic hydrocarbons or volatile coal tar products together are regulated. The World Health Organization has established $0.2\,\mu g\,l^{-1}$ as the limit for aromatic hydrocarbons in a domestic water supply. The US Occupational Safety and Health Administration limit in workplace air (coal tar volatiles) is $0.2\,mg\,m^{-3}$. The US Environmental Protection Agency weight-of-evidence classification for benz[*a*]anthracene is B2, a probable human carcinogen, for both oral and inhalation exposure based on adequate animal evidence and no human evidence.

Environmental Fate

As benz[*a*]anthracene is a universal product of combustion of organic matter, it is released into air and water and is associated with particulate matter. Biodegradation of benz[*a*]anthracene will occur very slowly with a half-life of approximately a year. Benz[*a*]anthracene will bioconcentrate in aquatic organisms. In the atmosphere, benz[*a*]anthracene is found both as the free vapor and adsorbed to particulate matter.

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

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Benzene

Stephen R Clough

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- Chemical Abstracts Service Registry Number: CAS 71-43-2
- Synonyms: Cyclohexatriene; Benzol; Coal naphtha; Benzole; Phenyl hydride
- Chemical/Pharmaceutical/Other Class: Aromatic hydrocarbon
- Chemical Formula: C_6H_6
- Chemical Structure:

Uses

The toxicological properties of benzene, particularly its ability to adversely affect blood forming elements in bone marrow and the subsequent classification of benzene as a human carcinogen, have substantially reduced its industrial use. Benzene, however, is still a minor component of some petroleum products such as gasoline and diesel fuel. In the past, benzene was used as a solvent for oils, resins, rubber, varnishes, lacquers, and waxes; as a chemical intermediate in the manufacture of pharmaceuticals, adhesives, and coatings; and as a solvent for dyes and inks. Its current use is primarily limited to the production of synthetic organic chemicals and plastics. Products in which benzene is used as a raw material include polystyrene plastics, polyester resins, synthetic rubber, phenol, nylon, aniline, detergents, and chlorobenzenes.

Exposure Routes and Pathways

Human exposure to benzene may occur as a result of exposure to petroleum products. Occupational exposures are now, however, very limited because the hazards to human health are known and measures are taken to protect against inhalation or skin exposures. Benzene is volatile at room temperatures so, for

the general population, pumping gasoline and the subsequent inhalation of gas fumes represent the primary source and route of exposure, respectively. A lesser number of people may be inadvertently exposed as a result of petroleum spills contaminating groundwater. Residents using private wells containing contaminated groundwater may be exposed orally by drinking the water or by inhalation after benzene volatilizes from groundwater into air during showering or migrates from soil gas into basement air. If exposure in the workplace did occur it would mainly occur via inhalation and dermal contact. Oral exposure can occur from accidental or intentional consumption of benzene-containing products.

Toxicokinetics

Benzene is lipid soluble and highly volatile at room temperature. As such, benzene readily crosses the alveolar membranes and is taken up by circulating blood in pulmonary vessels. The lung also serves as an excretion pathway for unmetabolized benzene, particularly following acute exposures. Benzene can also be readily absorbed from the gastrointestinal tract and from intact skin. Circulating benzene is preferentially taken up by lipid-rich tissues such as adipose and nervous tissue. Benzene has also been detected in the bone marrow, liver, kidneys, lungs, and spleen.

The human liver can metabolize benzene through a number of metabolic pathways. The major endproducts of benzene metabolism include phenol (hydroxybenzene), catechol (1,2-dihydroxybenzene), and quinol (1,4-dihydroxybenzene). These metabolic products are subsequently conjugated with inorganic sulfate and glucuronic acid in various degrees before being excreted in the urine. A small fraction of the catechol derived from benzene metabolism is oxidized to hydroxyhydroquinol or transformed to mucuronic acids.

Mechanism of Toxicity

Benzene can be irritating to mucus membranes. Dermal exposures defat the skin's keratin layer and can result in erythema, vesiculation, and dry, scaly dermatitis. Acute exposures to high concentrations can produce pulmonary irritation and edema, and gastrointestinal irritation (if consumed). Chronic exposure to benzene produces bone marrow depression. Experimental evidence indicates that benzene's bone marrow toxicity is mediated by one or more of its metabolites. For example, inhibition of benzene metabolism by administration of toluene or partial hepatectomy protects bone marrow against benzene damage, and benzene metabolites, such as 1,2-dihydroxybenzene (catechol), 1,4-dihydroxybenzene (quinol), and 1,2,4-trihydroxybenzene(hydroxyhydroquinol), have been shown to inhibit cell mitosis.

Acute and Short-Term Toxicity (or Exposure)

Animal

The literature on the toxicological properties of benzene in laboratory animals is extensive. Benzene can cause severe eye irritation and moderate skin irritation. When given orally, benzene is moderately toxic. The oral LD_{50} in rats and mice is 3400 and 4700 mg kg^{-1}, respectively. The median lethal dose through inhalation has been evaluated in rats, mice, dogs, and cats. In these laboratory species, the LC_{50} ranges from 31 887 in mice to 170 000 mg m^{-3} in cats.

Human

The literature on the toxicity of benzene in humans is extensive. The acute effects of benzene exposure generally differ markedly from the chronic effects. Acute exposure to high doses of benzene in air (at concentrations in excess of 3000 ppm) causes symptoms typical of organic solvent intoxication. Symptoms may progress from excitation, euphoria, headache, and vertigo, in mild cases, to central nervous system depression, confusion, seizures, coma, and death from respiratory failure in severe cases. The rate of recovery depends on the initial exposure time and concentration, but, following severe intoxication, the symptoms may persist for weeks.

Chronic Toxicity (or Exposure)

Animal

The effects of lifetime exposure to benzene have also been evaluated in laboratory animals. Chromosomal abnormalities in bone marrow cells have been reported to appear in rats, rabbits, mice, and amphibians as a consequence of experimental benzene exposure.

Human

The major toxicological manifestation of chronic benzene exposure in humans is bone marrow depression. Clinical manifestations include anemia, leucopenia, and thrombocytopenia. In severe cases, bone marrow aplasia develops. Later stages of toxicity are manifested by pancytopenia and aplastic anemia. Death may result from aplastic anemia or from leukemia. The US Environmental Protection Agency (EPA) and International Agency for Research on Cancer classify benzene as a known human carcinogen.

This classification was given to benzene in view of strong epidemiological and experimental evidence.

Clinical Management

The victim should be removed from the contaminated atmosphere. Contaminated clothing should be removed and the affected area should be washed with soap and water. Supportive treatment should be provided. In cases of ingestion, vomiting should not be induced. Benzene or organic solvents containing benzene can cause acute hemorrhagic pneumonitis if aspirated into the lungs. Activated charcoal can be given to minimize absorption from the gastrointestinal tract. Charcoal can be given in a slurry or mixed with sorbitol or a saline cathartic. The recommended doses of activated charcoal are 30–100 g for adults, 15–30 g for children, and 1 or 2 g kg^{-1} for infants. The indicated doses can be prepared in a slurry by mixing charcoal in a diluent at a rate of 10 g charcoal per 80 ml of diluent.

Environmental Fate

Benzene has a short half-life in surface water because it is so volatile. Detection of benzene in natural waters would therefore only be seen in areas adjacent to grossly contaminated waste sites. It would also tend not to bioaccumulate into fish tissue or biomagnify up the food chain.

Ecotoxicology

The US EPA ECOTOX database reports that *Ceriodaphnia* and *Daphnia* species are the most sensitive freshwater organisms following acute (48 h) exposure to benzene, with respective EC_{50} values of 130 and 400 ppb. Most organisms, however, can tolerate acute concentrations higher than this (in the 1–10 mg l^{-1} range). Following chronic exposures (4–7 day exposures), fish are relatively unaffected at concentrations up to 5 mg l^{-1} (at higher concentrations fish start to show adverse narcotic effects).

Exposure Standards and Guidelines

The odor threshold for benzene is 30 ppm, but the current American Conference of Governmental Industrial Hygienists Threshold Limit Value considered safe for occupational exposure (8 h day) is below that threshold at 0.5 ppm. The Occupational Safety and Health Administration permissible exposure limit (PEL) is 1 ppm, with a short-term exposure limit (STEL) of 5 ppm. The National Institute for Occupational Safety and Health recommends an exposure limit (recommended exposure limit) of 0.1 ppm with a STEL of 1 ppm.

See also: Blood; Carcinogenesis; Neurotoxicity; Pollution, Water; Respiratory Tract; Skin.

Further Reading

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Zhang L, Eastmond DA, and Smith MT (2002) The nature of chromosomal aberrations detected in humans exposed to benzene. *Critical Reviews in Toxicology* 32(1): 1–42.

Relevant Website

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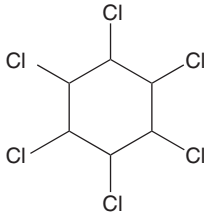
Benzene Hexachloride, Mixed Isomers

Madhusudan G Soni

- Chemical Abstracts Service Registry Number: CAS 608-73-1
- Preferred Name: BHC (technical-grade BHC is a mixture of eight isomers)
- Synonyms: HCCH; HCH; 1,2,3,4,5,6-Hexachlorocyclohexane; Hexachlor; Hexachloran; Benzahex; Benzex; Hexator; Kotol; Lindane
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbon. Technical-grade BHC consists of 65–70% α-BHC, 6–8% β-BHC, 12–15% γ-BHC, and ~10% of other isomers and compounds

- Chemical Structure: Isomers differ on the spatial positions of the chlorine atoms on the boat and chair forms



Uses

The only known use of benzene hexachloride (BHC) is as an insecticide.

Exposure Routes and Pathways

More than 90% of BHC intake in humans originates from food.

Toxicokinetics

BHC is absorbed through all portals including the intact skin. Different isomers of BHC are reported to be absorbed rapidly from the gastrointestinal tract and transferred exclusively to blood. Metabolism of BHC mainly takes place in the liver by four enzymatic reactions. Dehydrogenation, dechlorination, and hydroxylation are via P450 mixed-function oxidases, whereas dehydrochlorination is carried out by cytosolic enzymes. The end products of biotransformation are di-, tri-, tetra-, penta-, and hexachloro compounds. Within a few hours of uptake, BHC is distributed to all organs and tissues. The highest concentrations are found in adipose tissues and skin. In a long-term high-level BHC feeding study, it was shown that adipose tissue retains more α-isomer than β- and γ-isomers. BHC is excreted rapidly in urine and feces after metabolic degradation. The excreted metabolites are either free or conjugated forms of glucuronic or sulfuric acids of *N*-acetyl cysteine.

Mechanism of Toxicity

BHC produces a variety of neurological effects in insects and mammals. However, at both levels of the nervous system (peripheral and central), the mechanism of toxic action of BHC is poorly understood. Central nervous system stimulation appears to be due to blockade of the effects of γ-aminobutyric acid. *In vitro* BHC isomers are reported to increase the calcium uptake of isolated rat brain synaptosomes. In addition, γ-isomer of BHC has been shown to inhibit the uptake of chloride ions at inhibitory synapses in the brain, and it is this mode of action that is now widely considered to account primarily for the convulsant activity of this insecticide. The results of studies on initiation–promotion, on mode of action, and on mutagenicity indicate that tumorigenic effects of BHC in mice result from nongenetic mechanisms.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity of BHC has been investigated in numerous studies in a variety of species and strains via different routes. Signs of acute poisoning in rats include diarrhea, hypothermia, epistaxis, and convulsions; death is due to respiratory failure. Animal studies suggest a decreased ability to reproduce when fed moderate to high levels of BHC.

Human

Clinical signs of intoxication can appear from a few minutes to some hours after BHC ingestion. Ingestion of large (unspecified) doses of BHC has led to muscle and kidney necrosis and in one case to pancreatitis. The symptoms of poisoning include nausea, restlessness, headache, vomiting, tremor, ataxia, and tonic–clonic convulsion. Digestive tract inflammation, hemorrhage, coma, and death have also been reported after poisoning.

Chronic Toxicity (or Exposure)

Animal

Feeding of BHC (10–1600 mg kg^{-1} diet) for life span to rats resulted in decreased body weight and an increase in mortality at 800 mg kg^{-1} and above. Fatty degeneration and focal necrosis of the liver were observed at higher doses. Chronic nephritis with glomerular fibrosis and hyaline deposits was seen in rats fed 800 mg kg^{-1} diet BHC.

Human

Chronic liver damage (cirrhosis and chronic hepatitis) were observed in liver biopsies from eight workers heavily exposed to BHC, DDT, or both for periods ranging from 5 to 13 years. Several case reports indicate a relationship between exposure to BHC and the occurrence of aplastic anemia. It is not clear if BHC affects the ability of people to reproduce or if it causes birth defects in humans.

In Vitro Toxicity Data

γ-BHC did not induce unscheduled DNA synthesis in human cells *in vitro* and did not induce micronuclei or chromosomal aberrations in cultured rodent cells. It induced DNA strand breaks but not unscheduled DNA synthesis. β-BHC was not mutagenic to yeast, but the γ-BHC isomer induced gene conversion. Neither γ- nor β-BHC were mutagenic to bacteria, and did not cause DNA damage in bacteria.

Clinical Management

Gastric decontamination by lavage and saline cathartics should be carried out. Oil laxatives should not be used because they promote BHC absorption. Pentabarbital or phenobarbital in adequate amounts or calcium gluconate intravenously in conjunction with anticonvulsants may be used in the control of convulsions.

Exposure Standards and Guidelines

The exposure limit for γ-BHC in most countries is $0.5\,\mathrm{mg\,m^{-3}}$. The US Food and Agricultural Organization/World Health Organization acceptable daily intake of γ-BHC is $0.008\,\mathrm{mg\,kg^{-1}}$ body weight. The US Environmental Protection Agency has set a limit in drinking water of 0.2 ppb of BHC in water.

Environmental Fate

Residues of BHC on eight types of soils were found to be decreased by 40–80% per year. When sprayed on the surface, the half-life of BHC was 4–6 weeks with 90% gone in 30–40 weeks. The typical half-life for BHC was 400 days. BHC can be washed off and into the soil, especially when humus content is low. BHC is very stable in water. It will disappear from the water by adsorption on sediment, biological breakdown by microflora and fauna, and adsorption by fish through gills, skin, and food.

See also: Pesticides.

Further Reading

Agency for Toxic Substances and Disease Registry (ATSDR) (1999) *Toxicological Profile for Alpha-, Beta-, Gamma-, and Delta-Hexachlorocyclohexanes*. Update (Final Report), p. 313. Atlanta, GA: ATSDR, Public Health Service, US Department of Health and Human Services.

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Relevant Website

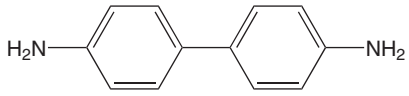
http://www.inchem.org – International Program on Chemical Safety, Hexachlorocyclohexane (Mixed Isomers).

Benzenedicarboxylic Acid, 1-2 *See* Phthalate Ester Plasticizers.

Benzidine

C Vaman Rao

- Chemical Abstracts Service Registry Number: CAS 92-87-5
- Synonyms: *p*-Diaminodiphenyl; 4,4′-Diaminobiphenyl; 4,4′-Diaminodiphenyl; [1,1′-Biphenyl]-4,4′-diamine
- Chemical/Pharmaceutical/Other Class: Benzidine is a diamine, manufactured synthetic aromatic hydrocarbon with two benzene rings covalently bonded to one another (1,1), substituted by amino group at 4,4′. It is a crystalline (sandy or sugar-like) solid that may be grayish-yellow, white, or reddish-gray in color. In the environment, benzidine is found in either its 'free' state (as an organic base) or as a salt (benzidine dihydrochloride or benzidine sulfate).
- Chemical Formula: $C_{12}H_{12}N_2$
- Chemical Structure:



Uses

Benzidine is used as an intermediate in the production of azo dyes, sulfur dyes, fast color salts, naphthol, and other dye compounds. However, it has not been

marketed or sold in the United States since the mid-1970s and US dye companies no longer manufacture benzidine-based dyes. However, a small amount of benzidine may still be manufactured or imported for scientific research in the United States, but in some countries it is still being manufactured. To date, more than 250 benzidine-based dyes have been reported. These dyes are primarily used for dyeing textiles, paper, and leather products.

Exposure Routes and Pathways

The primary routes of potential human exposure to benzidine are inhalation, ingestion, and dermal contact. The general population is not likely to be exposed to benzidine through contaminated air, water, soil, or food. People living near a hazardous waste site are likely to get exposed to benzidine through contaminated drinking water or by breathing contaminated air or by swallowing contaminated dust and soil. Benzidine can also enter the body by passing through the skin through contaminated clothing and gloves. People working at or near a hazardous waste site may get exposed to benzidine in a similar manner.

Toxicokinetics

Benzidine is rapidly absorbed through the skin in solid and vapor form. It is also quickly absorbed through the lungs on inhalation and from the gastrointestinal tract on consuming contaminated water and food. Generally, it will take only few hours for most of the benzidine to get into the body through the lungs and intestine. Breathing, eating, or drinking benzidine-based dyes may also expose a person to benzidine because the intestinal bacteria can break down these dyes into benzidine. It is a lipophilic substance, hence easily stored in fat tissues, and it firmly binds to cell receptors. Benzidine is metabolized to aromatic amine by intestinal microflora or liver azoreductase. The liver is the chief organ of metabolism where benzidine is converted to more reactive, toxic, and mutagenic (carcinogenic) *N*-hydroxyarylamides and *N*-hydroxylamine is considered to be a proximate carcinogen. *N*-Hydroxylamides are converted to the ultimate carcinogens through conjugation with sulfuric, acetic, or glucuronic acids. *N*-Acetoxyarylamines are also produced as metabolites and are highly reactive mutagens and carcinogens. Glutathione transferase plays an important role in the elimination of reactive metabolites of benzidine. Sulfonation, carboxylation, deamination, or substitution of an ethyl alcohol or an acetyl group for the hydrogen in the amino groups leads to a decrease in mutagenicity of benzidine metabolites as well as to easy elimination, primarily through urine and feces.

Mechanism of Toxicity

Benzidine is metabolized to highly toxic, reactive metabolites, such as *N*-hydroxyarylamides and *N*-hydroxyarylamines, which act as procarcinogens and are more mutagenic than parent compounds. The metabolites act as DNA adducts and bind to cell receptors. The metabolites on conjugation with sulfuric, acetic, and glucuronic acids form ultimate carcinogens. Acetylated benzidine metabolites such as *N*-acetoxyarylamines are known to cause bladder cancer in dye industry workers.

Chronic Toxicity (or Exposure)

Animal

There is sufficient evidence from animal studies that benzidine is a carcinogen. When administered in the diet, benzidine induced bladder cancer in dogs, multiple mammary carcinomas in rats and liver cell tumors in hamsters of both sexes. When administered by the subcutaneous route to mice of both sexes, it induced malignant tumors of the Zymbal gland (ear) and hepatocellular carcinoma; hepatomas, malignant tumors of the Zymbal gland, and local sarcomas in male rats; and malignant tumors of the Zymbal gland, mammary adenocarcinomas, and amyloid leukemia in female rats. When administered by intraperitoneal injection, benzidine induced Zymbal gland adenomas and carcinomas and malignant mammary tumors in female rats. The lethal dose in dogs is $400\,mg\,kg^{-1}$ by the subcutaneous route and $200\,mg\,kg^{-1}$ by the oral route. Dyes made from benzidine, such as Direct Blue 6, Direct Black 38, and Direct Brown 95 have been shown to cause cancer in animals. The Department of Health and Human Services (DHHS) has determined that Direct Black 38 and Direct Blue 6 cause cancer in animals, and the International Agency for Research on Cancer (IARC) has also determined that Direct Black 38, Direct Blue 6, and Direct Brown 95 cause cancer in animals.

Human

Benzidine can cause cancer in humans. This has been shown in studies of workers who were exposed for many years to levels much higher than the general population would be. An IARC study on dye industry workers reported that there is a direct correlation between the incidence of bladder cancer in the occupationally benzidine-exposed workers and the incidence of this cancer decreasing in workers after reduction in occupational exposure. Some evidences indicate that dyes made from benzidine, such as Direct Blue 6, Direct Black 38, and Direct Brown 95 may cause cancer in humans. Benzidine poisoning

causes vomiting, nausea, hemolysis, liver and kidney damage, and hematuria (bloody urine). Benzidine is considered to be acutely toxic to humans by ingestion, with an estimated oral lethal dose of between 50 and 500 mg kg^{-1} for a 70 kg person. Symptoms of acute ingestion exposure include cyanosis, headache, mental confusion, nausea, and vertigo. Dermal exposure may cause skin rashes and irritation.

Clinical Management

There is no antidote for benzidine poisoning. Since it produces reactive metabolites, administration of free radical scavengers would alleviate the toxicity. A complex of benzidine metabolites with copper and hydrochloride is known to decrease its mutagenic effects.

Environmental Fate

Industries release benzidine into the environment in the form of liquid waste and sludges. Benzidine may also be released into the environment due to spillage during transport. In air, benzidine is found bound to suspended particles or as a vapor, which may be brought back to the earth's surface by rain or gravity. Very small amount of benzidine dissolves in water at moderate environmental temperatures. When released into waterways, it sinks to the bottom and becomes part of the bottom sludge. In soil, most benzidine is strongly attached to soil particles, so it does not easily leach into underground water from the waste dumps.

Benzidine is slowly destroyed in the environment by light, certain other chemicals and microorganisms. Accumulation in the food chain has not been recorded so far but it is documented that water life may take up and store very small amounts of benzidine.

Further Reading

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Relevant Websites

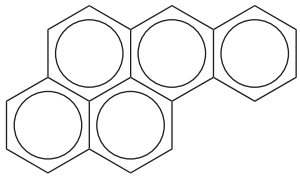
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Benzidene.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Benzidene.

Benzo(*a*)pyrene

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 50-32-8
- Synonyms: BAP; B(*a*)P; BP; 3,4-Benzopyrene; 6,7-Benzopyrene; 3,4-Benzpyrene; 3,4-Benz(*a*)-pyrene
- Chemical/Pharmaceutical/Other Class: Polycyclic aromatic hydrocarbon
- Chemical Formula: $C_{20}H_{12}$
- Chemical Structure:



Uses

In research, benzo(*a*)pyrene (BP) is used extensively as a positive control in a variety of laboratory mutagenicity and carcinogenicity short-term tests. It is not produced commercially in the United States.

Exposure Routes and Pathways

The primary routes of exposure to BP are inhalation and ingestion.

Toxicokinetics

Polycyclic aromatic hydrocarbons (PAHs) are absorbed following ingestion, inhalation, and dermal exposure. Following absorption, PAHs enter the lymph and then the bloodstream. BP is readily absorbed from the intestinal tract and tends to localize primarily in body fat and fatty tissues such as the breast. Disappearance of BP from blood and liver of

rats following a single intravenous injection is very rapid, having a half-life in blood of less than 5 min and a half-life in liver of 10 min. In blood and liver, the initial rapid elimination phase is followed by a slower disappearance phase, lasting 6 h or more. A rapid equilibrium is established between BP in blood and that in liver. The fast disappearance of the compound from blood is due to metabolism and distribution in tissues. BP is known to cross the placenta in mice and rats. ^{14}C metabolites were secreted into the bile of rats within 7 min of receiving an intravenous dose of ^{14}C BP. Pretreatment of animals with this carcinogen enhanced biliary secretion of ^{14}C radiolabel. PAHs are primarily metabolized enzymatically in the liver and kidneys. Additional sites of PAH metabolism include the adrenal glands, testes, thyroid, lungs, skin, and sebaceous glands. PAHs are metabolized by aryl hydrocarbon hydroxylase. The ultimate carcinogen of CYP450 metabolism of BP is 7,8-dihydro-7,8-diol-9,10-epoxide. The predominant metabolites of BP in mammals are 3- and 9-hydroxy BP, BP-1,6-quinone and BP-3,6-quinone, BP-4,5-dihydrodiol, BP-7,8-dihydrodiol, and BP-9,10-dihydrodiol. Human liver microsomal fractions were characterized for differences in the metabolism of BP. Pronounced interindividual differences in the composition of microsomal proteins in the molecular weight range of 49 000–60 000 were found. Large variation among human liver microsomal samples was also seen in BP metabolism. The results indicate the presence of seven or eight different forms of CYP450 in human liver microsomes and interindividual variations seen in metabolism may partly be explained by variations in the distribution of these isozymes.

Acute and Short-Term Toxicity (or Exposure)

Animal

Mild hepatotoxicity and nephrotoxicity have been observed in rats exposed to PAHs. Intraperitoneal administration of BP to rats produced an immediate and sustained reduction in growth rate of young rats. A single topical exposure of BP in acetone increased the mitotic rate of epidermal cells. Single oral administration of 100 mg BP to 50-day-old Sprague–Dawley rats produced mammary tumors. Single intraperitoneal administration of 10 mg BP produced two mammary and two uterine carcinomas among 10 Wistar rats within 1 year.

Human

In general, PAHs have a low order of acute toxicity in humans. BP may cause skin irritation with rash, redness, and/or a burning sensation. Exposure to sunlight and the chemical together can increase these effects. BP can irritate and/or burn the eyes on contact.

Chronic Toxicity (or Exposure)

Animal

In rats chronically fed PAHs, agranulocytosis, anemia, leukopenia, and pancytopenia have been observed. There is sufficient evidence suggesting that BP is carcinogenic to experimental animals. Exposure to BP caused a dose-dependent increase in the pulmonary tumor burden of mice administered B16F10 melanoma cells intravenously 1 day after the last of a 14 day exposure to BP. Biweekly administration of BP in oil by stomach tube produced papillomas of the stomach in hamsters. Biweekly painting with 0.3% solution of BP in benzene for 400 days produced one carcinoma and 10 papillomas among 10 rabbits. A possible causal relation between BP/diribonucleoside adduct formation and papilloma formation in Sencar mice was found. Among rats fed 1 mg BP per gram of diet during pregnancy many resorptions and dead fetuses were observed but only one malformed fetus was noted from seven litters. BP has been shown to be embryotoxic and teratogenic in mice. A reduction in fertility in male and female offspring was observed in mice following exposure *in utero*.

Human

Long-term health effects can occur at some time after exposure to BP and can last for months or years. BP is a probable carcinogen in humans. There is some evidence that it causes skin, lung, and bladder cancer in humans and animals. BP has caused cancer in the offspring of animals exposed to the substance during pregnancy. Many scientists believe that there is no safe level of exposure to a carcinogen. Cancer is the most significant toxicity associated with PAHs. The first occupational cancer described was that of scrotal cancer in chimney sweeps exposed to PAHs in soot and ash. Studies have noted increased lung cancer and a suggestion of increased gastrointestinal cancer incidence in the coal carbonization and coal gasification industries. BP has been observed to produce epithelial hyperplasia and inhibition of connective tissue growth on human fetal lung cultures. Since tobacco smoke contains BP, smoking may increase the risk of lung cancer with exposure to BP. BP on the skin in the presence of sunlight and/or ultraviolet light also increases the risk of skin cancer. Persistent nodules diagnosed as squamous epithelioma developed in a man who had been exposed to BP

for 3 weeks while carrying out an experiment on mice. BP may damage the developing fetus. There is some evidence that BP may affect the sperm and the testes. BP may be transferred to nursing infants through mother's milk. Repeated exposure to substances that contain BP can cause skin changes such as thickening, darkening, and pimples. Later skin changes include loss of color, reddish areas, thinning of the skin, and warts. Bronchitis may result from repeated exposure to BP-containing mixtures. Coke oven workers exposed to BP had significantly depressed levels of IgG and IgA compared to cold-rolling mill workers.

Clinical Management

Because of the low acute toxicity associated with PAHs, induced emesis is not recommended. Activated charcoal/cathartic may be used. On inhalation exposure, the patient should be moved to fresh air and monitored for respiratory distress. If cough or difficulty in breathing develop, evaluation for respiratory tract irritation, bronchitis, or pneumonitis should be performed. Humidified supplemental oxygen (100%) should be administered with assisted ventilation as required. On ocular exposure, the eyes should be irrigated for at least 15 min with tepid water. On dermal exposure, the affected area should be washed thoroughly with soap and water. Patients developing dermal hypersensitivity reactions may require treatment with systemic or topical corticosteroids or antihistamines. Treatment of gastric, lung, or skin cancer is no different from that for the same cell type.

Environmental Fate

Released BP is moderately persistent in the environment. It readily binds to soils and should not leach to groundwater, though it has been detected in some groundwater. If released into water, it will adsorb strongly to sediments and particulate matter. In most waters and sediments it will resist breakdown by microbes and reactive chemicals. BP is expected to bioconcentrate in aquatic organisms that cannot metabolize it, including plankton, oysters, and some fish.

Exposure Standards and Guidelines

A class I, type B biological safety hood should be used when working with BP in a laboratory. The following work practices are recommended: (1) Contaminated clothing should be removed immediately and laundered by individuals who have been informed of the hazards of exposure to BP. (2) Eye wash fountains should be provided for emergency use. Emergency shower facilities should be available if there is a possibility of skin exposure. On skin contact, affected skin should be washed immediately to remove the chemical. (3) Eating, smoking, or drinking should be prohibited where BP is handled. (4) Protective clothing (suits, gloves, footwear, and headgear) should be donned before work. Workers in industries that produce coal or coal tar products and those who tar road surfaces and roofs are at maximum risk.

Under Resource Conservation and Recovery Act (RCRA), BP must be managed as a hazardous waste according to federal and/or state regulations. The US Environmental Protection Agency (EPA) federal drinking water standard is $0.2\,\mathrm{mg\,l^{-1}}$. The National Institute for Occupational Safety and Health occupational exposure recommendations are $0.1\,\mathrm{mg\,m^{-3}}$ for cyclohexane extractable fraction and $0.1\,\mathrm{mg\,m^{-3}}$, 10 h time-weighted average for coal tar products.

The maximum contaminant level for BP has been set to 0.2 ppb by the EPA.

Miscellaneous

BP has a faint aromatic odor. It has a boiling point of greater than 360°C at 760 mmHg and a melting point of 179–179.3°C. It has a specific gravity of 1.351. Crystals of BP may be monoclinic or orthorhombic.

On contact with strong oxidizers, BP may cause fire or explosion. BP is light labile and is oxidized by chromic acid and by ozone.

BP is found in fossil fuels and occurs in products of incomplete combustion. It is present in charcoal, chimney sweepings, and coal tar.

See also: Immune System; Occupational Toxicology; Pollution, Water; Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

Miller AP and Ramos KS (2001) Impact of cellular metabolism on the biological effects of benzo(*a*)pyrene and related hydrocarbons. *Drug Metabolism Reviews* 33: 1–35.

Relevant Websites

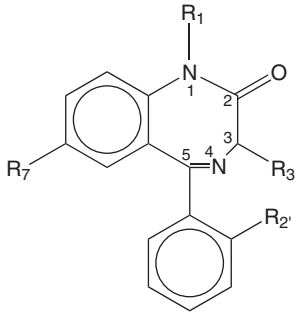
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Benzo(*a*)pyrene.
http://www.inchem.org – Benzo(*a*)pyrene (1983) IARC Summary and Evaluation, vol. 32.

Benzodiazepines

Christopher P Holstege

This article is a revision of the previous print edition article by Gregory P Wedin, volume 1, pp. 139–141, © 1998, Elsevier Inc.

- Representative Chemicals: Alprazolam; Chlordiazepoxide; Clonazepam; Clorazepate dipotassium; Clorazepate monopotassium; Diazepam; Estazolam; Flunitrazepam; Flurazepam; Halazepam; Lorazepam; Midazolam; Nitrazepam; Oxazepam; Prazepam; Quazepam; Temazepam; Triazolam
- Chemical Abstracts Service Registry Numbers: CAS 28981-97-7 (alprazolam); CAS 439-14-5 (diazepam); CAS: 604-75-1 (oxazepam)
- Synonyms:
  - Alprazolam – Xanax; $C_{17}H_{13}ClN_4$; 8-Chloro-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
  - Chlordiazepoxide – Librium; $C_{16}H_{14}ClN_3O$; 7-Chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine-4-oxide
  - Clonazepam – Klonopin, Rivotril, Clonapam; $C_{15}H_{10}ClN_3O_3$; 5-(2-Chlorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepin-2-one
  - Clorazepate dipotassium – Tranxene; ClorazeCaps; ClorazeTabs; $C_{16}H_{11}ClK_2N_2O_4$
  - Clorazepate monopotassium – Azene; $C_{16}H_{11}ClKN_2O_4$
  - Diazepam – Valium, Vivol, E-Pam; $C_{16}H_{13}ClN_2O$; 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one
  - Estazolam – ProSom; $C_{16}H_{11}ClN_4$; 8-Chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
  - Flunitrazepam – Rohypnol; $C_{16}H_{12}FN_3O_3$; 5-(2-Fluorophenyl)-1,3-dihydro-1-methyl-7-nitro-2*H*-1,4-benzodizepin-2-one
  - Flurazepam – Dalmane, Somnol, Som Pam; $C_{12}H_{23}ClFN_3O$; 7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepine-2-one
  - Halazepam – Paxipam; $C_{17}H_{12}ClF_3N_2O$; 7-Chloro-1,3-dihydro-5-phenyl-1-(2,2,2-trifluoroethyl)-2*H*-1,4-benzodiazepin-2-one
  - Lorazepam – Ativan; $C_{15}H_{10}ClN_2O_2$; 7-Chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one
  - Midazolam – Versed; $C_{18}H_{13}ClFN_3$; 8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidadazo[1,5-*a*][1,4]benzodiazepine
  - Nitrazepam – Mogadon; $C_{15}H_{11}N_3O_3$; 1,3-Dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one
  - Oxazepam – Serax; $C_{15}H_{11}ClN_2O_2$; 7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one
  - Prazepam – Verstran; $C_{19}H_{17}ClN_2O$; 7-Chloro-1-(cyclopropylmethyl)-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one
  - Quazepam – Doral; $C_{17}H_{11}ClF_4N_2S$; 7-Chloro-5-(2-fluorophenyl)-1,3-dihydro-1-(2,2,2-trifluoroethyl)-2*H*-1,4-benzodiazepine-2-thione
  - Temazepam – Restoril; 3-Hydroxydiazepam; $C_6H_{13}ClN_2O_2$; 7,-Chloro-l,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one
  - Triazolam – Halcion; $C_{17}H_{12}C_{l2}N_4$; 8-Chloro-6-(2-chlorophenyl)-1-methyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
- Chemical/Pharmaceutical/Other Class: 5-Aryl-1,4-benzodiazepines
- Chemical Structure:



Uses

The benzodiazepines are primarily administered for their sedative–hypnotic effects. Benzodiazepines are commonly used as anxiolytics, muscle relaxants, anticonvulsants, and to treat alcohol withdrawal, insomnia, and agitation. They are administered preoperatively for their anterograde amnesia effects and are combined frequently with other medications for conscious sedation before procedures. They are also utilized as drugs of abuse.

Exposure Routes and Pathways

The most common route of exposure to the benzodiazepines is ingestion of oral dosage forms. Several of these agents are also available for parenteral administration (intramuscular or intravenous). Diazepam may be administered through an

endotracheal tube; aerosolized diazepam is under investigation.

Toxicokinetics

The benzodiazepines are generally well absorbed from the gastrointestinal tract. The time to peak concentration of the benzodiazepines ranges from 0.5 to 6 h after ingestion. The benzodiazepines are all extensively metabolized by microsomal enzyme systems in the liver. The metabolites of many benzodiazepines are pharmacologically active and are biotransformed much more slowly than the parent compounds. The benzodiazepines that are not biotransformed to active metabolites include clonazepam, estazolam, lorazepam, nitrazepam, oxazepam, temazepam, and triazolam. The benzodiazepines and their active metabolites are widely distributed into body tissues and readily cross the blood–brain barrier and placenta. All are highly bound to plasma proteins. The elimination half-lives of the benzodiazepines range from 1 to 70 h at therapeutic doses. The half-lives of active metabolites, however, may be as long as 120 h. These metabolites are ultimately conjugated, largely with glucuronic acid, to inactive compounds that are excreted primarily in the urine.

Mechanism of Toxicity

Benzodiazepines exert their action by potentiating the activity of γ-aminobutyric acid (GABA). GABA is the major inhibitory neurotransmitter in the central nervous system (CNS). They bind to a specific receptor on the GABA receptor complex, which facilitates the binding of GABA to its specific receptor site. Benzodiazepine binding causes increased frequency of opening of the chloride channel. Chloride channel opening results in membrane hyperpolarization, thereby inhibiting cellular excitation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may be affected by the benzodiazepines much in the same way as humans. Lethargy, coma, shallow respirations, incoordination, and depressed reflexes may occur. Dogs may show a contradictory response (CNS excitement) following exposure. Standard supportive measures should be employed.

Human

There is a broad spectrum of signs and symptoms associated with acute benzodiazepine toxicity. Lethargy, ataxia, nystagmus, diplopia, amnesia, slurred speech, confusion, hypotonia, hypotension, hypothermia, coma, respiratory depression, and death have been reported. Rarely, paradoxical excitation may occur at lower doses. Toxic doses for each agent have not been clearly established. When large doses of lorazepam have been infused chronically, there are multiple reports of the development of a syndrome consisting of a hyperosmolar state with metabolic acidosis and cardiovascular compromise. This syndrome has been attributed to propylene glycol, the diluent in lorazepam.

Chronic Toxicity (or Exposure)

Animal

Chronic dosing in pregnant rats has resulted in increased rates of cleft palate formation as well as decrease in serum thyroxine levels. Prenatal exposure to benzodiazepines in rats describes learning and memory deficits in pups as well as absence of usual startle responses.

Human

Tolerance and physical dependence may develop in persons who chronically use benzodiazepines. Abrupt discontinuation of chronic benzodiazepine therapy may result in a withdrawal syndrome consisting of anxiety, agitation, insomnia, tremors, headache, and myalgias. In more severe cases nausea, vomiting, diaphoresis, hyperpyrexia, psychosis, seizures, and death may occur.

In Vitro Toxicity Data

In vitro studies examining the effects of diazepam in the immune system have shown mixed results. Several cultured cell models have demonstrated diazepam-induced inhibition of cell proliferation.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Gastrointestinal decontamination procedures should be used as appropriate based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used to adsorb the benzodiazepines. The patient's level of consciousness and vital signs should be monitored closely. Obtunded patients with reduced gag reflex should be intubated to prevent pulmonary aspiration. Respiratory support, including oxygen and ventilation, should be provided as needed. If hypotension occurs it should be treated with standard

measures including intravenous fluids, Trendelenburg positioning, and dopamine by intravenous infusion. Forced diuresis, hemoperfusion, and hemodialysis are of no value in benzodiazepine toxicity. If withdrawal signs and symptoms develop, treatment should focus on either benzodiazepine or phenobarbital therapy with a gradual dose reduction.

Flumazenil (Romazicon) is a benzodiazepine antagonist that can reverse the CNS depressant effects of these agents. It should be used with caution in acute intentional benzodiazepine overdoses. Because acute benzodiazepine overdoses generally result in only mild toxicity, it has limited clinical utility in this setting. Flumazenil's use in the acute benzodiazepine intoxicated patient may lead to an unnecessarily long observation period after fumazenil's infusion. This observation is necessary to be certain that reoccurrence of benzodiazepine toxic effects do not occur after flumazenil is metabolized. Flumazenil must be used with caution in mixed drug overdoses as seizures can develop, particularly if tricyclic antidepressants have been coingested. Also, it can induce potentially serious benzodiazepine withdrawal in dependent patients.

See also: Diazepam; Levothyroxine; Propylene Glycol.

Further Reading

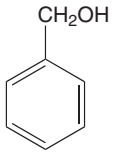
Finkle BS, McCloskey KL, and Goodman LS (1979) Diazepam and drug-associated deaths. *Journal of the American Medical Association* 242: 429–434.

Gaudreault P, Guay J, and Thivierge RL (1991) Benzodiazepine poisoning: Clinical and pharmacological considerations and treatment. *Drug Safety* 6: 247–265.

Benzyl Alcohol

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 100-51-6
- Synonyms: Benzene carbinol; Benzene methanol; Benzoyl alcohol; Phenyl carbinol; Phenyl methanol; Hydroxymethyl benzene; Hydroxy toluene
- Chemical/Pharmaceutical/Other Class: Alcohol
- Chemical Formula: $C_6H_5CH_2OH$
- Chemical Structure:



Uses

Benzyl alcohol is primarily used as a solvent and an antimicrobial preservative, but it has also found use as an antiseptic and local anesthetic. It is also used as a raw material of various esters, used in the soap, perfume, and flavor industries. Acceptable daily intakes were established at $5\,mg\,kg^{-1}$ for benzyl alcohol by the World Health Organization. In 1998, benzyl alcohol was reported by the US Food and Drug Administration as being used in 322 cosmetic formulations, belonging to 43 cosmetic categories.

Toxicokinetics

Body tissue possibly takes up benzyl alcohol rapidly and releases it slowly into the bloodstream. Rabbits when given 1 g (subcutaneously) of benzyl alcohol eliminated 300–400 mg of hippuric acid within 24 h. Rabbits eliminated 65.7% of a dose of 0.4 g of benzyl alcohol as hippuric acid in the urine. The plasma half-life of benzyl alcohol administered as a 2.5% solution in saline was found to be ~1.5 h in dogs injected intravenously at doses of 52 and $105\,mg\,kg^{-1}$.

Benzyl alcohol is oxidized by the liver alcohol dehydrogenase. Humans readily oxidize benzyl alcohol to benzoic acid, which, after conjugating with glycine, is rapidly eliminated as hippuric acid in the urine. Within 6 h after taking 1.5 g of benzyl alcohol orally, human subjects eliminated 75–85% of the dose in urine as hippuric acid. Benzyl alcohol yields benzaldehyde in rabbits and phenol in guinea pigs. If the dose is sufficiently high to allow the rate of formation of benzoic acid to exceed that of hippuric acid some of the benzoic acid is excreted as benzoylglucuronide.

Mechanism of Toxicity

Benzyl alcohol is oxidized by the liver to benzoic acid, and then conjugated with glycine to form hippuric acid. Metabolic acidosis can be explained by a direct effect of benzoic acid and/or secondary lactic acid production through depression of cellular metabolism. Benzyl alcohol is a weak local anesthetic with disinfectant properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

When injected into chickens, benzyl alcohol produced birth defects of the central nervous system (CNS) and skeleton. Doses of 0.2 ml kg^{-1} or more to dogs by stomach tube induced emesis and defecation. This was apparently due to irritation of the gastric mucosa. Diuresis was more pronounced in the rabbit than in the dog after administration of benzyl alcohol by various routes. Mice suffered respiratory stimulation, respiratory and muscular paralysis, convulsions, and CNS depression following a subcutaneous injection. A decrease in arterial blood pressure of rabbits, cats, and dogs was seen following intravenous injection of benzyl alcohol. No such decrease in arterial blood pressure was noted following oral administration to dogs. Benzyl alcohol displayed antiarrhythmic, antifibrillatory effects when injected intravenously into dogs and rats with spontaneous and drug-induced arrhythmias. Instillation of pure benzyl alcohol into rabbit conjunctival sac produces corneal necrosis, which is resolved after several weeks. The undiluted material when applied to depilated skin of guinea pigs for a period of 24 h caused moderately strong primary irritation, and there was evidence of systemic symptoms with death from applications of less than 5 ml kg^{-1}.

Because its primary effects are expected to be irritation and perhaps mild CNS depression, benzyl alcohol is in class I for general toxicity (may cause reversible effects which are generally not life-threatening). From the one study in chickens, it is in class B for reproductive hazard (few effects in animals but no human data). Some differences between control and benzyl alcohol-treated populations were noted in one reproductive toxicity study using mice, but these were limited to lower maternal body weights and decreased mean litter weights. Another study also noted that fetal weight was decreased compared to controls, but a third study showed no differences between control and benzyl alcohol-treated groups. The actual human reproductive hazard is unknown.

Human

High doses of benzyl alcohol cause nausea, vomiting, diarrhea, CNS, depression, and vertigo. Dilute solutions (1%) produce local anesthesia and slight irritation when instilled into the eye. Pure benzyl alcohol produces corneal necrosis. Following acute exposure lethargy, seizures, intraventricular hemorrhage, and neurological sequelae (cerebral palsy, developmental delay) have been seen in neonates with parenteral benzyl alcohol toxicity. Metabolic acidosis was a common finding with parenteral toxicity in neonates. Thrombocytopenia was a delayed feature of parenteral toxicity in neonates. Deaths associated with intravenous or endotracheal administration of benzyl alcohol-containing solutions in neonates were preceded by symptoms of respiratory distress progressing to gasping respirations, metabolic acidosis, CNS depression, hypotension, renal failure, and occasionally seizures and intracranial hemorrhage. Thrombocytopenia was a delayed feature of parenteral toxicity in neonates. Severe striated keratopathy, progressing to chronic edema of cornea, was noted following intraocular use of a sodium chloride solution containing 2% benzyl alcohol.

Chronic Toxicity (or Exposure)

Animal

There was no evidence of carcinogenic activity of benzyl alcohol for male or female F344/N rats dosed with 200 or 400 mg kg^{-1}. There was no evidence of carcinogenic activity of benzyl alcohol for male or female B6C3F1 mice dosed with 100 or 200 mg kg^{-1} for 2 years.

Human

Chronic exposure to benzyl alcohol would presumably produce effects similar to those from acute exposure. No other industrial illness is known from benzyl alcohol. No reproductive effects on humans are known.

In Vitro Toxicity Data

Benzyl alcohol was not mutagenic when tested by the preincubation protocol in the presence or absence of exogenous metabolic activation in the *Salmonella* assay. A significant increase in chromosomal aberrations was observed after exposure to benzyl alcohol in the presence, but not absence of S9.

Clinical Management

Treatment is supportive following exposure. The victim should be monitored for CNS and respiratory depression, metabolic acidosis, and hypotension. Ipecac-induced emesis is not recommended. On ocular exposure, the eyes should be irrigated for at least 15 min with tepid water. On dermal exposure, the exposed area should be washed with soap and water. If irritation, pain, swelling, lacrimation, or

photophobia persists, the victim should be seen in a health care facility.

Environmental Fate

When released into the soil, benzyl alcohol is expected to leach into groundwater. When released into the soil, this material may evaporate to a moderate extent and biodegrade to a moderate extent. When released into the water, benzyl alcohol is not expected to evaporate significantly and may evaporate to a moderate extent. It has an estimated bioconcentration factor of less than 100 and is not expected to significantly bioaccumulate. When released into air, benzyl alcohol is expected to have a half-life between 1 and 10 days and may be removed from the atmosphere to a moderate extent by wet deposition.

Exposure Standards and Guidelines

The odor threshold for benzyl alcohol is 5.5 ppm.

Miscellaneous

Benzyl alcohol is a water-white liquid with a faint aromatic odor and a sharp burning taste. It has a molecular weight of 108.13 and a specific gravity of 1.045. Aqueous solution of benzyl alcohol is neutral. Benzyl alcohol decomposes to benzaldehyde slowly when exposed to air. For this reason, storage tanks should be blanketed with nitrogen. Benzyl alcohol is incompatible with oxidizing agents. Problems may occur when polystyrene syringes are used with certain types of drug products containing benzyl alcohol since these agents can extract and dissolve the plastic. At times the rubber tip may release a constituent to the drug product.

See also: Fragrances and Perfumes; Respiratory Tract.

Further Reading

CIR (2001) Cosmetic Ingredient Review Expert Panel, final report on the safety assessment of benzyl alcohol, benzoic acid and sodium benzoate. *International Journal of Toxicology* 20(Suppl. 3): 23–50.

Nair B (2001) Final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate. *International Journal of Toxicology* 20(Suppl. 3): 23–50.

NTP (1989) Toxicology and Carcinogenesis Studies of Benzyl Alcohol (CAS No. 100-51-6) in F344/N Rats and B6C3F1 Mice (Gavage Studies). National Toxicology Program Technical Report Series 343, pp. 1–158.

Relevant Website

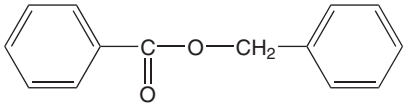
http://www.intox.org – Chem Info. Chemical Profiles Created by CCOHS.

Benzyl Benzoate

Jamaluddin Shaikh

This article is a revision of the previous print edition article by Sushmita M Chanda, volume 1, pp. 143–144, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 120-51-4
- Synonyms: Ascabin; Ascabiol; Ascarbin; Benzylate; Scabanca; Tenutex; Vanzoate; Venzoate; Benzoic acid phenylmethyl ester; Benzy alcohol benzoic ester
- Chemical/Pharmaceutical/Other Class: Benzoic acid phenylmethyl ester
- Chemical Structure:



Uses

Benzyl benzoate is used as an acaricide, scabicide, and pediculicide in veterinary hospitals and as a repellent for chiggers, ticks, and mosquitoes.

Exposure Routes and Pathways

Dermal exposure is the most common route of exposure. Benzyl benzoate occurs naturally in balsams of Peru and Tolu and other essential oils. It is also available in liquid, emulsion, and lotion dosage forms.

Toxicokinetics

Benzyl benzoate is rapidly absorbed from the stomach. It is rapidly hydrolyzed to benzoic acid and benzyl alcohol, which is subsequently hydrolyzed to benzoic acid. Benzoic acid is conjugated with glycine to give benzoylglycine or hippuric acid and with glucuronic acid to give benzoylglucuronic acid. The

conjugates are rapidly eliminated in urine in varying ratios depending on species and dose.

Mechanism of Toxicity

Benzyl benzoate is a local irritant.

Acute and Short-Term Toxicity (or Exposure)

Animal

Benzyl benzoate has low acute toxicity in laboratory animals. The oral LD_{50} value for rats is greater than $1\,g\,kg^{-1}$. If applied too frequently or to a large area, it can induce systemic signs of toxicity including salivation, piloerection, muscular incoordination, tremors, progressive paralysis of hindlimbs, prostration, violent convulsions, dyspnea, and death. Cats are especially susceptible to such toxicity. In contrast, dogs are highly resistant to acute benzyl benzoate toxicity. When given in large doses to laboratory animals, benzyl benzoate can cause hyperexcitation, incoordination, ataxia, convulsions, and respiratory paralysis.

Human

Benzyl benzoate is a slightly toxic compound when used topically. It may cause slight allergenic responses, which may disappear after the end of exposure. If used as an acaricide, it may cause peristalsis of the intestine, diarrhea, intestinal colic, enterospasm, pylorospasm, spastic constipation, contraction of the seminal vesicles, hypertension, and bronchospasms.

Chronic Toxicity (or Exposure)

Human

Relatively little is known about the chronic effects of benzyl benzoate. Contact dermatitis may occur with repeated use (blistering, crusting, oozing, reddening, or scaling of skin).

In Vitro Toxicity Data

Benzyl benzoate was negative in an *in vitro* screen for estrogenic stimulation.

Clinical Management

Basic life-support measures for respiratory and cardiovascular functions should be utilized. Dermal decontamination should be accomplished by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min.

Ecotoxicology

Benzyl benzoate is toxic to some aquatic organisms. The LC_{50} (4 day) was $4.5\,mg\,l^{-1}$ in fish.

See also: Pesticides.

Relevant Website

http://www.inchem.org – International Programme on Chemical Safety.

Beryllium

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst, Shirley B Radding, and Kathyrn A Wurzel, volume 1, pp. 144–145, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-41-7
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Be^{2+}

Uses

Beryllium is an important industrial metal because of its material properties, that is, it is lighter than aluminum and six times stronger than steel. Often alloyed with other metals such as copper, beryllium is a key component of materials used in the aerospace and electronics industries. Beryllium has a small neutron cross-section, which makes it useful in the production of nuclear weapons and in sealed neutron sources. Specifically, beryllium is used in nuclear reactors as a neutron reflector or moderator, and in the aerospace industry in inertial guidance systems; beryllium alloys (consisting of copper or aluminum) are also used in structural material. Beryllium oxide is used as an additive in glass, ceramics, and plastics and as a catalyst in organic reactions. In the past, beryllium was widely used in the manufacture of fluorescent lights and neon signs. Alloyed with copper, aluminum, or nickel, beryllium imparts excellent electrical and thermal conductivity.

Background Information

Beryllium was discovered as an element in 1798. Its use in metallurgy and electrical components were largely developed in the 1920s.

Exposure Routes and Pathways

The primary exposure pathway for beryllium is inhalation. Inhalation, ingestion, and dermal contact are possible exposure pathways in workplace settings.

Exposure to small amounts of beryllium occurs with ingestion of some foods and drinking water. Beryllium enters the air, water, and soil as a result of natural and human activities. Emissions from burning coal and oil increase beryllium levels in air. Beryllium enters waterways from the wearing away of rocks and soil. Most of the man-made beryllium that enters waterways comes when industry dumps waste water and when beryllium dust in the air from industrial activities settles over water. Beryllium, as a chemical component, occurs naturally in soil; however, disposal of coal ash, incinerator ash, and industrial wastes may increase the concentration of beryllium in soil. In air, beryllium compounds are present mostly as fine dust particles. The dust eventually settles over land and water.

Toxicokinetics

Beryllium is not well absorbed by any route; oral absorption of beryllium is less than 0.01% and probably only occurs in the acidic stomach environment. About half of inhaled beryllium is cleared in $\sim$2 weeks; the remainder is cleared slowly and the residual becomes fixed in the lung (granulomata). The half-life of beryllium in rat blood is $\sim$3 h. Beryllium is distributed to all tissues. High doses generally go to the liver and then are gradually transferred to the bone. Most beryllium concentrates in the skeleton. Beryllium is excreted in the urine; however, the fraction of administered dose excreted in urine is variable.

Mechanism of Toxicity

Beryllium compromises the immune system. Enzymes catalyzed by magnesium or calcium can be inhibited by beryllium; succinic dehydrogenase is activated. Beryllium exposure leads to a deficiency in lung carbon monoxide diffusing capacity. Hypercalcemia (excess of calcium in the blood) can occur.

Acute and Short-Term Toxicity (or Exposure)

Animal

The pulmonary effects of inhaled beryllium have been evaluated in a variety of laboratory animal species. Monkeys, for example, exposed to relatively high concentrations of beryllium compounds developed symptoms and histopathological findings consistent with acute beryllium disease.

Human

The major toxicological effects of beryllium are on the lung. Acute exposure to soluble beryllium compounds (e.g., fluoride, an intermediate in the ore extraction process) irritates the entire respiratory tract, may produce acute chemical pneumonitis, and can result in fatal pulmonary edema. Hypersensitivity, which appears to be mediated by the immune system, may also occur following exposure. This means that future exposure to beryllium may produce health effects at concentrations lower than those generally associated with the effect (the individual becomes much more sensitive to beryllium).

The acute disease in humans is also marked by conjunctivitis, nasopharyngitis, tracheobronchitis, and dermatitis.

Chronic Toxicity (or Exposure)

Animal

Although beryllium produces cancer in more than one animal species (lung cancer in rats and monkeys; osteogenic sarcoma in rabbits), it does not appear to be teratogenic.

Human

Chronic exposure to insoluble beryllium compounds, particularly the oxide, leads to berylliosis (a chronic granulomatous disease), which begins with a cough and chest pains. In most cases, these symptoms soon lead to pulmonary dysfunction. The latency period ranges from months to 25 years. Diagnosis based on clinical, radiographic, and lung function evidence has been found to be difficult.

Other effects of beryllium exposure include enlargement of the heart (which can lead to congestive heart failure), enlargement of the liver, and kidney stones. Finger 'clubbing' is often seen with berylliosis.

Skin lesions are the most common industrial exposure symptom. Three distinct skin lesions have been noted following exposure to beryllium: dermatitis, ulceration, and granulomas. There appears to be an immunological component to chronic beryllium disease, including the dermal responses.

Although available information from epidemiological studies is insufficient to confirm human carcinogenesis, the data strongly suggest beryllium is associated with cancer in humans, and it is categorized

as a B1 (probable human carcinogen) by the US Environmental Protection Agency.

In Vitro Toxicity Data

In vitro studies indicate beryllium will induce morphological transformations in mammalian cells, but beryllium is not mutagenic in bacterial systems.

Clinical Management

Treatment of the acute disease includes bed rest, oxygen therapy, mechanical ventilation when needed, and corticosteroids. Chelation has been used to treat beryllium toxicity; however, no one agent is recommended over another. Aurin tricarboxylic acid has been used to protect primates from beryllium overdose, but human trials have not been conducted.

Ecotoxicology

Fish do not accumulate beryllium from water into their bodies to any great extent. A major portion of beryllium in soil does not dissolve in water but remains bound to soil, so it is not very likely to move deeper into the ground and enter groundwater. In the environment, chemical reactions can change the water-soluble beryllium compounds into insoluble forms. In some cases, water-insoluble beryllium compounds can change to soluble forms. Exposure to water-soluble beryllium compounds in the environment, in general, will pose a greater threat to human health than water-insoluble forms.

No evidence was found to substantiate that biomethylation or any other environmental process results in the volatilization of beryllium into the atmosphere from water or soil.

Beryllium is extremely toxic to warm water fish in soft water. The degree of toxicity decreases with increasing water hardness. Bioconcentration of beryllium in fish to high levels is not likely due to the low uptake of beryllium from water by aquatic animals. A measured bioconcentration factor (BCF) of 19 was reported for beryllium in bluegill fish. Other investigators have reported a BCF of 100 for freshwater and marine plants, invertebrates, and fish. Chemicals with BCFs <1000 will not bioaccumulate significantly in aquatic organisms. It is possible that bottom-feeding crustaceans, such as clams and oysters, could accumulate beryllium from sediment and show higher bioconcentration than freshwater fish. No evidence for significant biomagnification of beryllium within food chains was found.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, 8 h time-weighted average is $0.002\,\mathrm{mg\,m^{-3}}$ for beryllium and beryllium compounds and ACGIH classifies beryllium as a suspected human carcinogen.

See also: Metals; Respiratory Tract.

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Beta Blockers

Michael Wahl

- Representative Chemicals: Acebutolol; Atenolol; Betaxolol; Bisoprolol; Carteolol; Esmolol; Labetalol; Metoprolol; Nadolol; Penbutolol; Pindolol; Propranolol; Sotalol; Timolol
- Synonyms: Sectral (CAS 37517-30-9); Tenormin (CAS 29122-68-7); Kerlone (CAS 63659-19-8); Zebeta (CAS 66722-44-9); Cartrol (CAS 51781-21-6); Brevibloc (CAS 81161-17-3); Normodyne; Trandate (CAS 32780-64-6); Lopressor (CAS 37350-58-6); Corgard (CAS 42200-33-9); Levatol (CAS 38363-32-5); Visken (CAS 13523-86-9); Inderal (CAS 318-98-9); Betapace (CAS 959-24-0); Blocadren (CAS 26921-17-5)
- Chemical/Pharmaceutical/Other Class: Beta adrenergic blockers

Uses

Beta blockers are used in the treatment of hypertension, angina pectoris, supraventricular arrhythmias, supraventricular tachycardia, sinus tachycardia, ventricular tachycardia, myocardial infarction, pheochromocytoma, migraine headache, and essential tumor.

Exposure Routes and Pathways

Ingestion is the most common route for both accidental and intentional exposures to the beta blockers. Esmolol, labetalol, metoprolol, and propranolol are all available for parenteral administration; therefore, toxicity can occur via this route. Beta blockers are also administered as ocular medications and systemic toxicity can occur following administration by this route.

Toxicokinetics

The extent of absorption varies widely from 30% (nadolol) to 100% (labetalol, betaxolol). The rate of absorption is rapid for nonsustained release preparations. Sustained release preparations are more slowly absorbed and can have delayed and prolonged clinical effects following poisoning/overdose. The degree of protein binding has a wide range from 0% (sotalol) to 98% (penbutolol). Most of the beta blockers have significant hepatic metabolism (e.g., at least 50%). Atenolol, nadolol, and sotalol are principally excreted unchanged in the urine. Absolute bioavailability is often limited by significant first-pass metabolism. Esmolol is metabolized by esterases in the cytosol of red blood cells. Both renal and fecal eliminations occur. Elimination half-life ranges from 0.15 h (esmolol) to 24 h (nadolol).

Mechanism of Toxicity

The toxicities of the beta blockers are directly related to their pharmacologic effects. These agents block the effects of catecholamines such as epinephrine and norepinephrine on the beta-1 and beta-2 receptors. Beta-1 receptors are located in the heart, kidneys, and eyes. Toxicity is most often due to antagonism of the cardiac beta-1 receptors.

Acute and Short-Term Toxicity (or Exposure)

Human

Cardiac beta-1 stimulation results in increases in sinoatrial rate, myocardial contractility, and increased atrial, atrioventricular node, and ventricular conduction velocity. Beta blockers decrease heart rate, contractility, and conduction. Beta-2 receptors are found in the bronchioles, vasculature, intestines, uterus, pancreas, adipose tissue, and the liver. Stimulation of bronchial and vascular beta-2 receptors causes smooth muscle relaxation with resultant bronchial dilation and vasodilation. Blocking beta-2 receptors can cause contraction of bronchial smooth muscle and result in emergence of worsening asthma in asthmatic patients.

Chronic Toxicity (or Exposure)

Animal

No evidence of carcinogenicity has been documented in rats at doses of atenolol up to 300 mg kg^{-1} day^{-1}. Doses of up to 200 mg kg^{-1} day^{-1} have not shown decreased fertility in rats. However, dose related fetal resorptions were noted at doses of greater than 50 mg kg^{-1} day^{-1}. Chronic propranolol dosing at 100 mg kg^{-1} to newborn rats resulted in decreased weight gain and growth. The effects were reversible once the propranolol was discontinued.

Human

The primary clinical effects observed in beta blocker toxicity are cardiovascular in nature. Direct cardiac effects include bradycardia (sinus, atrioventricular node, and ventricular), all degrees of atrioventricular block, bundle branch blocks, and asystole. Ventricular arrhythmias may occur secondary to bradycardia. Torsades de pointes has been associated with chronic toxicity from sotalol. Hypotension occurs and is due to decreased cardiac output and/or vasodilation. Central nervous system effects of these drugs including lethargy, coma, and seizures are secondary to the cardiovascular toxicities. Seizures and coma may be secondary to hypoglycemia. Bronchospasm can occur secondary to beta-2 blockade. Hypoglycemia and hyperkalemia can occur.

In Vitro Toxicity Data

Ames testing of propranolol by different laboratories has demonstrated equivocal results.

Clinical Management

Advanced life-support measures should be instituted as deemed appropriate. A baseline 12-lead electrocardiogram should be obtained. Continuous cardiac and blood pressure monitoring should be initiated. Gastric decontamination procedures should be initiated based on the history of the ingestion and the patient's neurologic status. Consider charcoal, up to 1 gm kg^{-1} for recent ingestions. Whole bowel irrigation may be useful following ingestions of sustained release preparations. Bradyarrhythmias and conduction

disturbances should be managed with atropine and a pacemaker. Isoproterenol can be effective in increasing heart rate and contractility, but should be used with caution due to its arrhythmogenic and vasodilatory potential. Ventricular arrhythmias should be managed with class IB antiarrhythmics (e.g., lidocaine) and overdrive pacing. Class IA and IC antiarrhythmics should be avoided due to their potential to interfere with conduction. Hypotension should be managed initially with normal saline solution. If decreased cardiac output is responsible for hypotension, dobutamine, amrinone, or isoproterenol can be used. Glucagon has been effective in increasing myocardial contractility in beta blocker toxicity. Glucagon stimulates production of cyclic adenosine monophosphate, which enhances contraction. Initial intravenous doses of 50–100 $\mu g\,kg^{-1}$ have been used. These are followed by infusions of 70 $\mu g\,kg^{-1}\,h^{-1}$. If cardiogenic shock is resistant to traditional measures, use of insulin and glucose (to maintain euglycemia) has been successful in small numbers of patients as well as in animal models of beta blocker toxicity. For patients who fail all other therapies, an intra-aortic balloon pump and cardiopulmonary bypass should be considered. If systemic vascular resistance is low, vasopressors such as dopamine and norepinephrine should be administered. Hemodialysis or hemoperfusion may be effective in removing acebutolol, atenolol, nadolol, and sotalol.

See also: ACE Inhibitors; Calcium Channel Blockers; Cardiovascular System.

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Bhopal

Pallavi B Limaye and Harihara M Mehendale

Background Information

Bhopal, the capital city of the state of Madhya Pradesh in central India is the site of possibly the greatest industrial disaster in history till date. On the night of December 2, 1984, a disastrous accident at Union Carbide plant led to a massive leakage of methyl isocyanate (MIC) gas and other related by-products. MIC was used in production of carbaryl, an insecticide. The available evidence suggests that inadvertent seepage of water into the storage tank containing over 40 metric tons of MIC led to a violent exothermic reaction resulting in emission of MIC and a number of other toxic decomposition by-products that could not be contained by safety valves. The exact nature and the constituents of the gas mixture are not known. This Union Carbide plant was shut down after the accident.

Estimated Total Release of MIC and Estimated Individual Exposure

It has been estimated that ~27 tons of MIC escaped from the plant in a period of 1–2 h. Due to lack of planning, air monitoring for MIC was not possible, nor was it subsequently attempted. The Central Water and Air Pollution Control Board, India estimated MIC concentration to be ~27 ppm, which is ~1400 times that of the US Occupational Safety and Health Administration workplace standard of 0.02 ppm calculated over an 8 h work day. The established limit for immediate danger to life or health for MIC is 3 ppm. The American Industrial Hygiene Association's Emergency Response Planning Guideline, level 2 (ERPG-2) limit, defined as maximum airborne concentration below which it is believed that nearly all persons could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair their abilities to take protective action is determined to be 0.5 ppm. This indicates that the MIC concentration released after the accident was ~50 times that of the accepted limit.

Mechanism of Toxicity

Before the accident not much was known about MIC toxicity. Even today, mechanisms of MIC-induced toxicity are not clearly understood. It is known that MIC is a corrosive agent for the eyes, respiratory tract, and skin. Acute exposure to high vapor concentrations may cause severe pulmonary edema and injury to the alveolar walls of the lung, severe

corneal damage, and death. MIC may cross the placenta and enter a developing fetus.

Acute and Short-Term Toxicity (or Exposure)

Animal

The Indian Council of Agricultural Research's report indicates that a large number of cattle (~4000), as well as dogs, cats, and birds were killed due to exposure to the toxic gases released from the Bhopal plant.

Human

The gas leak had devastating effects on the exposed population. Over 200 000 residents (that comprised about one-fourth of the total population of city of Bhopal) were exposed to MIC and other related toxic gases released from the plant. Most of these residents were from the poor class and were living in the immediate surroundings of the Union carbide plant. The human mortality is estimated to be between 2500 and 5000 from this accident. Respiratory failure due to MIC inhalation was the principal cause of death. MIC caused bronchial necrosis and pulmonary edema. Within the first 24 h after the accident, ~90 000 patients were admitted in local hospitals and clinics with multiple symptoms of respiratory distress, breathlessness, choking, cough, chest pain, and hemoptysis. Acute ophthalmic effects were also reported with severe eye irritation and watering of the eyes.

Reproductive and gynecological effects were evaluated by retrospective cohort studies. In an epidemiological survey conducted nine months after the accident, revealed that 43% of 865 pregnancies amongst exposed women suffered fetal loss, as compared to 6–10% among the general Bhopal population. The spontaneous abortion rate was highest among those exposed during their first trimester. A study conducted by Shilotri NP and coworkers after 105–110 days of the accident showed a higher incidence of abnormal uterine bleeding and abnormal Pap smears amongst exposed women in the childbearing age.

Few immunological toxicity studies of MIC have been reported. A study of humoral and cell mediated immunity, in exposed subjects two months after exposure, found that cell-mediated immunity was suppressed, and that MIC-specific antibodies persisted for several months after the accident.

Long-Term Health Effects

Human

Till this date, Bhopal accident has claimed more than 6000 lives, and ~50 000 survivors are estimated to be suffering from long-term health effects that are termed as 'Bhopal syndrome' due to lack of information on the exact constituents of the gas cloud. The Indian Council for Medical Research established a field office called as Bhopal Gas Disaster Research Centre (BGDRC) immediately after the accident. In addition, International Medical Commission on Bhopal (IMCB) was established in 1993 comprising 15 professionals from 12 different countries. BGDRC and IMCB have reported that after 15 years of exposure, the affected population is still suffering from multisystemic toxicities. The major long-term health effects observed are shortness of breath, chest pain, muscle/bone pain, asthma, increased spontaneous abortions, and certain psychological problems. A randomized retrospective cohort study undertaken 10 years after the exposure by Cullinan *et al.* indicates the presence of persistent small airways obstruction. The lung examination carried out among the survivors several months later exhibited presence of obliterative bronchiolitis and interstitial fibrosis. Thirty-nine percent of 783 patients examined showed ventilatory impairment.

A recent study published in *Journal of the American Medical Association* in October of 2003 indicates that even the second generation of the exposed population is adversely affected. According to this study, significant growth retardation has been observed in boys who were either exposed to the gases as toddlers or born to exposed parents. Interestingly, no significant effects have been observed in girls.

Prevention and Management Measures

To prevent and manage such disasters in the future, international guidelines such as the United Nations Environment Program Awareness and Preparedness for Emergencies at the Local Level and Organization for Economic Cooperation and Development Guiding Principles for Chemical Accident Prevention, Preparedness and Response have been established. Few of the important recommendations proposed by these committees are to institute Local Emergency Planning Committees that will make the community aware of the dangerous substances used locally by industries and also try to prepare the local medical personnel, emergency first responders, and municipal administrators for the management of unexpected toxic substance release into local community by an industry. These measures have been effectively undertaken in every city and township in the United States today.

Litigations

The Indian Government filed a lawsuit against the Union Carbide, which was settled out of court. The Union Carbide paid $470 million in compensation.

See also: Carbaryl; Methyl Isocyanate.

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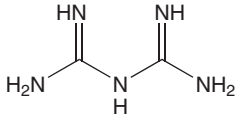
Biguanides

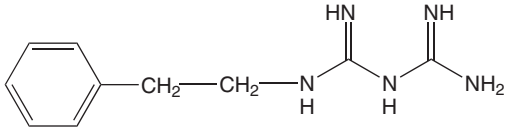
C Vaman Rao

- Chemical Abstracts Service Registry Numbers: CAS 5188-42-1 (chromate salt); CAS 6272-66-8 (dinitrate salt)
- Synonyms: Guanyl guanide; Diguanide; Amidinoguanidine; Phenformin; Metformin; Buformin; Glucophage (brand name)
- Chemical/Pharmaceutical/Other Class: Synthetic aliphatic amino alkanes with a large number of amino substituted groups
- Chemical Structures:

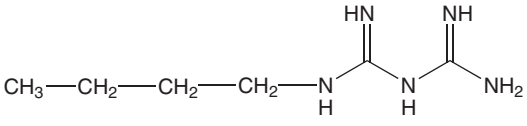


Biguanide

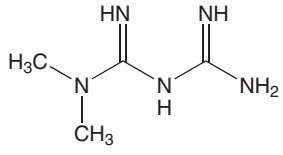
Modified form of biguanides:



Phenformin (β-Phenethyl biguanide)



Metformin (n-butylbiguanide)



Buformin (1,1-dimethyl biguanide)

Uses

Biguanides are used as an oral drug for the management of mild to moderately severe, noninsulin-dependent (type II) diabetes mellitus in obese patients who are usually above 40 years of age. It is important that for the administration of this drug the disease should have adult onset.

Polymeric biguanides were originally developed as a presurgery antimicrobial scrub and in 1977 it was introduced in the market for treating pools and spas as a disinfectant under the trade name Baquacil. It has Environmental Protection Agency (EPA) approval as the only nonhalogen sanitizer of pools and spas. The biguanide itself is combined with algecides and hydrogen peroxide for periodic oxidation of pools and spas. Biguanides are incompatible with chlorine, ozone, detergents, and ionizers, but are compatible with water ion balancing chemicals. Biguanides reduce the surface tension of water, which gives it a smoother feeling. They are stable in sunlight and temperature. At recommended concentrations when used in pools and spas, biguanides do not irritate the skin or eyes and do not corrode the pool equipment.

Exposure Routes and Pathways

The exposure to this drug is through oral route and absorption through the gastrointestinal tract.

Toxicokinetics

Phenformin is ~50% absorbed from the gastrointestinal tract. Its protein binding ability is very poor, which is ~20%. Phenformin is distributed throughout the major organs and it is mainly metabolized in the liver by hydroxylation. On hydroxylation, it produces *N-P*-hydroxy-*B*-phenyl-ethyl biguanide as

a metabolite. About 66% of the biguanide is excreted unchanged and the remaining 33% as a metabolite. Phenformin's half-life in the plasma is 7–15 h versus metformin's 1.5 h and buformin's 4–6 h. Metformin and buformin are excreted largely in an unchanged manner. The renal clearance of buformin, metformin, and phenformin are 393, 440, and 42–262 ml min^{-1}, respectively.

Biguanides are known to show interaction with furesemide, nifedipine, and cationic drugs.

Mechanism of Toxicity

A modification of the basic biguanide structure results in difference in potency, metabolism, excretion, and probably toxicity. The drug has a two-fold mechanism of action: it enhances the peripheral muscle glucose uptake and utilization, and inhibits glucose release from the liver. Biguanides induce an increase in peripheral gluconeogenesis and a decrease in intestinal absorption of glucose, vitamin B_{12}, and bile acids. Biguanides do not usually decrease blood sugar in a normal individual unless ethanol or another hypoglycemic agent is simultaneously administered or there is severe hepatic insufficiency. Biguanides are known to cause decreased absorption of vitamin B_{12} and folic acid. In the medical registry, it has been advised to avoid biguanide treatment to patients having hepatic insufficiency, renal insufficiency, peripheral vascular disease, and coronary diseases.

Phenformin generally lowers the blood sugar level in diabetics and nutritionally starved patients. Phenformin appears initially to produce a gastric mucosal irritability, which may predispose a person to a number of gastrointestinal symptoms, including gastric hemorrhage. Phenformin may act on the cell membrane to decrease oxidative phosphorylation, produce tissue anoxia, increase peripheral glucose uptake (Pasteur effect), and lead to lactic acidosis (accumulation of lactic acid) by inhibition of lactic acid metabolism.

Chronic Toxicity (or Exposure)

Animal

No systematic animal study has been reported to date.

Human

Biguanides are known to cause vomiting, nausea, abdominal cramps, gastrointestinal intolerance, anorexia, epigastric fullness, photosensitivity, dyspepsia, and dysgeusia (metallic taste), confusion, and lethargy.

Phenformin is the only biguanide to have been marketed in the United States and removed from the market by the US Food and Drug Administration (FDA) in 1977 because of its association with the development of lactic acidosis, a metabolic aberration that results in mortality in 50–75% of cases. Ethanol intake before the administration of phenformin therapeutic doses or excessive dose appears to predispose the patient to the development of lactic acidosis with a serious outcome. Phenformin and its other relative biguanides are still sold in European and other countries worldwide.

Daily therapeutic doses recommended for humans for three different biguanides are as follows: buformin, 100 mg; metformin, 500 mg ($\times 3$) and 850 mg ($\times 2$); phenformin, 25 mg.

Clinical Management

Biguanide toxicity is primarily managed by supportive means. Its elimination from the body could be enhanced by dialysis or enhancing excessive urination.

Environmental Fate

There is no official report on the environmental fate of biguanides.

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Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents

James M Madsen*

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Introduction and Classification

Toxins are toxic chemicals that can be elaborated by a biological organism. The word 'toxin' is often loosely used to mean poison but should be reserved for its more restricted definition; toxicant is a better synonym for poison. Several of the less complex toxins can now be synthesized in the laboratory or produced by other organisms following gene insertion, but synthetic toxins identical to their naturally occurring counterparts are still by definition toxins. Related terms include phycotoxins (toxins from algae), mycotoxins (fungal toxins), phytotoxins (plant toxins), and venoms (toxins from animals, especially vertebrates). Endotoxins are lipopolysaccharide toxins in the cell walls of certain gram-negative bacteria, and enterotoxins are toxins, such as cholera toxin, that damage intestinal mucosal cells. An exotoxin is a toxin that an organism releases into the environment. The actual toxin secreted by cells has in some cases been altered from the protoxin initially formed within the cells. Toxins usually do not perform crucial metabolic functions within their organisms of origin but act as offensive or defensive reactions to other organisms.

More than 400 toxins are known. They may be grouped according to size: low-molecular weight (LMW) toxins, which may be either peptides or nonpeptide organic compounds such as domoic acid, weigh less than 1 kDa and, if peptides, have no more than ~10 amino acids; heavier (larger) toxins are called protein toxins. Classification by organism of origin leads to the division of toxins into bacterial, algal, fungal, plant, marine dinoflagellate, marine soft coral, arthropod, molluscan, and vertebrate toxins. Toxins of similar chemical structure can be grouped together. Pathophysiologically, toxins comprise at least three major groups depending upon their toxicodynamics, or mechanisms of action. Neurotoxins, which affect neurotransmission, include botulinum toxin (which blocks the release of acetylcholine from cholinergic neurons), anatoxin, saxitoxin, and many animal venoms, some of which act presynaptically and others of which act postsynaptically. Membrane-damaging toxins include ricin, microcystin (which is also a hepatotoxin), certain venoms (such as the hemolytic snake venoms), and the trichothecene mycotoxins. Superantigen toxins such as staphylococcal enterotoxin B, toxic shock syndrome toxin-1, and streptococcal pyrogenic exotoxins exert pronounced systemic effects by activating the immune system in a nonspecific way.

Bioregulators are potent low-molecular peptides and proteins that modulate a wide variety of physiological processes such as inflammation, blood clotting, and neurotransmission. Unlike most toxins, they have definite roles in the normal physiology of their hosts. Bioregulators are not normally considered poisons but at toxicological doses may produce dramatic effects on blood pressure, body temperature, and other physiological parameters.

As chemicals produced by biological organisms, toxins and bioregulators occupy a zone that lies between chemical and biological agents and overlaps them to some extent. Saxitoxin and ricin are listed as chemical agents in the Chemical Weapons Convention, and toxins are listed separately from biological agents in the Biological and Toxin Weapons Convention (BTWC). The usual practice is to group toxins with biological agents. This is natural and appropriate from the perspectives of production, storage, and treaty issues, since toxins are generally produced by and often stored near their biological agents of origin. However, from a clinical standpoint, both toxins and bioregulators resemble other chemicals in that they do not replicate inside their hosts, are not transmissible, and are amenable to a chemical-based approach to clinical management. The term mid-spectrum agents (or mid-spectrum chemical warfare agents) has been proposed to refer to toxins and bioregulators along with synthetic viruses and genocidal agents produced by recent advances in biotechnology. **Table 1** displays one classification scheme for these compounds; the agents that are underlined will receive particular attention in this entry. Agents discussed as separate entries in this encyclopedia are also so indicated.

History

For millennia, indigenous South Americans deliberately used plant-derived arrow poisons such as curare and also toxins from poison-dart frogs, although these preparations were used mainly for hunting; similar toxins were used in Africa. The military use

*The conclusions and opinions expressed in this document are those of the author and do not necessarily reflect the official position of the United States Government, the Department of Defense, the United States Army Medical Research Institute of Chemical Defense, or the Uniformed Services University of the Health Sciences.

Table 1 Toxins and other mid-spectrum agents relevant to warfare and terrorism: a classification scheme

TOXINS
Bacterial toxins
Phycotoxins (algal toxins)
Botulinum toxin (CDC Category A)
Epsilon toxin from *Clostridium perfringens* (CDC Category B)
Staphylococcal enterotoxin B (SEB) (CDC Category B)
Diphtheria toxin
Tetanus toxin
Shigatoxin (veratoxin)
Mycotoxins (fungal toxins)
Aflatoxins
Ergot alkaloids (historical)
Trichothecene mycotoxins
  Stachybotrotoxins, including satratoxin H
  T-2 mycotoxins
Marine toxins
Phycotoxins (algal toxins)
  Algal toxins (blue-green algal) toxins
    Anatoxin-A (AnTx-a)
    Microcystins and nodularins
    Saxitoxins (STX), causing paralytic shellfish poisoning (PSP)
  Diatom toxin
    Domoic acid, causing amnesic shellfish poisoning (ASP)
  Dinoflagellate toxins
    Brevetoxins (PbTx), causing neurotoxic shellfish poisoning (NSP)
    Ciguatoxins (CTX) and maitotoxins (MTX), causing ciguateric fish poisoning (CFP)
    Diarrheic shellfish toxins (DST), causing diarrheic shellfish poisoning (DSP)
     Okadaic acid
    Palytoxin (concentrated by corals)
Conotoxins (from cone snails)
Scombrotoxins (mainly histamine)
Tetrodotoxin (TTX)
Phytotoxins (plant toxins)
(numerous alkaloids, including curare)
Type 2 ribosomal-inhibitory-protein (RIP) toxins
  Ricin (CDC Category B)
  Abrin
  Eranthis hyemalis lectin (EHL) from winter aconite
  Modeccin
  Viscumin
  Volkensin
Venoms from land animals
Invertebrate toxins, mostly from arthropods
Vertebrate toxins
  Amphibian toxins, including batrachotoxin
  Snake and lizard venoms
  Bird toxins (mainly batrachotoxin)

BIOREGULATORS
Cytokines
Early-phase proinflammatory cytokines (endogenous pyrogens)
  Interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α)
  IL-6
  IL-18
  Interferon gamma (IFN-γ)
Chemokines
  IL-8

Table 1 Continued

Eicosanoids (prostanoids and leukotrienes)
Prostaglandin D_2 (PGD_2), leukotrienes C_4 (LTC_4), LTD_4, LTE_4
LTB_4
Neurotransmitters and hormones
Catecholamines (e.g., epinephrine, norepinephrine, serotonin, dopamine)
Amino acid neurotransmitters (e.g., glutamate, aspartate, glycine, and γ-aminobutyric acid, or GABA)
Neuropeptides
  Neuropeptide Y
  Opioids (endorphins and enkephalins)
  Tachykinins
    Neurokinins A and B
    Substance P
Insulin
Vasopressin
Cholecystokinin
Somatostatin
Neurotensin
Bombesin
Vasoactive plasma proteases
Kallikreins and bradykinins
Tissue factor and thrombin

SYNTHETIC VIRUSES
Poliovirus
Other viruses identical to their natural counterparts
Genetically modified or combined synthetic viruses

GENOCIDAL AGENTS
Toxins, bioregulators, synthetic viruses, or traditional agents modified to enhance virulence
Toxins, bioregulators, synthetic viruses, or traditional agents modified to target specific genotypes

of toxins dates from at least the sixth century BC, when Assyrian soldiers poisoned enemy wells with ergot-contaminated rye. The ancient Greeks, for whom *toxikon* meant 'arrow poison', tipped arrows with aconite, and this practice continued into medieval Europe and persisted into the seventeenth century in Spain and Portugal. Japanese scientists in the infamous Unit 731 investigated tetrodotoxin during World War II, and suspicions have surfaced that the bomb used in the assassination of Reinhard Heydrich in Czechoslovakia in 1942 contained botulinum toxin. After World War II, ricin saw use as an injectable assassination weapon. More recently, Iraq had a weapons program that included the development of botulinum toxin, epsilon toxin from *Clostridium perfringens*, and aflatoxin. Militia groups in the United States and terrorist groups throughout the world have used ricin for political purposes. Toxins could be used on the battlefield or by terrorists to generate large numbers of military or civilian casualties as a mass-casualty weapon (MCW), to spread panic, for assassinations, or, in the case of toxins that damage crops, to create damage to an economy.

Concepts Relevant to Military or Terrorist Use

Toxicity

Toxins include several of the most acutely toxic chemicals known. For example, botulinum toxin is generally conceded to be the most lethal poison in existence. However, agent toxicity by itself is only a beginning variable that is subsequently modified by environmental and host factors. Because most toxins are far less toxic than botulinum toxin, tons of those toxins may be needed to cover a desired area on the battlefield. Many groups of toxins can thus be assumed to be at low risk of use as mass casualty weapons, or MCWs (but not necessarily as tools of assassins or for terrorist use in buildings), simply because of their relatively low toxicities. Others can be excluded on the basis of difficulties with isolation, synthesis (in general, chemical synthesis of only selected LMW toxins is currently feasible), production, storage, or dissemination; and still others degrade too rapidly in the environment. In general, the highest toxicities (lowest LD_{50} values, or lethal doses for 50% of a group) are associated with high-molecular weight (HMW) bacterial toxins such as botulinum toxin (MW 150 000 Da; LD_{50} 0.001 $\mu g\,kg^{-1}$), tetanus toxins (MW 150 000 Da; LD_{50} 0.002 $\mu g\,kg^{-1}$), and shigatoxin (MW 55 000 Da; LD_{50} 0.002 $\mu g\,kg^{-1}$). On the other end of the scale, aconitine (MW 647 Da; LD_{50} 100 $\mu g\,kg^{-1}$) is of the same order of toxicity as the nerve agent sarin; and T-2 mycotoxin (MW 466 Da; LD_{50} 1210 $\mu g\,kg^{-1}$) is approximately as toxic as the vesicating chemical agent sulfur mustard. Nonetheless, exceptions do occur; and the potency of a given toxin depends not only upon its size and structure but also upon its formulation (e.g., particle size in an aerosol) and, notably for most toxins, upon the route of entry.

States in the Environment and Routes of Entry

Although toxins do not volatilize as do many (but not all) chemical agents, this difference is moot as long as aerosolized toxin is capable of being inhaled. The preferred mode of dissemination for toxins used as MCWs would usually be the generation of appropriately sized particles in an aerosol, and the most pertinent route of exposure would be inhalation during the initial dispersion of agent. Primary aerosolization and inhalation have been conclusively demonstrated to be practical for botulinum toxin, staphylococcal enterotoxin B, ricin, T-2 mycotoxin, aflatoxins, brevetoxins (naturally, from wave-induced sprays of algal blooms), domoic acid, saxitoxin, tetrodotoxin, and others. Once an agent has settled onto environmental surfaces, its ability to reaerosolize largely determines its likelihood for subsequent inhalation. This property varies from toxin to toxin and from formulation to formulation and also depends upon environmental conditions such as humidity and temperature. Secondary aerosolization is presumed to be negligible for many toxins, but this assertion has not been rigorously demonstrated for all of these compounds. Many toxins are stable for long periods on environmental surfaces and in water; trichothecene mycotoxins and palytoxin are particularly persistent, but even botulinum toxin can remain in nonmoving water and in food for weeks. Personnel decontamination can usually be accomplished by gentle flushing using water with or without soap.

Most toxins are neither absorbed through intact skin nor dermally active, but there are exceptions, to include T-2 mycotoxin, lyngbyatoxin, and other blue-green algal toxins (cyanobacterial toxins). Ricin and abrin are among toxins that may incite an allergic contact response. Certain toxins and certain bioregulators, especially if heavily glycosylated, can survive ingestion to be absorbed from the gastrointestinal tract, and parenteral absorption of injected ricin was responsible for the death of Bulgarian dissident Georgi Markov in London in 1978 and was suspected in several other assassinations. This kind of attack, depending upon surreptitious employment of an umbrella modified to inject a ricin-filled pellet, would appear to be more suited to isolated assassinations than to use as a mass casualty weapon, but saxitoxin, which is particularly stable even under high temperatures, has been considered for coating bullets.

Threat Estimates

Risk assessment of possible use of toxins and related mid-spectrum agents on the battlefield or as terror weapons is an inexact science at best. Even if a toxin or a bioregulator is extremely potent, it may not be easily weaponizable, it may not survive long in the environment (particularly for bioregulators), or it may be so rare that the resources required to isolate it from natural sources or (in the case of LMW compounds) to synthesize it would be prohibitive. However, a determined individual, terrorist cell, or state with available time, personnel, equipment, and natural product may overcome these limitations, particularly if the goal is to use a little-known agent that will be low on the list of differential diagnoses for the effects produced. The rapid development of new techniques in biotechnology, toxicogenomics, and proteomics may also help to open the door to the

production, storage, and dissemination of exotic mid-spectrum agents. The Centers for Disease Control and Prevention (CDC) in the United States has established three threat categories for biological agents and toxins; botulinum toxin appears in category A (the highest-threat group), and ricin, epsilon toxin from *C. perfringens*, and staphylococcal enterotoxin B (SEB) all reside in category B. Abrin does not appear on the list, perhaps because it is less readily available than ricin. However, its toxicity is almost an order of magnitude higher than that of ricin. T-2 mycotoxin is asserted to have been used in Southeast Asia from 1975 to 1981, and despite continuing controversy regarding these claims, its potential for use appears to be significant despite its absence from the CDC list. Iraq stockpiled aflatoxin at one point for possible use, and saxitoxin, domoic acid, and tetrodotoxin have been mentioned as toxins capable of weaponization as MCWs. It seems safe to assert that although the most likely toxins to be used in warfare or terrorism include botulinum toxin, ricin, staphylococcal enterotoxin B, and perhaps trichothecene mycotoxins, all of which have been seriously investigated for military use, several other candidates exist and should not be excluded from consideration. The likelihood of development and use of bioregulators, synthetic viruses, and genocidal agents is even less predictable but should be expected to rise with new advances in biotechnology. Space prohibits discussion of each of the agents in **Table 1**, but a brief overview of each of the underlined toxins in **Table 1** will be presented.

Representative Agents

Botulinum Toxin

Chemical Abstracts Service Registry Number: CAS 93384-43-1. Botulinum toxins comprise a series of seven related protein neurotoxins that prevent fusion of synaptic vesicles with the presynaptic membrane and thus prevent release of acetylcholine. Exposure in a battlefield or terrorist setting would most likely be to inhaled aerosolized toxin. The clinical presentation is that of classical botulism, with descending skeletal muscle weakness (with an intact sensorium) progressing to respiratory paralysis. A toxoid vaccine is available for prophylaxis, and a pentavalent toxoid can be used following exposure; its effectiveness wanes rapidly, however, after the end of the clinically asymptomatic latent period. Because treatment is supportive and intensive (involving long-term ventilatory support), the use of botulinum toxin has the potential to overwhelm medical resources especially at forward echelons of care.

Ricin

Chemical Abstracts Service Registry Number: CAS 9009-86-3. Ricin, easily extracted from the castor bean plant (*R. communis*), is a globular glycoprotein membrane-damaging toxin with an A chain and a B chain separated by a disulfide bond. The A chain binds to the 28S unit of ribosomes to impair protein synthesis. The clinical presentation is very much dependent upon the route of entry: ingestion produces predominantly gastrointestinal effects, inhalation causes airway necrosis and damage to alveolar-capillary membranes leading to diffuse necrotizing pneumonitis and pulmonary edema, and parenteral exposure (from injection or from contamination of wounds) generally spares the respiratory tract but leads to necrosis of lymph nodes, gastrointestinal mucosa, the liver, the kidneys, and the spleen and to disseminated intravascular coagulation. Local cutaneous reactions and absorption may also follow contact with intact skin. The results of active prophylaxis with toxoid have been encouraging in animal studies, but treatment in humans remains empirical and supportive.

Abrin

Chemical Abstracts Service Registry Number: CAS 1393-62-0. Abrin is a toxalbumin similar in structure, absorption, and mechanism of action to ricin but is found not in castor beans but rather in jequirity beans. No reports of its use as a battlefield or terrorist agent exist, but in mice it is 75 times more potent than ricin. No specific treatment is available. Both ricin and abrin are type 2 ribosomal inhibitory proteins (RIPs): the other potent toxins in this class are *Eranthis hyemalis* lectin (EHL) from winter aconite, modeccin and volkensin from African succulents, and viscumin from mistletoe.

Epsilon Toxin from *Clostridium perfringens*

Clostridium perfringens has at least six serotypes and produces over 20 toxins. Epsilon toxin, along with alpha, beta, and iota toxins, is dermonecrotic and lethal. It is produced by some strains of type B and especially type D as a protoxin that is then converted to an active, mature, heat-labile toxin. The resulting toxin binds to cell membranes and forms a membrane complex that promotes the efflux of intracellular potassium. Because the usual route of entry is the gastrointestinal tract, the resulting pathology is an increase in intestinal permeability that enhances absorption of more toxin and ensures systemic toxemia. In animals, increased vascular permeability leads to enterotoxemia, 'pulpy kidney', altered hepatic function, and cerebral edema and necrosis.

Aerosolized alpha toxin from *C. perfringens* causes serious pulmonary damage with vascular leakage, hemolysis, thrombocytopenia, and liver damage and could easily be lethal, but the effects in humans of epsilon toxin, especially from inhalation, are unclear. However, the Iraqi biological agent program included the study not only of *Bacillus anthracis* and *Clostridium botulinum* but also of *C. perfringens*, including its epsilon toxin. Theoretically, this toxin could be genetically combined with another agent to increase the absorption of both. Animal toxoids exist but have not been evaluated for safety or efficacy in humans.

Staphylococcal Enterotoxin B (SEB)

Chemical Abstracts Service Registry Number: CAS 11100-45-1. SEB, the toxin that after ingestion causes sudden-onset staphylococcal food poisoning, is one of seven enterotoxins elaborated by *Staphylococcus aureus*. It is resistant to both heat and freezing. As a superantigen toxin, its mechanism of action involves binding to receptors for T-cell antigens and to major histocompatibility complex class II molecules, bypassing normal routes for antigen recognition and leading to antigen-nonspecific activation of the immune system and a massive release of bioregulatory cytokines to include not only histamine and leukotrienes (responsible for the intestinal response) but also interferon gamma, interleukin-6, and tumor necrosis factor alpha (responsible for systemic effects). Inhalation of aerosolized SEB leads to incapacitating respiratory signs and symptoms, although deaths at high doses may occur from pulmonary edema. Inadvertently swallowed toxin may also produce nausea and vomiting.

In a military or a terrorist setting, SEB could be added to unguarded food or water or could be disseminated by aerosol. The resulting incapacitation may be a desirable goal either on the battlefield or for terrorism. Human trials of a pre-exposure toxoid and of post-exposure passive immunization are underway but have not yet led to approved products.

T-2 Mycotoxin

Chemical Abstracts Service Registry Number: CAS 21259-20-1. T-2 mycotoxin is a trichothecene toxin, so-called because of two particular chemical moieties in its structure. Many otherwise unrelated groups of fungi produce a rich variety of trichothecene mycotoxins, each with its own toxicological profile. T-2 mycotoxin has been associated with disease in animals and, in the 1930s in the Soviet Union, with a largely gastrointestinal condition called alimentary toxic aleukia, a chronic intoxication from repeated consumption of contaminated bread. This toxin was also found in autopsy specimens from one of the Khmer Rouge casualties associated with the yellow, green, red, or white smoke that came to be called yellow rain in Laos and Cambodia (now Kampuchea) in the 1970s. Whether the T-2 toxin acted in concert with other mycotoxins found in the victim and to what extent it was responsible for the observed results remain matters of controversy, even though laboratory exposures to the toxin created similar cutaneous, ocular, and systemic effects. The toxicity of T-2 mycotoxin, which is also one of the few toxins capable of creating small vesicles on the skin after direct contact, is roughly comparable to that of the chemical agent sulfur mustard, and relatively large quantities would be needed to cause casualties over a large area. The cytotoxicity of T-2 toxin is thought to be related to lipid peroxidation of plasma membranes, inhibition of electron (proton) transport in mitochondria, and especially RNA inhibition and consequent disruption of protein synthesis in ribosomes. Treatment is supportive, supplemented with steroids.

Aflatoxin

Chemical Abstracts Service Registry Number: CAS 1402-68-2. Aflatoxins are toxic, immunosuppressive, mutagenic, and carcinogenic mycotoxins produced by the mold *Aspergillus flavus* and commonly contaminating cereals, oilseeds, tree nuts, and spices. They are quite resistant to dry heat but gradually deteriorate under conditions of moist heat. They are also inactivated by food additives such as sodium bisulfite. Aflatoxin was first recognized as a toxin for animals following a severe outbreak of 'Turkey X' disease in the United Kingdom in 1960. Since that time, outbreaks of human disease have been reported, including one from contaminated maize in Kenya during May 2004; the case fatality rate for this outbreak approached 50% in one of the affected districts. Iraq is known to have included aflatoxin in its arsenal; it is unclear whether this toxin was intended to be used to cause acute effects or to cause cancer years later in survivors (or both) is unclear. In the body, cytochrome P450 converts the toxin (usually after ingestion) to an epoxide that reacts with RNA and DNA, inhibits protein and DNA synthesis in the liver and bone marrow, and can lead to mutations and eventually cancer. The acute clinical manifestations are protean and include vomiting, abdominal pain, gastrointestinal hemorrhage, fatty change of the liver, pulmonary edema, convulsions, and cerebral edema; chronic effects include liver cancer. Treatment is supportive.

Domoic Acid

Chemical Abstracts Service Registry Number: CAS 14277-97-5. Domoic acid, a glutamic acid analog that is resistant to temperature extremes, is an excitatory neurotoxin produced by a diatom and concentrated in shellfish. Ingestion leads to amnesic shellfish poisoning, which can also include seizures. Its relevance to use in warfare and terrorism, apart from its being unfamiliar to most disaster-response personnel, is that it is also easily absorbed by inhalation and across mucous membranes. No specific antitoxin is available, and treatment is supportive.

Saxitoxin (STX)

Chemical Abstracts Service Registry Number: CAS 35523-89-8. Saxitoxin, a heat-stable neurotoxin produced by blue-green algae, is associated with paralytic shellfish poisoning. It leads to weakness and paralysis by blocking sodium channels in neurons. It is a potential agent for use on the battlefield or in terrorism because of its increased potency via inhalation, its fast onset and progression, and its proposed use for coating projectiles such as bullets. No toxoid or antitoxin is available.

Tetrodotoxin (TTX)

Chemical Abstracts Service Registry Number: CAS 4368-28-9. Tetrodotoxin, a neurotoxin produced by several species of starfish, crabs, salt-water fish, octopi, newts, and salamanders, blocks sodium channels within neurons. In a terrorist scenario, tetrodotoxin could be inhaled as an aerosol or ingested in contaminated food or water. Mortality may reach 50%.

Bioregulators

All of the bioregulators listed in **Table 1** are potentially weaponizable, although not all with present-day technology. Their attractiveness as weapons of assassination or to produce mass casualties is tied to several possible advantages. They are not on most standard lists of agents to be expected in warfare or terrorism, they are easily purchased (partly because they are used extensively in research), they are rapid in onset (making them useful assassination agents) but relatively nonspecific in their clinical effects (thus not arousing suspicion), and no vaccines are available against them. However, the costs of production or purchase may be high, they may not be available in large enough quantities to be effective, and neither their aerosolizability nor their environmental persistence has been characterized thoroughly. Nevertheless, since enteral absorption is significant for many of these compounds, they could also be added to foodstuffs.

Synthetic Viruses

Simple viruses such as the polio virus, consisting of a single strand of RNA, have already been successfully assembled using commercially available reagents. Larger and more complex viruses will undoubtedly be synthesized in the near future, and the relative ease with which this can be done, and the possibility of designing and testing novel viral structures not found in nature, could lead to large quantities of completely new agents. Since these compounds can be synthesized in a laboratory setting but can then replicate within hosts, they are prototypical mid-spectrum agents.

Genotoxic Agents

The ability to synthesize viral genomes is part of the burgeoning development of biotechnology, which uses high-speed data processing, microarrays, and the new sciences of genomics and proteomics to alter genetic code and to affect the expression of that code. Bioengineered viruses and other organisms could be targeted toward individuals or populations with specific genotypes. Toxicogenomics could be used in a similar way for chemical agents and for mid-spectrum agents such as toxins and bioregulators.

Summary

Toxins, which are chemical poisons produced by living organisms, and bioregulators, which are LMW molecules involved in physiological processes within the body, are biological in origin but are noninfectious and nonreplicating. As mid-spectrum agents, they occupy a position between and overlap the traditional dichotomy of mass-casualty agents as chemical versus biological agents. This part of the spectrum may also be said to include synthetic viruses and genotoxic agents. All of the mid-spectrum agents have potential for use in small-scale or large-scale operations against military forces, civilians, or both. An appreciation of the unique position of these agents and of the threat that they pose and a heightened level of suspicion for their use are necessary in order to recognize their use and institute appropriate preventive and treatment measures.

See also: Aflatoxin; Algae; Botulinum Toxin; Castor Bean; Ciguatoxin; *Clostridium perfringens*; Marine Organisms;

Mold; Mycotoxins; Ricin and Other Toxalbumins; Saxitoxin; Scombroid; *Staphylococcus aureus*; Tetrodotoxin.

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Biocides

Amy Merricle

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The word ‘biocide’ encompasses a broad class of chemical agents and literally means an agent that destroys life. The United States Environmental Protection Agency defines the term ‘biocide’ as follows:

> A diverse group of poisonous substances including preservatives, insecticides, disinfectants, and pesticides used for the control of organisms that are harmful to human or animal health or that cause damage to natural or manufactured products.

This broad definition includes terms and topics covered in this encyclopedia and other literature, including pesticides, which encompasses herbicides, insecticides, miticides, rodenticides, algaecides, etc. Biocides have sometimes been considered a subcategory of pesticides. When the term is used in this context, it refers specifically to the control or destruction (killing) of microorganisms, typically in nonagricultural applications. Biocides as nonagricultural pesticides encompass a wide range of applications, including disinfectants and sanitizers, preservatives and microbicides, antifouling products, wood preservatives, and structural treatments.

Biocides are used widely in industry. There are at least three main classes of industrial chemical biocides. The first class includes the oxidizing and bleaching agents, such as chlorine dioxide, hydrogen peroxide, and sodium hypochlorite. The oxidizing action may directly kill bacteria or fungi or weaken the cell walls so that they are more susceptible to other classes of biocides (see below). Sodium hypochlorite (like all hypochlorites) is a salt of hypochlorous acid. In solution, it splits into the sodium cation (Na^+) and the hypochlorite anion (ClO^-). The oxidizing power of the latter causes the bleaching and disinfecting effect. Chemicals with oxidizing and bleaching properties have been under scrutiny in recent years. This is largely because of the toxicity of reaction by-products, particularly chlorine and its derivatives. There is a high probability of the formation of toxic gases (chloramine gas) and mutagenic and/or carcinogenic halogen-containing organic substances (e.g., trihalomethanes) during water treatment activities and when these chlorine-containing compounds are released into the environment. As a result, there has been an increase in the use of oxygen, hydrogen peroxide, and other oxygenated compounds in bleaching applications, and a sharp decline in the demand for chlorine and the hypochlorites.

A second class of industrial chemical biocides involves highly toxic organic chemicals. Subclasses of toxic biocides include thiazoles, thiocyanates, isothiazolins, cyanobutane, dithiocarbamate, thione, and bromo-compounds. As the names imply, many of the toxic biocides contain sulfur (‘thio’-).

A third class of industrial chemical biocides consists of agents with the ability to inhibit biological film formation, also called ‘surfactants’. The term surfactant originates from the phrase surface active agent. Surfactants fall into four broad categories: anionic (e.g., soaps, alkyl benzenesulfonates, alkyl sulfonates, alkyl phosphates), cationic (e.g., quaternary ammonium salts), nonionic (e.g., alkyl polyglycosides, alcohol ethoxylates, alkylphenol ethoxylates), and zwitterionic.

See also: Consumer Products; Organochlorine Insecticides; Pesticides.

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Biocompatibility

Samantha E Gad

The biological evaluation of medical devices is performed to determine the potential toxicity resulting from contact of the component materials of the device with the body. The device materials should not produce adverse local or systemic effects, be carcinogenic, or produce adverse reproductive and developmental effects, either directly or through the release of their material constituents. Systemic testing must ensure that the benefits of the final product will outweigh any potential risks produced by device materials.

In 1986, the US Food and Drug Administration (FDA), Health and Welfare Canada, and Health and the United Kingdom (UK) Social Services issued the 'Tripartite Biocompatibility Guidance' #G87-1 for Medical Devices. This guidance was used by the FDA and device manufacturers until July 1, 1995, when the new blue book memorandum #G95-1 entitled *Use of International Standard ISO-10993, Biological Evaluation of Medical Devices Part-1: Evaluation and Testing*, became effective by direction of the ODE (Office of Device Evaluation). The 'ANSI/AAMI/ISO' Standard 10993 *Biological Evaluation of Medical Devices* is accepted in Europe and Asia and is also referred to as ISO 10993/EN 30993. Also, since 1995, medical devices marketed and sold within the European Union (EU) have been required to comply with safety assessment requirements stated in EU Medical Devices Directive 93/42/EEC. The European Committee for Standardization (CEN) is now in the process of adopting ISO 10993. Japanese procedures for sample preparation and testing are slightly different from either United States Pharmacopoeia (USP) or ISO tests. The unofficial translation of the Japanese toxicological testing requirements is available as *Guidelines for Basic Biological Tests of Medical Materials and Devices*. In addition, FDA has recognized standard developed by the USP and by the American Society for Testing and Materials (ASTM).

For use in the United States, the blue book memorandum includes an FDA-modified matrix designating the type of testing required for various medical devices and also a flowchart entitled 'Biocompatibility Flow Chart for the Selection of Toxicity Tests for 510(k)s'. The matrix also consists of two tables: Table 1 – Initial Evaluation Tests for Consideration; and Table 2 – Supplementary Evaluation Tests for Consideration. In general, the agency does not have a list of approved materials.

An ISO standard, it should be noted, is a document that undergoes periodic review and is subject to revision. Recently, the FDA, more specifically the Center for Devices and Radiological Health (CDRH), has been considering the use of international consensus standards for the toxicological evaluation of medical devices.

FDA notes that the ISO standard acknowledges certain kinds of discrepancies. It states "due to diversity of medical devices, it is recognized that not all test identified in a category will be necessary and practical for any given device. It is indispensable for testing that each device shall be considered on its own merits: additional tests not indicated in the table may be necessary." It is necessary to consider the properties of device materials and the nature degree, frequency, and duration of exposure to the body when determining appropriate tests for a particular device. Material found to be safe for one intended use in a device might not be in a device intended for another use. The final assessment must be not only made for all components but more importantly on the finished product. Generally, the tests include: acute, subchronic, and chronic toxicities; irritation to the skin, eyes, and mucosal surfaces; sensitization; hemocompatibility; genotoxicity; carcinogenicity; and effects on reproduction

including developmental effects. Depending on the characteristic and intended uses of the device these tests may not be necessary or sufficient. Neurotoxicity and immunotoxicity, among other tests, may be necessary for some devices. The specific clinical application and the materials used in the manufacture of a device determine which tests are appropriate. Some materials that have been well characterized both chemically and physically in published literature and which have a long history of safe use may prove unnecessary to complete all tests if substantial equivalence to marketed products under 510(k) is shown. In this case the manufacturer must document the use of a particular material in a legally marketed predicate device or a legally marketed device with comparable patient exposure.

The FDA has made several modifications to the tests required by Part 1 of the ISO 10993 standard for the category of surface devices that permanently contact mucosal membranes. The ISO does not require acute, subchronic, or chronic implantation tests as does FDA. FDA requires irritation, systemic toxicity, acute, subchronic, and chronic tests for external communicating devices, tissue/bone/dentin with prolonged and permanent contact. Device manufacturers are advised to consider tests to detect chemical components of device materials that may be pyrogenic. This matrix is a framework and not a checklist and it is stressed by the FDA that necessary safety testing will be decided on a case-by-case basis.

ISO-10993 includes the following sections:

1. Guidance on Selection of Tests
2. Animal Welfare Requirements
3. Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicity
4. Selection of Tests for Interactions with Blood
5. Tests for Cytotoxicity – *In Vitro* Methods
6. Tests for Local Effects after Implantation
7. Ethylene Oxide Sterilization Residuals
8. Clinical Investigation of Medical Devices
9. Degradation of Materials Related to Biological Testing
10. Test for Irritation and Sensitization
11. Test for Systemic Toxicity
12. Sample Preparation and Reference Materials
13. Identification and Quantification of Degradation Products from Polymers
14. Identification and Quantification of Degradation Products from Ceramics
15. Identification and Quantification of Degradation Products from Coated and Uncoated Metals and Alloys
16. Toxicokinetic Study Design for Degradation Products and Leachables
17. Establishment of Allowable Limits for Leachable Substances

The following tests are recommended:

- cytotoxicity,
- acute systemic toxicity,
- sensitization,
- genotoxicity,
- skin irritation,
- implantation,
- intracutaneous reactivity, and
- hemocompatibility.

Subchronic and Chronic toxicities and also carcinogenicity may be appropriate.

When designing a medical device it is important to first select appropriate materials and then a sterilization method before searching for relative information on the materials and beginning testing. It is advisable to test individual components of a device prior to testing the complete device in case one component has toxic properties. Premarket Approval (PMA) applicants often use another party's product or facility in the manufacture or their device. Many manufacturers keep data on qualified materials used in their products. This information regarding the product is pertinent to its review; the third party may choose to submit confidential information directly to the FDA in a device master file. This is not a marketing application and additional testing or information may be necessary.

It may be necessary to repeat biocompatibility tests when modifying a device based on the changes made (see the flow chart given in the Appendix for conditions necessary in order not to have to repeat testing). If available, clinical data can be used to satisfy some biological effects categories from the ISO 10993-1 test selection matrix. Medical device toxicity problems are most often caused by leachable or extractable toxins. Extracts of materials are often tested for biocompatibility. Section 17 of 10993 entitled *Establishment of Allowable Limits of Leachable Substances*, gives guidance on the use of analytical data (e.g., extraction studies) to reduce biocompatibility test requirements. The extraction media should be comprised of a series of media with various polarities to capture results found in different solubilities. The most common extraction media include physiological saline, vegetable oil, dimethylsulfoxide, and ethanol; other less common ones include polyethylene glycol or aqueous dilutions of ethanol. The temperature at which the

extraction should be carried out varies throughout various guidelines, but it is generally recommended that extraction be performed at approximate body temperature for 72 h. For *in vitro* cytotoxicity testing, complete cell-culture medium is most commonly utilized, with extraction performed at 37°C for 24 h. Inexpensive nonanimal studies such as cytotoxicity and hemocompatibility tests can be used to screen device materials.

Biological control tests are recommended to determine sources of possible contamination and to ensure safety of the final product. Microbiological tests to determine the status of the final product (e.g., sterility, bacteria, contaminants, and microbial count limits) are necessary. Devices should be tested for endotoxins as cell wall lipopolysaccharides (from gram-negative bacteria) may be present even after sterilization. Assessment of nonspecific toxicological effects should be performed by intravenous injection of device eluate in mice. It has also become more common since the general acceptance of ISO 10993 that device materials be more rigorously characterized analytically and also that more extensive genotoxicity testing be performed.

The following are special considerations that must be considered when testing devices and their component materials for safety.

Color additives: A color additive is a dye, pigment, or other substance, whether derived from a vegetable, animal, mineral, or other source, which imparts a color when added to a food, drug, cosmetic, or the human body. The US Food, Drug and Cosmetic (FD&C) Act states devices containing a color additive are considered unsafe, and therefore adulterated, unless one is in effect listing the color additive for such use. The FD&C Act limits applicability of these color additives for devices that directly contact the body for a significant period of time (undefined by FDA). Manufacturers of devices should choose a color additive listed for use in foods, drugs, or cosmetics as a starting point but keep in mind that these may not be appropriate for devices. The color listing regulation may permit the use of the color additives or may place limitations on its use; PMA applicants must demonstrate their safety. Color additives listed for use in medical devices are provided in 21 CFR 73 (Color additives exempt from batch certification) and 21 CFR 74 (Color additives subject to batch certification).

Combination products: A combination product is a product consisting of two or more regulated components (drug/biologic/device, etc.) that are combined as a single entity or is a product labeled for use with a separate device or biologic where both are required to achieve the intended use, indication, or effectiveness. Intercenter agreements have been made within FDA to review and oversee these categories. More information can be found at FDA website for the CBER (Center for Biologics Evaluation and Research) and CDRH (Center for Devices and Radiological Health) Intercenter agreement, and the CDER (Center for Drug Evaluation and Research) and CDRH Intercenter agreement.

In vitro diagnostic (IVD) products: These are medical devices which analyze human body fluids, such as blood or urine, to provide information for the diagnosis, prevention, or treatment of a disease. Classification for these devices can be found under 21 CFR 862, 21 CFR 86?, and 21 CFR 866.

Radiation emitting products: Electronic product radiation means any ionizing or nonionizing electromagnetic or particulate radiation, or any sonic, infrasonic, or ultrasonic wave, which is emitted from an electronic production of the operation of an electronic circuit in such product. If a medical device emits electronic product radiation, additional requirements apply through the Radiation Control for Health and Safety Act (RCHSA). Additional information concerning radiation-emitting products can be found at the FDA website.

Software: If a device contains software, the PMA submission must include documentation of software testing appropriate to the level of risk of the device.

The FDA recognizes certain consensus standards of conformance when making regulatory decisions. In addition, sterility assurance is necessary, and FDA validated method for sterilization should be used and included in the PMA.

When positive biocompatibility results are reported, development discontinuation is not the only option. First it should be confirmed that no mistakes were made in the testing laboratory, including the testing of the proper article and formulation. In addition, it should be made certain that the article was properly manufactured, cleaned, stored, and tested (e.g., the extractant used, the testing conditions, and the procedure). Finally, reproducibility of positive biocompatibility results should be confirmed. In a certain situation where the possible benefits outweigh the risks, or when quality of life is a factor, a level of toxicity may be acceptable.

Appendix

Biocompatibility Testing Flow Chart

Material Characterization/Risk Assessment

Flow Chart for the Selection of Toxicity Tests for 510(k)s

Category		*Examples (not exclusive)*	*Contact duration* **(A) Limited** *≤ 24 h* **(B) Prolonged** *24 h to 30 days* **(C) Permanent** *> 30 days*	*Biological effect*									
				Initial evaluation tests								*Other*	
				Cytotoxicity	*Sensitization*	*Irritation*	*Systemic Tox*	*Subchronic Tox*	*Genotoxicity*	*Implantation*	*Hemocompatibility*	*Chronic Tox*	*Carcinogenicity*
Surface devices	Skin	Devices that contact intact skin surfaces only (electrodes, external prostheses, fixation tapes, compression bandages)	A	X	X	X							
			B	X	X	X							
			C	X	X	X							
	Mucous membrane	Devices communicating with intact mucosal membranes (contact lenses, urinary catheters, intraintestinal devices, endotracheal tubes)	A	X	X	X							
			B	X	X	X	F	F		F			
			C	X	X	X	F	X	X	F		F	
	Breached or compromised surfaces	Devices that contact breached or otherwise compromised external body surfaces (wound dressings, healing devices, occlusive patches)	A	X	X	X	F						
			B	X	X	X	F	F		F			
			C	X	X	X	F	X	X	F		F	
External communicating devices	Blood path indirect	Devices that contact the blood path at one point and serve as a conduit for entry into the vascular system (solution administration sets, extension sets, blood administration sets)	A	X	X	X	X				X		
			B	X	X	X	X	F			X		
			C	X	X	F	X	X	X	F	X	X	X
	Tissue/bone/dentin communicating	Devices communicating with tissue (includes fluids and subcutaneous spaces), bone, and pulp/dentin system (lacroscopes, arthroscopes, draining systems, dental cements, dental filling materials, skin staples, surgical instruments)	A	X	X	X	F						
			B	X	X	F	F	F	X	X			
			C	X	X	F	F	F	X	X		F	X

continued

Category		*Examples (not exclusive)*	*Contact duration* **(A) Limited** *⩽24 h* **(B) Prolonged** *24 h to 30 days* **(C) Permanent** *>30 days*	*Biological effect*									
				Initial evaluation tests								*Other*	
				Cytotoxicity	*Sensitization*	*Irritation*	*Systemic Tox*	*Subchronic Tox*	*Genotoxicity*	*Implantation*	*Hemocompatibility*	*Chronic Tox*	*Carcinogenicity*
	Circulating blood	Devices that contact circulating blood (intravascular catheters, temporary pacemaker electrodes, oxygenators, dialyzers, hemodsorbents, and immunoadsorbents)	A	X	X	X	X		F[1]		X		
			B	X	X	X	X	F	X	F	X		
			C	X	X	X	X	X	X	F	X	X	X
Implant devices	Tissue/bone	Devices principally contacting bone, tissue and tissue fluid (orthopedic pins, plates, replacement joints, pacemakers, drug supply devices, neuromuscular sensors and stimulators, replacement tendons, breast implants, ligation clips)	A	X	X	X	F						
			B	X	X	F	F	F	X	X			
			C	X	X	F	F	F	X	X		X	X
	Blood	Devices principally contacting blood (pacemaker electrodes, heart valves, vascular grafts and stents, internal drug delivery catheters, and ventricular assist devices)	A	X	X	X	X			X	X		
			B	X	X	X	X	F	X	X	X		
			C	X	X	X	X	X	X	X	X	X	X

ISO Recommended Initial Tests

Noncontact devices: These are devices that do not contact the patient's body directly or indirectly (*in vitro* diagnostic devices). Regulatory agencies rarely require biocompatibility testing for these devices.

X: ISO evaluation tests for consideration.

F: Additional Tests which the FDA may require.

[1]For all devices used in extracorporeal circuits.

In addition to these tests, pyrogenicity, reproductive and developmental, and biodegradation should be considered depending on the nature and intended use of the device.

See also: Foreign Body Response; Implant Studies.

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Bioinformatics

Kartik Shankar and Harihara M Mehendale

Advances in the fields of cellular and molecular biology coupled with the technological advances in genome and proteome scale investigations have led to an explosive increase in biological information. An efficient way to organize and utilize these data has become a necessity. Bioinformatics is the field that utilizes computer science and information technology to organize, view, and mine biological information. Bioinformatics till recently has been organization of large genome-scale data in public and private databases. Databases allow the storage and management of these data sets. A biological database is a large, organized body of persistent data, usually associated with computerized software designed to update, query, and retrieve components of data stored within the system. For example, the National Center for Biotechnology Information maintains several large databases containing genome information, protein sequences, transcription factors, promoters, and single nucleotide polymorphisms. However, in the 'postgenomic' era the emphasis of bioinformatics will be on data mining and knowledge-discovery. Consequently, bioinformatics is increasingly being integrated with bench-based science as hypotheses generated *in silico* are tested *in vitro* and *in vivo*. Some publicly available databases and bioinformatic software for several biomedical researches are listed in the 'Relevant websites' section. It should be noted that an extensive summary of the tools available for the interested researcher is not provided in the 'Relevant websites' section, but only a primer to the more popular bioinformatic tools.

See also: Genomics, Toxicogenomics; Microarray Analysis; Proteomics.

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http://www.ncbi.nlm.nih.gov – Database/software – dbEST; Application – Database of expressed sequence tags.

http://www.ncbi.nlm.nih.gov – Database/software – Unigene database; Application – Database of expressed sequence tags.

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http://www.gene-regulation.com – Database/software – TRANSFAC; Application – Transcription factor database.

http://wwwmgs.bionet.nsc.ru – Database/software – Transcription Regulatory Regions Database (TRRD); Application – Transcription factor database.

http://www.genomatix.de – Database/software – MatInspector; Application – Transcription factor binding site search engine.

http://www.ebi.ac.uk – Database/software – ClustalW; Application – Multiple sequence alignment program.

Biological Exposure Index

Alan J Weinrich

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Human biological exposure indices are guidance levels of determinants for assessing worker dose from occupational exposures. They differ from other occupational exposure limits (OELs) for chemicals, which typically are measured in air, in that their determinants are measured in biological materials from the workers. BEIs consider the dose that has entered a worker's body by all routes. Thus, these measurements can provide more complete estimates of exposure, especially for chemicals that may be absorbed by routes other than inhalation and when inhalation rates are altered because, for example, of increased work rates.

Defining Biological Exposure Indices

Most BEIs are defined as concentrations of determinants or biomarkers anticipated in biological specimens collected from healthy workers whose exposure to certain chemicals by all routes is equivalent to that of workers with inhalation only exposure at the OEL. Others measure reversible effects on the body, and still others are those that are below the concentrations associated with health effects. However, other definitions are common. For example, the German biological tolerance values (BAT) can be defined as rates of excretion of the chemical or its metabolites, or the maximum possible deviation from the norm of biological parameters induced by these substances in exposed humans. BEIs for some chemicals use other criteria, such as direct comparison with a measurable toxic effect, like carboxyhemoglobin in blood for carbon monoxide.

The most commonly used Biological Exposure Indices are known by the abbreviation, BEI®, which is a trademark of the American Conference of Governmental Industrial Hygienists (ACGIH®). Like most, ACGIH defines BEIs as guidance values for assessing human biological monitoring results. ACGIH indicates that most of its BEIs are bioequivalent to its airborne OELs, the threshold limit values (TLVs®): a "BEI generally indicates a concentration below which nearly all workers should not experience adverse health effects." It also asserts that BEIs should not be used as measures of adverse health effects or for diagnosing occupational illness. In addition to the ACGIH BEIs and German BATs, other sources of BEGs include the Finnish Institute of Occupational Health, United Kingdom Health and Safety Executive, Italian Society of Reference Values, and the Japan Society for Occupational Health.

The determinant for a biological exposure index can be the chemical itself, one or more metabolites, or a characteristic biochemical change induced by the chemical. The specimen used for biological monitoring usually is urine, blood, or exhaled air. For example, the BEI for trichloroethylene includes four determinants:

- a metabolite, trichloroacetic acid, in urine;
- another metabolite, trichloroethanol, in blood;
- the parent compound, trichloroethylene, in blood; and
- trichloroethylene in end-exhaled breath.

The latter two determinants are recommended as confirmatory tests to document exposure to trichloroethylene, since other chemicals also can be metabolized to trichloroacetic acid and trichloroethanol.

Basis for Biological Exposure Indices

While most BEIs are based on overall exposures equivalent to inhalation exposures at an OEL, several provide the basis for the corresponding airborne OEL. For example, airborne OELs for carbon monoxide, acetylcholinesterase inhibitors, certain solvents like hexane, and most heavy metals represent inhalation exposures that are expected to cause measurable biological concentrations or changes that available data indicated should be safe for most workers. For substances with low potential for inhalation exposure that are readily absorbed through the skin, there is likely to be little correlation between airborne concentrations and measurement of biological determinants. BEIs for these substances are based on the relationships between health effects and the biological concentrations of the determinants.

While most BEIs are quantitative, data sometimes support only a screening-type guideline that is nonquantitative or semiquantitative. Such guidelines typically are used for substances on which there are good qualitative data on human exposure and the biological determinant concentration, but poor quantitative data relating exposure to the determinant. They most commonly are used for substances that cause chronic, systemic health effects when absorbed through the skin. Nonquantitative determinants are useful especially for substances, like 4,4′-methylene bis(2-chloroaniline) (MBOCA), that meet these criteria and

for which there is a long lag time from exposure to health outcome and low or no background level of the determinant in the unexposed population. While there are several good methods to measure either MBOCA or its metabolites in urine, none of these measurements relates well enough to exposure or risk of health effects to determine a quantitative biological exposure index. So, for example, the ACGIH BEI for MBOCA is a nonquantitative guideline for total MBOCA in urine.

Applying Biological Exposure Indices

In addition to providing comprehensive estimates of recent exposures, in many cases biological monitoring can allow health professionals to do one or more of the following:

- measure body burden of a chemical;
- supplement air monitoring to document exposures;
- detect small exposures;
- distinguish nonoccupational exposures;
- identify unknown or undiscovered exposures, especially from noninhalation sources such as dermal absorption or ingestion;
- examine effectiveness of engineering controls, work practices, and personal protective equipment;
- follow trends of exposure over time;
- reconstruct past exposures; and
- enhance individual or group risk assessments.

As is the case for all types of OELs, credible BEIs are explained and supported by documents that critically review the scientific criteria on which they are based and often provide practical information for their application. These documentations generally describe the following types of pertinent information:

- scientific rationale;
- sampling and analytical methods;
- quality control measures;
- issues related to specimen collection and storage;
- potential for confounding exposures;
- typical background concentrations of the determinant;
- quality of the relevant database;
- other limitations; and
- research needs.

Like all occupational exposure values, BEIs should be used by knowledgeable health professionals who understand their bases and how they are intended to be applied.

See also: American Conference of Governmental Industrial Hygienists; Occupational Toxicology; Occupational Exposure Limits.

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Biomarkers, Environmental

Lee R Shugart

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Introduction

Ecotoxicology is a relatively new scientific discipline and is a branch of toxicology that studies the subtle toxic effects that pollution exerts on living organisms by investigating the fate and effects of chemicals and natural substances on ecosystems. The term ecotoxicology merges the fields of ecology and toxicology. A major difficulty with the merging of these two fields is that each focuses on a different organizational level. Toxicology is concerned with adverse effects of chemicals on living organisms, whereas ecology is focused at the population, community, or even ecosystem level. Ecotoxicology is sometimes used synonymously with environmental toxicology; however, the latter also encompasses the effects of environmental pollution on humans.

There are many tests available to the ecotoxicologist to measure toxicity of chemicals but the problem becomes one of the extrapolation of data from a few species to many and from laboratory or

limited field tests to effects on natural communities and ecosystems. Although pollution can produce stress at the ecosystem level, the response observed is latent and so far removed from the initial event of exposure at the organismal level that causality is almost impossible to establish.

Biological Markers

Definition/Classification

The use of biological responses (biological markers or biomarkers) in organisms exposed to toxic substances is an approach that may help resolve the problems of causality. Chemicals and physical agents are known to elicit measurable and characteristic biological responses in exposed organisms and such evidence can provide a link between exposure and effect.

The definition of biomarker used here is 'a biological response to a chemical(s) that gives a measure of exposure and sometimes also of toxic effect in an organism'. Biological responses measured above the organism level are considered as 'ecological indicators'. Thus, biomarkers are any of a series of biochemical or molecular responses to compounds that have entered an organism, reached sites of toxic action, and are exerting an effect on the organism. In this context, the organism functions as an integrator of exposure, accounting for abiotic and physiological factors that modulate the dose of toxicant taken up from the environment. Because of the commonality of biochemical and cellular structure and function among all living organisms, biomarkers are potentially applicable over a broad range of species and across most ecosystem types.

Four classes of biomarkers have been proposed: exposure, effect, exposure/effect, and latent effect. The scientific journal *Biomarkers* makes the following division: biological markers of disease and of response, exposure, and susceptibility to drugs and other chemicals. The latter category, susceptibility, is considered to include genetic factors and changes in biological receptors, which alter the susceptibility of an organism to exposure to a chemical substance. These subdivisions seem artificial since all biomarkers are, by definition, biomarkers of exposure. Whether they are also biomarkers of effect depends on the state of our scientific knowledge.

Specific and Nonspecific Biomarkers

The specificity of biomarkers to chemicals varies greatly. Both specific and nonspecific biomarkers have their place in environmental assessment. A nonspecific biomarker can tell one that a pollutant is present in a meaningful concentration but does not tell one as to which chemical is present. Based on this information a more detailed chemical investigation can be justified. In contrast, a specific biomarker tells one about which chemical is present, but gives no information on the presence of other chemicals.

Criteria for Evaluating Biomarkers

A list of criteria for evaluating biomarkers that should be given consideration are

1. *Biological specificity*. It is important to know which classes of organisms the biomarker may be used on. The inhibition of the enzyme acetyl choline esterase (AChE) by organophosphates and carbamates can be applied throughout the animal kingdom whereas the induction of vitellogenin is confined to those vertebrates that lay eggs.
2. *Clarity of interpretation*. How clear cut is the endpoint as an indicator of exposure to anthropogenic stress? Can the endpoints be clearly distinguished from natural stresses? It is valuable to know the mechanism of response to the chemical in assessing this point.
3. *Time of responses*. The temporal expression of different biomarkers can vary widely from nearly instantaneous to years. Depending on the type of study, slow or rapid manifestation maybe desirable.
4. *Permanence of response*. Similarly, it is important to know how long the response lasts. If it is transient, it may readily be missed. The inhibition of AChE, especially in blood, is a transient response and thus it is necessary to know when the exposure occurred to assess the importance of the degree of inhibition. In contrast, the inhibition the enzyme amino levulinic acid dehydratase by lead is only slowly reversed.
5. *Reliability*. This can be considered under two headings: (1) environmental influences that modulate the organism's response to a chemical, and (2) inherent variation in the biological response to a given exposure. It is important to know the extent of all variations in order to have a reliable biomarker.
6. *Methodological considerations*. Important considerations here are precision (analytical reproducibility of the method), cost, and ease of the analysis. Although many reliable assays have been developed there is a need for standardization, along the lines used in analytical chemistry, so that results from different laboratories are comparable.

7. *Relative sensitivity*. It is important that the biomarker be sensitive when compared to other endpoints, such as mortality or reproductive impairment, and it is important to know the relative sensitivity of this comparison.
8. *Validation in the field*. For a biomarker to be useful in environmental assessment, it must be validated in the field. Organisms in the field are subjected to a wide range of variables that are usually accounted for or controlled in laboratory experimentation.
9. *Linkage to higher-level effects*. A biomarker is more useful if there is clear linkage to effects at higher levels of organization. Studies on invertebrates have been particularly fruitful as population changes occur more rapidly than in higher species.

Biological Monitoring

At the present time there is a strong drive to clean up the environment; however, since costs increase rapidly with the degree of cleanup, there is a pressing need to have a practical, defensible strategy that provides information for establishing both priorities for environmental restoration and endpoints for regulatory compliance. The study of biological responses in living organisms to contaminants in their environment (i.e., biological monitoring) is an informative, cost-effective, and logical complement to chemical monitoring of toxicants of many environmental monitoring programs.

Ecological Application

In order to predict and avoid unacceptable health risks associated with environmental pollution such as disease in humans, mass mortality, and loss of commercially or ecologically important species, an understanding of the critical cellular events between exposure at the organismal level and effects expressed at higher levels of biological organization must be established. In this context, it is often necessary to regress along the conceptual sequence of responses to toxicant exposure and select appropriate biological responses that are causally related to, or predictive of, longer term effects. The biochemical and physiological consequences of toxicant exposure of most concern to ecotoxicologists are those that might affect reproductive health. There is a need to relate biomarker responses to changes in Darwinian fitness parameters in individuals so that population and other effects at higher levels can be predicted. In this regard there is considerable evidence that some toxicants may potentiate any number of measurable biological mechanisms (i.e., mutational events in embryonic tissue, impaired growth, and change in genetic diversity) that eventually manifested this toxicity at the population level.

Concept of Meaningful Exposure

Biomarkers have the advantage over chemical analysis in that they can demonstrate whether or not an organism is meaningfully exposed. For some classes of persistent organic chemicals, especially the organochlorines, detection limits are now down to parts per trillion. Thus, in almost all samples these man-made chemicals can be detected, but the physiological significance is rarely known.

With biomarkers it is possible to determine if the physiology of the organism is significantly different from normal. If it is, then the organism is considered to be meaningfully exposed. Equally important, if the physiology is not significantly different, then the organism is considered not to be meaningfully exposed even though the chemical(s) can be detected. The ability to determine whether or not an organism is meaningfully exposed is important in making the decision whether regulatory action should be taken and also in making the decision whether or not remedial action has been successful. The following criteria need to be met before the concept of meaningful exposure can be used. These are

1. Data must be available on what is normality for each biomarker. Because of the diversity of species involved, this is a good deal more complex than in the case where biochemical levels are used in the diagnosis of human health. Obviously, it is impossible to have data on all species. While there is a great deal of data available, there is a need for a centralized database to collect, verify, and validate this baseline information.
2. To adequately assess the impact of the major classes of chemicals of concern, biomarkers are needed that indicated the status of the important functions of the organism. While we have not reached this point yet, the rate of progress toward the goal is encouraging.

See also: Biomarkers, Human Health; Chemicals of Environmental Concern; Ecotoxicology.

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Biomarkers, Human Health

Rogene F Henderson

Introduction

In the field of environmental health research, it is often difficult to determine if exposures to a specific chemical or to a chemical mixture have induced an adverse health effect. In the 1980s, a promising field of research, focused on 'biological markers' (or 'biomarkers'), began to develop as an aid for linking toxicant exposures with potential health effects. A biological marker is defined by the National Research Council (NRC) of the National Academy of Sciences (NAS) as an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism. Biomarkers are always found within an organism and may be used to demonstrate the relationship between exposure, internal dose, dose to target organ, biologically effective dose, initial biological effects, and induced adverse health effects (**Figure 1**).

In the absence of biomarkers, epidemiology studies have relied on external indicators of exposure (area or personal monitors, questionnaires, histories) to establish associations between exposures and subsequent disease development. Such tools are not highly accurate, and most studies address only exposures to populations, not the dose received by individuals. New molecular techniques, such as measurements of chemical adducts formed by exogenous compounds with macromolecules in the body, have provided the tools for assessing the dose received by an individual; the same molecular techniques also provide information on the initial changes induced by the biologically effective dose, changes that may eventually lead to disease. These new biological markers take into account individual variability in processing of potential toxicants and show promise for providing more accurate information for the assessment of the effect of exposures to noxious agents on the induction of adverse health effects in individuals.

The most useful biomarkers are those that are chemical-specific, and: (1) quantitatively reflect the degree of the prior or ongoing exposure; or (2) quantitatively reflect, or predict, later developing disease.

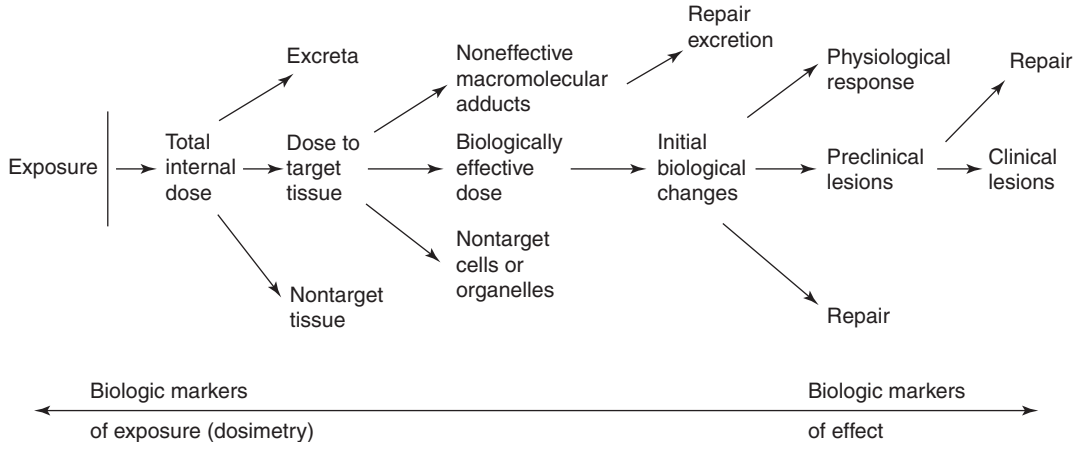

Figure 1 Biomarkers for risk assessment. Toward the left are biomarkers of exposure (dosimetry); most of these markers represent values obtained from toxicokinetic studies. Toward the right are biological markers of effect; many of these markers are standard signs and symptoms familiar to clinicians. The goal of biomarker research is to obtain more information on the link between biologically effective doses and the early, initial biological changes that can lead to disease; such values will come from studies on the mechanism of disease induction.

Ideally, the biomarker of the extent of exposure could also be used to predict the health outcome, but it is rare that sufficient information is available to make such predictions. Quantitation is needed when establishing regulations governing allowable exposures that are protective of health. If it is only important to determine if an exposure has occurred, the presence of a biological marker specific for the chemical of concern may be all that is needed. However, for the purposes of risk assessment – that is, determining the potential for a given exposure to an exogenous substance to cause adverse health effects – quantitation of the amount of biomarker present is required.

There are also practical limitations to the selection and use of biomarkers in human studies. The biomarker should be measurable in a relatively available tissue or fluid; for example, urine and breath. Sampling blood is an invasive process and so is more difficult to perform although it is done routinely. However, sampling liver tissues from humans for DNA adducts is much too invasive and would not be performed except at the time of autopsy. In addition, the assays for the marker of interest should not be so expensive that the cost of a study using the marker is prohibitive. Finally, the marker must be validated for its accuracy in quantitatively reflecting either exposure or health outcome. Otherwise, the results of the biomarker assays cannot be interpreted.

Biomarkers are generally divided into three categories: markers of exposure, markers of effects, and markers of susceptibility. Each of these types of biomarkers is described below, along with how they may be used in risk assessment.

Biomarkers of Exposure

As stated previously, the NRC/NAS has defined biological markers as exogenous substances or their metabolites or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism. Biomarkers of exposure are measures of internal substances, and thus reflect various manifestations of the internal dose that results from exposures. Markers of interest include those that provide measures of the: (1) total internal dose (such as blood, urine, or breath levels of a chemical); (2) dose to a target organ (which may be in the form of macromolecular adducts formed between the chemical or its metabolite and the organ tissue); or (3) biologically effective dose (which can only be measured if the mechanism of disease induction is known in sufficient detail to suggest what entities might represent the biological effect). An example of the last type of marker is a specific DNA adduct known to lead to a disease.

Some of these biomarkers are the same parameters measured in classical pharmacokinetic (or toxicokinetic) studies such as blood, urine, or breath levels of a substance. Other biomarkers have become available only recently due to the development of new techniques that allow detection, and sometimes quantitation of macromolecular adducts formed from the interaction of the chemical of interest or its metabolite with blood proteins or tissue DNA. Even if the biomarkers of exposure are not thought to lead to disease, such biomarkers can be useful if they can be linked quantitatively to other biomarkers that do lead to disease. For example, knowledge of the quantitative relationship between levels of hemoglobin adducts formed during exposure to a specific chemical (example of an adduct formed with a blood protein and an adduct that is not known to lead to disease) and levels of liver DNA adducts (example of an adduct formed in the target tissue and a biomarker of the biologically effective dose) from the same exposure to a liver carcinogen, could lead to the use of the more available blood adducts (vs. liver tissue adducts) as predictors of the biologically effective dose.

Strategies for Use of Biologic Markers of Exposure to Assess Prior Exposures

Many commonly measured pharmacokinetic values can be used as biomarkers of exposure. Examples include parent compound or metabolites in exhaled breath, blood, or urine and macromolecular adducts or their degradation products that appear in urine. To make quantitative assessments of the relationship of such markers to prior exposures, it is necessary to determine the rate of formation and removal (clearance) of the marker. From this information it is possible to predict the steady-state concentrations of the marker following various exposure scenarios. In addition, with information on the rate of formation and removal of a marker and knowledge of the factors that influence those rates (such as gender, dose, repeated exposures, route of exposure, rate of exposure), a mathematical model that describes the concentration of the marker under different exposure conditions can be developed. While the concentration of the marker cannot be used to identify a unique exposure scenario, the marker can indicate the types of exposure regimens that would produce the measured level of the biomarker.

From a practical viewpoint, human populations cannot be used to determine the rate of formation and clearance of markers and the influence of various factors on those rates. Therefore, most toxicokinetic studies are conducted in animal models. From detailed studies in animals, mathematical models are

derived based on the animal toxicokinetic data, animal physiological data, and the physical/chemical properties of the compounds of interest (such as partition coefficients). The models, which are often referred to as 'physiologically based pharmacokinetic models', can then be modified for use in making predictions for humans by substituting human physiological data into the model and using the results of metabolic rate studies conducted with human tissues *in vitro*. The validity of such modified models must then be verified by limited studies in humans.

A second strategy for the use of biomarkers in establishing prior exposures is to make use of a battery of biomarkers with differing half-lives. Some biomarkers of exposure have half-lives of minutes or hours (volatile parent compound in exhaled breath, some blood or urinary metabolites); other biomarkers may be present for days or weeks (some DNA adducts, blood albumin adducts); while others may accumulate over longer periods of time due to longer half-lives (blood hemoglobin adducts, some DNA adducts, products of DNA repair in the urine). There are also differences in the fraction of the internal dose of a chemical that is converted to each type of biomarker. In general, some markers with shorter half-lives, such as urinary metabolites, represent large fractions of the internal dose, while macromolecular adducts, many of which have longer half-lives, represent only a small fraction of the dose. By combining knowledge of the half-lives of markers and the amount of marker formed relative to the total dose, it is possible to obtain more information about a prior exposure using a battery of biomarkers rather than by using a single biomarker. For example, if multiple markers of a single chemical are determined in an individual, it should be possible to distinguish between someone who has had a recent exposure, someone who is receiving an ongoing exposure, and someone who was exposed repeatedly in the past but has had no recent exposures. If someone has had only a recent single exposure to a chemical, the shorter half-life, more abundant biomarkers in the form of urinary metabolites should be readily detectable, but there should be very little of the longer half-life, less abundant DNA adducts present. If the person has had an ongoing exposure for many years to the same chemical, there should be relatively high amounts of both the urinary metabolites and the DNA adducts. If the person was exposed some time ago but not recently, then only the longer-lived DNA adducts or hemoglobin adducts may be detectable.

Biomarkers of Effect

The NRC/NAS defines a biomarker of effect as any change in a biological system that is qualitatively or quantitatively predictive of health impairment or potential impairment resulting from exposure. While a distinction is made between biomarkers of exposure and biomarkers of effect, in practice, the two areas overlap. For example, DNA adducts may be biomarkers of exposure, but if they occur at specific sites known to induce mutations leading to cancer, the adducts also may be biomarkers of effect.

Many types of responses may be made to toxicants. Some of the induced effects may be merely physiological responses that are not deleterious. Other responses may be deleterious, but are quickly repaired. But some responses represent the earliest indicators of a change that, if persistent, can lead to an adverse health effect. The persistence and amplification of such a response leads to a clinical disease state. The most useful biomarker of an effect is one that is definitive for a specific adverse health effect, is quantitatively predictable for health outcome, and occurs early enough in the process that its detection allows intervention in the disease process. Signs and symptoms that occur in later stages of a disease process are the tools of clinical medicine; the goal of scientists working on biomarkers is to discover preclinical markers of the early stages of a disease process when intervention is still possible. Examples of early markers of a disease process might be a preneoplastic, proliferative lesion, an increase in a cytokine that is associated with a fibrotic process or the under- or over-expression of a gene known to be associated with a disease process.

New genomic techniques have been developed that can be used to measure the responses of the total gene array of a tissue in one assay. These responses can be considered biomarkers of either exposure or of effect. The challenge now is to assemble and analyze the tremendous amount of data made available by this new field of toxicogenomics and to interpret the meaning of the responses. In the future, it is hoped that the responses can be used to elucidate the pathways involved in the early stages of disease development.

To relate biomarkers of exposure to health outcomes, it is necessary to know which markers can be associated with the disease outcome and the degree of that association. That is, given the presence of a certain level of a biomarker, what is the probability of contracting a disease? This query is certain to be made by participants in any occupational or general population study in which biomarkers are assayed. Currently, very little information is available on which to base an answer. The inability to use biological markers of either exposure or effect to predict health outcome represents a major gap in knowledge and decreases the potential usefulness of the markers. What are needed are valid markers of risk.

Strategies for Improving the Ability to Link Biomarkers of Effect to Disease Outcome

Perhaps the most fruitful area of research for identifying biomarkers of exposure that can be linked to disease outcome is the study of mechanisms of disease induction. It is not possible to define a marker of a 'biologically effective dose' unless the mechanism by which the biological effect is induced is known. Likewise, the earliest biological events that lead to a disease cannot be determined unless the mechanism of disease induction is understood. Mechanistic studies should help to link the biologic markers represented by traditional toxicokinetic measurements and the biologic markers represented by traditional clinical markers of disease.

In addition to knowledge on the mechanism of disease induction, it is necessary to define the quantitative relationship between the level of the marker and the probability of progression to an adverse health effect. To accomplish this, pharmacodynamic or toxicodynamic modeling describing the kinetics of disease development, similar to the toxicokinetic modeling used to describe the kinetics of internal dosimetry, is required. For example, to use chemical-specific DNA adducts to predict cancer induction, the following pieces of information are required. First the various DNA adducts formed by the chemical must be identified. Then the biological half-lives of each adduct (How long will they be present before they are repaired?) and the mutagenic potential of each adduct (How much harm will the adducts cause if they are present?) must be determined. If adducts are formed that have relatively long half-lives and high mutagenic potential, it is possible to determine if the mutations induced by the adduct in *in vitro* studies are present in tumors induced by the chemical. Once enough is known about the disease induction to form a hypothesis for the process, intervention studies, in which the proposed path to disease is blocked, can be used to validate the path as the active disease generating process. Finally, toxicodynamic models can be generated that describe the quantitative relationship between adduct levels and cancer induction. Such models require knowledge of the cellular dynamics involved in tumor formation.

Biomarkers of Susceptibility

Indicators of individual or population differences that influence the response to environmental agents are called 'biomarkers of susceptibility'. These indicators might include such characteristics as an enhanced metabolic capacity for converting a chemical to its reactive, more toxic metabolite; an enhanced capacity to detoxicate reactive metabolites; or differences in number of receptor sites that are critical for a specific response. An example is the inherited deficiency in the enzyme, α-1-antitrypsin, which is associated with an increased susceptibility to development of emphysema. The new assays developed by researchers in the field of toxicogenomics allow detection of genetic polymorphisms that can affect susceptibility to pollutant exposures. Such markers can be quite valuable in providing information that can contribute to protection of susceptible populations. Knowledge of the mechanisms of susceptibility can also be important in designing therapy for a disease. However, the use of such markers is fraught with legal and ethical problems, because identification of persons with enhanced susceptibility to adverse health effects from exposure to chemicals could lead to discrimination against those persons in obtaining jobs and insurance.

Uses of Biomarkers

As mentioned in the beginning of this section, a major potential use for biological markers is to link environmental exposures causally and quantitatively to health effects in individuals or populations. If the whole chain of events illustrated in **Figure 1** can be defined in a quantitative fashion for a single toxicant, it may be possible to regulate the exposure to such a toxicant and to prevent adverse health effects with a reasonable degree of certainty. This would avoid over- or under-estimation of the risk from such a toxicant. In practice, this use of biomarkers is still in its infancy because of insufficient data to fill out all the information illustrated in **Figure 1**. Strategies for improving the use of biomarkers to quantitate the ability of environmental agents to produce adverse health effects have been discussed.

Another practical use for biomarkers is to detect and quantitate prior or ongoing exposures to specific chemicals; biomarkers have been successfully used in biological monitoring programs in industry but have only recently been used to monitor environmental exposures. Medical researchers are seeking biomarkers that can be employed to: (1) detect early stages of a disease to enhance successful intervention; (2) determine the effectiveness of intervention strategies; and (3) detect cells at risk from a toxicant. Finally, research is ongoing, particularly in the field of genetics, to find inherited biomarkers of susceptibility that can be used for the detection and protection of sensitive populations.

See also: Analytical Toxicology; Biomarkers, Environmental; Carcinogen–DNA Adduct Formation and DNA Repair;

Epidemiology; Mechanisms of Toxicity; Medical Surveillance; Molecular Toxicology–Recombinant DNA Technology; Pharmacokinetic Models; Pharmacokinetics/Toxicokinetics; Risk Assessment, Human Health.

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Biomonitoring

Chris Theodorakis

Environmental surveillance is the systematic collection of samples of air, water, soil, sediment, and living organisms to determine the magnitude, persistence, and severity of environmental contamination. Biomonitoring is the biotic component of environmental surveillance, in which living organisms, including humans, are used to assess environmental (or occupational, in humans) contaminant exposure and effects. Ecological applications of biomonitoring include: (1) ecological risk assessments; (2) determining the efficacy of pollution remediation ('cleanup'); (3) assessing the effects or persistence of 'environmental disasters' such as chemical/oil spills or accidental releases; and (4) assessing compliance with environmental regulations. In fact, any industry, company, or facility that discharges effluent (wastewater) into a natural body of water ('receiving water') must have a National Pollutant Discharge and Elimination System (NPDES) permit, as required by the Clean Water Act. Biomonitoring of the effluent and receiving waters is a mandatory requirement of all NPDES permit holders. Components of ecological biomonitoring may include: (1) collection of contaminated media for laboratory toxicity testing; (2) *in situ* exposures of organisms at contaminated sites; or (3) field collections and surveys of organisms from contaminated sites.

Contaminated media that are collected for laboratory toxicity tests may include contaminated water, soil, or sediment (the 'mud' at the bottom of lakes, rivers, streams, bays, etc.), or extracts of these media. Organisms used for conducting soil toxicity tests may include worms, insects, plants, or bacteria. In aquatic systems, effluent water is collected from discharge pipes and tested in whole effluent tests. The effluent is diluted with noncontaminated water in the laboratory to provide dose–response curves. Subchronic or acute toxicity tests are usually carried out with fish, crustaceans, or algae. Such tests may also be carried out on the receiving water. Sediment may also be collected from contaminated sites, and toxicity tests carried by placing freshwater or saltwater worms, clams, or sediment-dwelling crustaceans in contaminated sediment. Other soil/sediment toxicity tests include elutrate and pore water toxicity tests. Elutrate is an aqueous extract made by vigorously mixing soil or sediment with water, and removing solid material by centrifugation or filtration. Pore water is extracted from wet sediment by centrifugation at high speeds to collapse the sediment and squeeze out the water between sediment particles. Toxicity tests are then conducted with the elutrate or pore water. Besides toxicity endpoints such as survival, growth, reproduction, and seed germination, bioaccumulation of chemicals from the soil or sediment may also be measured.

Environmental contaminants are present as complex mixtures, so that if toxicity is found, procedures known as 'toxicity identification evaluation' may be carried out. This procedure identifies toxic components by systematically treating the effluent, elutrate, or pore water to remove various fractions – hydrophobic ('fat soluble') chemicals, metals, acids, volatile compounds, etc. – and retesting the toxicity after each extraction. Loss of toxicity after an extraction implicates the chemical that was extracted. This is confirmed by chemical analysis and toxicity tests on

the extracted fraction and the pure chemicals therein. After the toxic chemical has been identified and industrial processes have been modified to remove it or reduce its concentration to nontoxic levels, follow-up 'toxicity reduction evaluation' procedures are carried out to confirm that the toxicants have been removed or abated.

In contrast to the aforementioned toxicity tests, *in situ* toxicity tests involve exposing organisms to contaminants on-site. This provides for more environmental realism, but there is also less control over confounding variables that may affect toxicity (spatial or temporal variation in temperature, sunlight, nutrients, pH, etc.), or other factors that may disturb or disrupt the test (animals, winds, floods, vandalism, etc.). For these tests, animals may be placed in mesh cages or corralled by impermeable barriers, such as wood, metal, or plastic sheets, at various locations throughout the contaminated zone. Plants may be planted in plots of contaminated soils. Toxicity endpoints may include survival, sublethal effects, or accumulation of contaminants in body tissues. For these tests, organisms are also placed in less contaminated sites for comparison.

An alternative to *in situ* exposures or toxicity tests is to conduct field surveys, in which organisms that are indigenous to the contaminated sites are collected and analyzed. Endpoints that are examined may be concentrations of chemicals in living organisms ('body burden'), sublethal responses, ecological effects, abundance of indicator species, or biotic indices. Indicator species are those whose abundance or presence/absence is indicative of pollution stress. Examples of indicator species include (1) aquatic tubificid worms, which thrive in streams that are impacted by sewage effluent; (2) certain midge fly (Chrionomid) larvae tolerant of low dissolved oxygen in the water; and (3) many darter fish species, which are intolerant of pollution or heavy silt loads in the water. Biotic indices (e.g., the Index of Biotic Integrity) use a variety of metrics to calculate a score that can be used to compare the degree of pollution in different streams or rivers. Such metrics include species diversity, abundance of indicator species or pollution tolerant/sensitive species, prevalence of gross injuries (tumors, lesions, deformities, etc.), and relative abundance of organisms at different trophic levels (e.g., carnivores, herbivores, omnivores). Similarly, the 'EPT Index' uses the relative abundance of pollution-intolerant mayfly (Ephemeroptera), stonefly (Plecoptera), and caddisfly (Tricoptera) larvae as a measure of the relative degree of pollution in streams and rivers.

However, there are a variety of 'confounding variables' such as temperature, season, and water or soil chemistry, and other disparities between sites that have nothing to do with degree of contamination, but may affect relative differences between contaminated and reference (noncontaminated) sites. Therefore, in a variety of 'Criteria for Establishing Causality' have been developed in order to aid in distinguishing between natural variation and pollution impacts. Also, biomonitoring efforts that integrate a variety of different endpoints are also useful in differentiating between natural variation and effects of environmental contamination. For instance, sediment toxicologists often use a 'Sediment Quality Triad' to assess contaminant effects. This triad includes chemical analysis of pollutants, sediment toxicity tests, and determination of the diversity of aquatic invertebrate species in contaminated sediments.

Other recently developed biomonitoring technologies involve the use of biosensors or genetically engineered organisms (GEOs). Biosensors consist of both biotic and electronic sensing components enclosed in a chamber. The electronic sensing components include electrodes, video cameras, or fiber optic sensors. Examples of biotic components are isolated cellular constituents (enzymes, DNA, etc.), bacteria and other microbes, cultured cells, or multicellular organisms (fish, plants, clams, or other invertebrates, etc.). The concept behind biosensors is that a biotic response – for example, a change in enzyme activity, DNA integrity, metabolism, or animal behavior – is converted into an electronic signal that can be remotely recorded to detect release of environmental contaminants in real (or near real) time. Biosensors can be stationed at field sites, with the signal relayed to a recorder via electric cables or radio transmissions. Alternatively, air or water can be continuously collected from field stations, and organisms can then be exposed to these media in mobile or stationary labs located on site.

GEOs that have been developed for biomonitoring include microbes, cultured cells, fish, plants, rodents, and invertebrates. Some of these can produce colored, fluorescent, or luminescent ('glowing') substances when exposed to specific contaminants. Such changes can be detected visually with the naked eye or, if tissue sections, isolated cells, or small organisms are used, with the aid of a microscope. Alternatively, electronic devices such as spectrophotometers, colorimeters, fluorometers, or luminometers can be used to quantify the signal in whole cells, tissue sections, or tissue homogenates. GEOs can be used in the laboratory or as part of a biosensor. Other GEOs have been developed that have bacterial or viral DNA inserted into their genome. These genetic markers can be isolated from the DNA of the GEO and analyzed for mutations or other DNA damage.

Other endpoints often used in biomonitoring are 'biomarkers', which can be defined as alterations of physiological, cellular, biochemical, or molecular structures or processes that are indicative of contaminant exposure and effects. Examples of such biomarkers include histopathology, induction of contaminant detoxification enzymes, induction of enzymes that repair molecular or cellular lesions, damage to biological macromolecules (proteins, lipids), DNA damage (genotoxicity), inhibition of endogenous enzyme activity, patterns of gene expression, and metabolites of xenobiotic chemicals or endogenous compounds. Biomarkers of exposure – which include such endpoints as contaminant metabolites in body fluids – are unambiguous and specific indicators that an organism has been exposed to xenobiotic compounds. However, the consequences of such biomarker responses to overall health of the organism may be unclear. Biomarkers of effects (e.g., histopathology, gross lesions) are unequivocal indicators of contaminant-induced harm to the organism, but the causative agent may not be implicated. In reality, however, most biomarkers can be classified on a gradient between two extremes. Some biomarkers are specific indicators of exposure or effects of individual chemicals, others may be indicative of a class of chemicals (e.g., heavy metals, aromatic hydrocarbons, organophosphate pesticides), while still others are responsive to a wide array of chemicals. Recent advances in molecular techniques and gene expression assays, as well as genome sequencing projects, have contributed great opportunities for discovery and interpretation of biomarkers, as well as provided insight as to the mechanism of action of single chemicals and complex mixtures. Studies that use a large suite of biomarkers are also useful in discriminating between natural variation and pollution impacts, particularly if complex mixtures of pollutants are present, especially when used in combination with chemical analysis, ecological indicators, and other sublethal effects.

Biomonitoring for human health hazard surveillance typically involves collection of hair, expired air, bodily fluids (blood, urine, saliva, breast milk, semen), feces, epithelial scrapings, exfoliated cells, or, less frequently, tissue biopsies from people known or suspected of being exposed to potential chemical, physical, or biological hazards. These samples are analyzed for toxic chemicals, their metabolites, or biomarker responses. The most commonly used biomarkers in humans are those relating to DNA and chromosomal damage, because these effects contribute to cancer risk. Besides biomarkers of chemical exposure and effects, there are also human biomarkers of tumor formation and susceptibility to toxic chemicals or cancer. Biomarkers of tumor formation are proteins, metabolites, or malformed cells in biological samples that are indicative or highly suggestive that a tumor has formed in the body. Such biomarkers may be used by clinicians as early warning indicators and noninvasive methods for assessing carcinogenesis. Biomarkers of susceptibility include genetic markers or enzymatic activities for chemical detoxification and DNA repair systems in the body. These biomarkers indicate relative risk of effects from toxic chemical exposures in individual members of the population. Similar to ecological biomonitoring studies, human health biomonitoring may involve collection of environmental media (air, water, soil, food) or chemicals extracted from such media for determination of effects in human cell or tissue cultures and animal models of human health risk (rodents, rabbits, primates, etc.).

Human health biomonitoring may also use animal surrogates in the environment to assess potential health hazards to humans – the proverbial 'canary in the coal mine'. For example, chemical and biomarker analysis of bodily fluids or tissue biopsies from family pets, especially dogs, are sometimes used to assess potential chemical exposure and effects in children. This is because dogs often accompany children in the outdoor environment, and both have a tendency to (accidentally or intentionally) consume environmental media such as soil and surface water. There has also been an increasing trend to use native animals as sentinel species, that is, fish, wildlife, or invertebrates that are indicators of possible human health risks from environmental hazards. For example, increased incidences of tumors or endocrine disruption in fish may indicate the presence of compounds in the water that may cause cancer or reproductive dysfunction in humans. Concern has also been raised over the increased incidence of deformities in frogs, because these may indicate an increased level of chemicals in the environment, which can cause birth defects in humans.

Human health biomonitoring using biomarkers and chemical analyses are used in the following applications: (1) Health surveillance of persons who are known to have high occupational or environmental exposures to potentially toxic chemicals. This may include those who work with chemicals, radioactive materials, or biohazards as part of their occupation. Examples include factory workers, chemical industry employees, farmers, health care professionals, nuclear plant employees, and veterans of the Gulf War I. This may also consist of those who are involuntarily exposed to such hazards in their everyday surroundings. Some examples are people living near land fills, factories, hazardous waste sites, or environmental catastrophes such as the Chernobyl

nuclear plant explosion, chemical spills, and other accidental releases of hazardous materials. (2) Human health risk assessment of environmental or occupational health hazards. (3) Epidemiological studies of occurrence or potential for environmentally induced diseases. Such studies that use molecular biomarkers are termed 'molecular epidemiology' studies. (4) Assessment of potential health effects of certain behaviors such as smoking and alcohol consumption.

See also: Biomarkers, Environmental; Biomarkers, Human Health; Ecotoxicology; Environmental Health; Environmental Toxicology.

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Bioremediation

Lee R Shugart

Worldwide, the introduction of a wide variety of anthropogenic chemicals into waters and soils has caused a growing concern about the consequences of such practices. Public awareness concerning the vulnerability of the environment to pollution has only been heightened by major incidents such as the Union Carbide (DOW) Bhopal and the Seveso disasters, the Three Mile Island and the Chernobyl accidents, and the Amoco Cadiz and the *Exxon Valdez* oil spills.

Environmental pollutants are defined as chemicals of natural or synthetic origin that are released by various human activities into the environment where they have an undesirable effect on the environment and/or on humans. Heavy metal compounds are found as environmental contaminants. Organic compounds are the most common and range from slightly water-soluble organics such as aromatic and halogenated hydrocarbon solvents to hydrophobic organics such as polychlorinated biphenyls (PCBs) and aliphatic hydrocarbons. Organic solvents degrade only slowly, if at all, once they enter groundwater. Hydrocarbon compounds such as fuels, lubricating oils and creosote contain toxic components. Naphthalene and its methyl-substituted derivatives are some of the most acutely toxic, water-soluble components of crude oils. As the molecular size of hydrocarbons increases, their lipophilicty, environmental persistence and mutagenicity also increase. Many chemicals represent classes of molecules not previously investigated, some have no close structural analogs in nature, and there are those that were intentionally developed to be resistant to microbial attack and to persist in nature.

Over 80% of industrial wastes, much of which can be classified as hazardous, are disposed of in landfills. Many chemicals enter the environment directly as a result of accidents, spills, or leakage from industrial facilities and waste disposal sites. In the past, contaminated wastes were buried, burned, or chemically treated in place. These treatments are costly, have limited effectiveness, and are difficult to regulate. Landfill and *in situ* fixation do not destroy waste, and landfilling only changes the place of residence delaying future liability. Contamination of the environment has placed many of our vulnerable resources (e.g., groundwater, wet lands, fisheries, and agricultural lands) at risk.

The release by humans, intentionally or otherwise, of chemicals and other pollutants into the environment has forced government, industry, and the public to come to grips with the undesirable consequences to the environment and to human health. As a result, the US Federal Government enacted laws to ameliorate these problems and included the Safe Drinking Water Acts of 1974, the Resources Conservation Recovery Act of 1976 (RCRA), the Clean Water of 1977, the Comprehensive, Environmental Response, Compensation and Liability of 1980 (CERCLA), and the Superfund Amendments and Reauthorization Act of 1986 (SARA). These laws focus on problems associated with the cleanup of disposal sites and spills of toxic substances and also with the need to reduce the volume and toxicity of waste as well as to develop safe and effective alternatives for waste disposal. In this context, the biotechnology industry has embraced bioremediation as a safe approach for these problems. Bioremediation is the enhanced microbiological treatment of unwanted chemicals of natural or synthetic origin released by human activities. An old technology once primarily used in wastewater treatment, today bioremediation is routinely applied to a wide variety of environmentally contaminated sites. Bioremediation techniques are versatile and can be used for raw materials before processing, pipeline wastes, decontamination of soils and surface groundwater, and the cleanup of dumpsites. Its salient features include attractive economics, undisturbed environment, destroyed contaminants, and eliminated liability.

Natural attenuation in contaminated environments is accomplished by biochemical degradation, evaporation, adsorption, metabolism, and transformation by microorganisms. Microorganisms such as bacteria, actinomycetes, and fungi are capable of degrading a wide range of organic compounds via biodegradation. Indigenous microbial populations, those occurring naturally, are the chief agents involved in the metabolism of chemicals in waters and soils. Metabolism may result in mineralization, which is the complete biodegradation of an organic molecule to inorganic compounds. Microorganisms can also transform hazardous organic compounds into innocuous or less toxic organic metabolic products a process that may be promoted by cometabolism (i.e., growth of the microorganism on another substrate while the organic molecule is degraded coincidentally). Heterotrophic bacterial and fungi are responsible for most of the chemical transformations. Many aerobic bacterial found in soil and water can metabolize petroleum hydrocarbons, converting them to carbon dioxide and water. Anaerobic bacteria are important for the biodegradation of chlorinated pesticides and halogenated organics (e.g., trichloroethylene and pentachlorophenol).

Degradation in contaminated soil and water may be affect by environmental constraints; therefore, treatment generally consists of optimizing conditions of pH, temperature, soil moisture and oxygen content, and nutrient concentrations necessary for the stimulation of the growth of the desired microorganisms. If the locally occurring organisms are not effective for the given set of contaminants, inoculation with microbial isolates that have been selectively adapted or genetically altered to degrade these compounds can be used. For example, recombinant PCB-degrading microorganisms with improved stability and survivability in mixed populations of soil microorganisms have been developed. Use of microbes for bioremediation is not limited to the detoxification of organic compounds; selected microbes have been used to reduce the toxic cations of heavy metals to the less toxic and much less soluble elemental forms.

As versatile as bioremediation may seem, there are certain complex xenobiotic chemicals which have proven resistant to microbial degradation. Nevertheless, the state-of-the-art in bioremediation of inorganic and recalcitrant organic contaminants is rapidly advancing.

See also: Chemicals of Environmental Concern; Environmental Toxicology; Hazardous Waste.

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Biotransformation

Tanya C McCarthy and Christopher J Sinal

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Introduction

Biotransformation refers to the process by which lipophilic (fat-soluble), xenobiotic (foreign), or endobiotic (endogenous) chemicals are converted in the body by enzymatic reactions to products that are more hydrophilic (water-soluble). In this context, metabolism and metabolic transformation are synonymous with biotransformation. A xenobiotic is a relatively small (molecular weight <1000), nonnutrient chemical that is foreign to the species where metabolism occurs.

The major purpose of biotransformation is to chemically modify (metabolize) poorly excretable lipophilic compounds to more hydrophilic chemicals that are readily excreted in urine and/or bile. Without metabolism, lipophilic xenobiotics accumulate in biota, increasing the potential for toxicity. Examples of such compounds are highly halogenated polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (TCDD and dioxins) that occur as tissue residues in humans. On the contrary, biotransformation is normally not required for xenobiotics with high water solubility because of rapid excretion in urine.

Two or more sequential enzymatic reactions are routinely required to convert lipophilic chemicals to metabolites that are efficiently excreted. R.T. Williams, a pioneer in biotransformation studies, classified these pathways as phase I (oxidation, reduction, and hydrolysis reactions) and phase II (conjugation reactions; **Table 1**). Normally, a phase I reaction precedes its phase II counterpart, but some compounds contain functional groups that are sites for direct conjugation (e.g., $-$OH, $-$COOH, and $-NH_2$). Frequently, the biological activity of a chemical decreases (termed 'detoxication') during metabolism but this is not always the case. Both phase I and phase II reactions can function in 'toxication' or metabolic activation processes as well, and this is a fundamental mechanism for the formation of many chemical toxicants. Multiple classes of toxic compounds, including polycyclic aromatic hydrocarbon-derived carcinogens and mutagens, are formed by cytochrome P450-dependent oxidative metabolism, the most common toxication pathway.

The highest concentration of xenobiotic metabolizing enzymes is routinely found in liver, but epithelial cells of extrahepatic tissues, such as the lung, kidney, intestine, placenta, and eye, also have activity. Relative to liver, extrahepatic tissues do not normally play a major quantitative role in the biotransformation of foreign compounds, including drugs. Extrahepatic organs, however, can be extremely important in the metabolic activation of xenobiotics and resultant target organ toxicity because the ratio of activation to detoxication enzyme activity is frequently higher in these cells than in hepatocytes (i.e., bioactivation predominates over detoxication and results in the formation of concentrations of active metabolites that overwhelm the capacity of detoxication pathways). The contribution of intestinal flora to the *in vivo* metabolism of xenobiotics can also be significant, especially for chemicals that require anaerobic (oxygen-deficient) reduction as a quantitatively important pathway.

Table 1 Classification of major biotransformation pathways

Classification	*Enzymes*
Phase I	
Oxidation	Cytochrome P450
	Flavin-containing monooxygenase
	Alcohol dehydrogenase
	Aldehyde dehydrogenase
	Monoamine oxidase
	H_2O_2-dependent peroxidase
Reduction	Cytochrome P450
	NADPH-P450 reductase
	Carbonyl reductase
Hydrolysis	Epoxide hydrolase
	Carboxylesterase/amidase
Phase II	
Conjugation	UDP-glucuronosyltransferase
	Sulfotransferase
	Glutathione *S*-transferase
	Mercapturic acid biosynthesis
	Cysteine conjugate β-lyase/thiomethylase
	N-Acetyltransferase
	N-Methyltransferase
	O-Methyltransferase

Oxidation Reactions

Oxidation is the most common metabolic reaction for lipophilic xenobiotic and endobiotic compounds, in part because most mammalian tissues are well oxygenated.

Cytochrome P450 Monooxygenase System

The cytochrome P450-dependent monooxygenase system is concentrated in the endoplasmic reticulum

of cells and is referred to as a microsomal enzyme system. This P450 system is composed of multiple forms or isozymes of P450 belonging, in humans, to at least 18 distinct gene families as well as the flavoprotein, NADPH-P450 reductase. This monooxygenase system has been termed a 'universal' oxidase because it catalyzes the oxidation of a multitude of lipophilic compounds including both xenobiotics (antioxidants, carcinogens, drugs, environmental pollutants, food additives, hydrocarbons, and pesticides) and endobiotics (bile acids, cholesterol, eicosanoids, fatty acids, lipid hydroperoxides, retinoids, and steroid hormones).

With several classes of xenobiotic substrates, including chemical carcinogens such as benzo(*a*)pyrene or the mycotoxin, aflatoxin B1, some metabolites are more toxic than the parent chemical, a process termed toxication. Endogenous compounds can also be bioactivated by P450 to metabolites with greater biological activity . For example, arachidonic acid is metabolized to four isomeric epoxyeicosatrienoic acids which have potent physiological and/or pathobiological effects in multiple tissues and cell types. Consequently, the P450 system is extremely important in toxicology (toxication and detoxication of both endogenous and exogenous substances), pharmacology (rate-limiting step in the metabolism of many drugs, drug–drug interactions, and individual qualitative and quantitative differences in drug metabolism due to genetic differences), and physiology (formation and metabolism of endobiotics that function as intercellular and/or intracellular messengers).

The multiple forms of P450 vary in their substrate selectivity and level of expression in different tissues and cell types. In lung, for example, the highest concentrations of P450 are normally found in (epithelial) Clara and alveolar type II cells but lower amounts occur in ciliated, goblet, and vascular endothelial cells as well as alveolar macrophages. The selective modulation (relative increase or decrease in concentration) of P450 isozymes in a single tissue or cell type can have pronounced effects on the metabolism of both endogenous and exogenous substances and on chemical-mediated target organ and/or cell toxicity by altering the balance between toxication and detoxication reactions.

The overall oxidation of a substrate, RH, by P450 is summarized in **Figure 1**, in which reduced nicotinamide-adenine dinucleotide phosphate (NADPH) is shown as the required cofactor.

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

Figure 1 Overall reaction that occurs during the cytochrome P450-dependent oxidation of a substrate, RH.

Some of the important reactions catalyzed by the P450 monooxygenase system include aliphatic hydroxylation, aromatic hydroxylation, epoxidation, heteroatom (*N*-, *O*-, and *S*-)dealkylation, nitrogen oxidation, oxidative deamination, oxidative dehalogenation, oxidative denitrification, and oxidative desulfuration. Most of these reactions result from the initial oxidation of a carbon atom, another reason that P450 is so important in the oxidative biotransformation of lipophilic chemicals. Some P450-catalyzed oxidation reactions are illustrated in **Table 2**.

The microsomal P450 system is most highly concentrated in the liver, but it is also present in many extrahepatic tissues including the lung, kidney, placenta, small intestine, skin, adrenal, testis, ovary, eye, pancreas, mammary gland, aorta wall, brain, nasal epithelial membrane, colon, salivary gland, prostate, heart, lymph node, spleen, thymus, and thyroid. A second P450 monooxygenase system, localized to mitochondria of steroid-metabolizing tissues (adrenal, ovary, and testis), is primarily involved in the oxidative biosynthesis of endogenous steroids such as cholecalciferol, cortisone, and deoxycorticosterone. In contrast to the 'universal' oxidase properties of the microsomal system, the mitochondrial P450 tem has a much higher degree of substrate specificity.

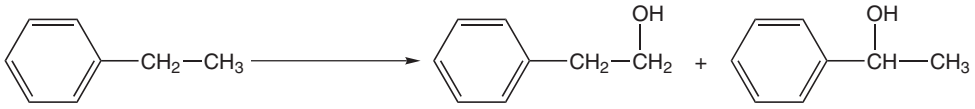
Flavin-Containing Monooxygenases

There is also a P450-independent monooxygenase enzyme family, termed the flavin-containing monooxygenases (FMOs); that is, localized in the endoplasmic reticulum of virtually all nucleated mammalian cells. Six distinct genes encoding FMOs have been identified in the human genome. These enzymes contain the coenzyme flavin adenine dinucleotide (FAD) and, similar to the P450 system, also require NADPH as a cofactor. A major difference between the FMOs and P450 is that the former do not oxidize carbon atoms. However, FMOs do oxidize many nitrogen-, sulfur-, selenium-, and phosphorus-containing xenobiotics (**Table 3**).

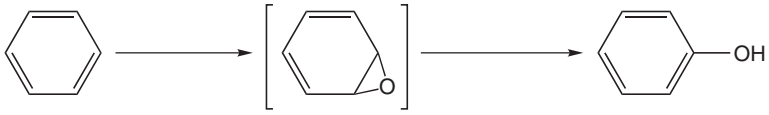
Since there are many drugs and environmental pollutants that contain sulfur, it is of considerable interest that FMO preferentially catalyzes the oxidation of sulfur in compounds containing both nitrogen and sulfur. Thus, FMO is an important enzyme system for the oxidation of selected classes of xenobiotics, and its spectrum complements that of the P450 system because the latter prefers oxidation of carbon atoms. Other ways in which FMO enzymes differ from many microsomal P450 isozymes include their

Table 2 Examples of important reactions catalyzed by the microsomal P450 monooxygenase system

Aliphatic hydroxylation

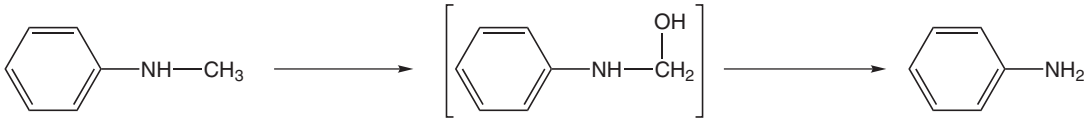


Aromatic hydroxylation

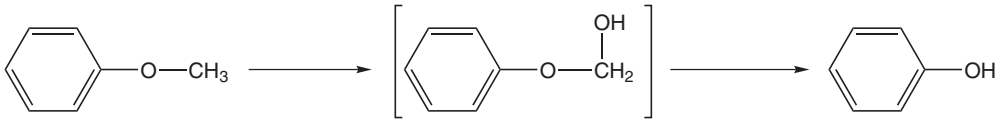


Epoxidation

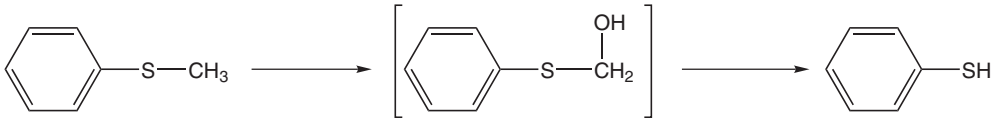
N-Dealkylation



O-Dealkylation

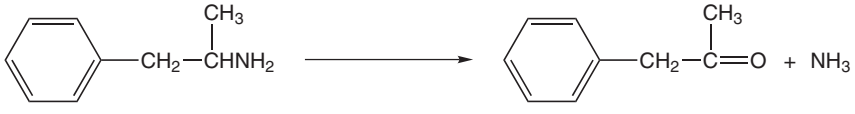


S-Dealkylation



Nitrogen oxidation

Oxidative deamination



Oxidative dehalogenation

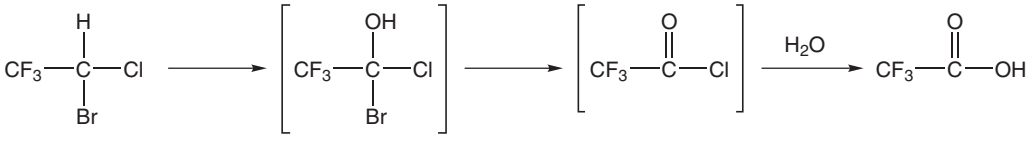
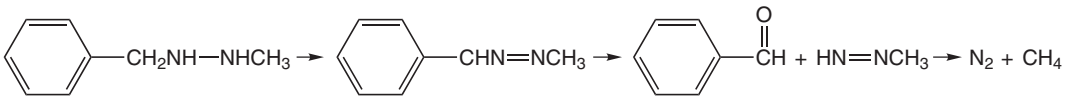


Table 2 Continued

Oxidative denitrification



Oxidative desulfuration

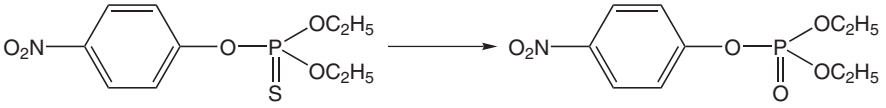
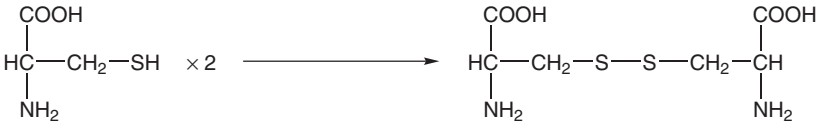


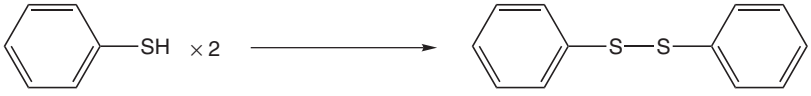
Table 3 Examples of important reactions catalyzed by microsomal flavin-dependent monooxygenases

Tertiary amine oxidation

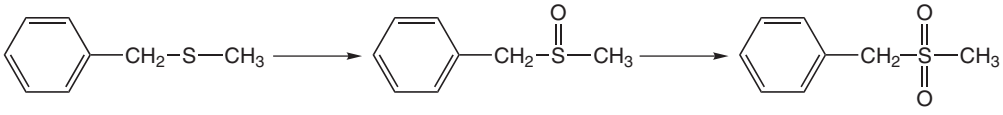
Alkyldisulfide formation



Aryldisulfide formation



Thioether oxidation



Phosphorus oxidation

apparent lack of induction (increased enzyme concentration) or repression (decreased enzyme concentration) by environmental factors and their more limited role in metabolic activation. Consequently, the P450 system is of greater significance in chemical toxicology.

Alcohol and Aldehyde Dehydrogenases

An extremely important metabolic pathway for alcohols and aldehydes is oxidation to aldehydes and ketones and to carboxylic acids, respectively. Mammalian liver alcohol dehydrogenases are a family

of zinc-containing, cytosolic NAD^+-dependent enzymes that catalyze the oxidation of primary and secondary aliphatic, arylalkyl, and cyclic alcohols. Aromatic alcohols (phenols), however, are not substrates for these enzymes. Alcohol dehydrogenases are widely distributed in mammalian tissues, with the highest concentrations occurring in the liver. As shown in **Figure 2**, alcohol dehydrogenases also catalyze the reverse reaction – reduction of aldehydes to primary alcohols in the presence of reduced nicotinamide adenine dinucleotide (NADH).

However, the *in vivo* reduction of aldehydes by this enzyme is not normally a quantitatively important reaction because aldehydes are rapidly oxidized to their corresponding carboxylic acid derivatives by aldehyde dehydrogenase. Alcohol dehydrogenase is a very important enzyme for the metabolism of ethanol.

Aldehyde dehydrogenases are also widely distributed in mammalian tissues, with the highest concentration in the liver. Both aliphatic and aromatic aldehydes are readily oxidized to carboxylic acids by this enzyme in the presence of NAD^+, the required cofactor (**Figure 3**).

Although this is a reversible reaction *in vitro*, the carboxylic acids formed are either converted rapidly to their ester glucuronide derivatives (a phase II reaction catalyzed by UDP-glucuronosyltransferase; see below) or, if polar enough, are excreted unchanged. Consequently, the reverse reaction is generally not of significance *in vivo*.

Monoamine Oxidases

The monoamine oxidases are localized in the outer membrane of the mitochondria of cells and are widely distributed in most mammalian tissues, with exceptions being the erythrocyte and plasma. This enzyme system catalyzes the oxidative deamination of a wide variety of xenobiotic and endobiotic (e.g., neurotransmitter) monoamines (**Figure 4**).

Monoamine oxidases are flavoproteins that contain one molecule of FAD per molecule. There are two major types of monoamine oxidase (A and B), whose relative concentration varies in tissues of the same species. In general, the A form of the enzyme is more active with endogenous neurotransmitter amines (serotonin, norepinephrine, and epinephrine), whereas the B form is more active toward xenobiotic amines such as 2-phenethylamine.

$$CH_3CH_2OH + NAD^+ \rightleftharpoons CH_3CHO + H^+ + NADH$$

Figure 2 Oxidation of ethanol and reduction of acetaldehyde by alcohol dehydrogenase and the appropriate form of NAD^+.

$$CH_3CHO + NAD^+ \rightleftharpoons CH_3COOH + H^+ + NADH$$

Figure 3 Oxidation of acetaldehyde and reduction of acetic acid by aldehyde dehydrogenase and the appropriate form of NAD^+.

H_2O_2-Dependent Peroxidases

Easily oxidized phenols and arylamines are excellent substrates for peroxidase-catalyzed one-electron oxidation reactions. These reactions are very important in toxicology because of the reactivity and toxicity of the free radicals (molecules with a highly reactive unpaired electron) formed. A well-studied example of this type is the cooxidation of xenobiotics catalyzed by the hydroperoxidase activity of prostaglandin H synthase. This enzyme, which converts arachidonic acid to prostaglandin (PG) H_2, has two distinct enzyme sites: cyclooxygenase, which oxidizes arachidonic acid to PGG_2, and hydroperoxidase, which reduces PGG_2 to PGH_2. PGG_2 reduction requires the donation of single electrons that can come from a xenobiotic and result in its conversion to a free radical. Many chemicals that are oxidized to toxic products, including acetaminophen, 2-aminofluorene, diethylstilbestrol, benzo(*a*)pyrene 7,8-dihydrodiol, and 4-phenetidine, are bioactivated to free radicals during reduction of PGG_2 to PGH_2 (**Figure 5**).

Prostaglandin H synthase activity is high in several extrahepatic sites that are targets for chemical-mediated toxicity but which contain very low amounts of P450 monooxygenase activity. These include skin, kidney medulla, lung of certain species, and platelets. It is now generally accepted that prostaglandin H synthase hydroperoxidase activity is important for the metabolic activation of amines and

$$RCH_2NH_2 + O_2 + H_2O \longrightarrow RCHO + NH_3 + H_2O_2$$

Figure 4 Oxidation of a substituted methylamine by monoamine oxidase. *R* can be an alkyl (CH_3^-) or aryl ($C_6H_5^-$) substituent.

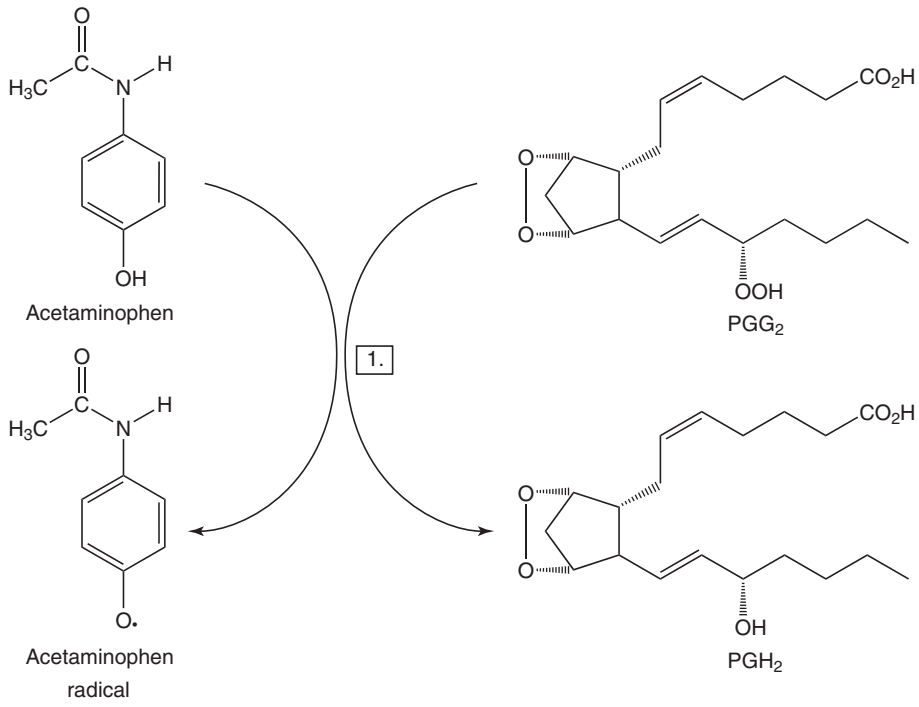


Figure 5 Conversion of acetaminophen to its reactive free radical by co-oxidation mediated by the hydroperoxidase activity of prostaglandin H synthase-catalyzed reduction of prostaglandin G_2 (PGG_2) to prostaglandin H_2 (PGH_2).

phenols, some of which are converted to potent mutagens and carcinogens, particularly in cells deficient in P450 monooxygenase activity but high in prostaglandin synthesis activity.

Other peroxidases are also involved in bioactivation of easily oxidized compounds. Oxyhemoglobin in erythrocytes can oxidize arylamines to products that cause methemoglobinemia; chloroperoxidase and myeloperoxidase of activated polymorphonuclear leukocytes and macrophages bioactivate certain drugs including various sulfonamides by *N*-oxidation to reactive nitroso products that contribute to adverse drug reactions; and diethylstilbestrol, a transplacental carcinogen, is oxidized by estrogen-inducible peroxidases in the reproductive tract.

These few examples emphasize that H_2O_2-dependent peroxidases can activate aromatic alcohols (phenols) and aromatic amines to reactive free radicals, which are often very toxic.

Reduction Reactions

Several functional groups, including nitro, azo, tertiary amine *N*-oxide, aldehyde, ketone, sulfoxide, and alkyl polyhalide, are reduced by mammals *in vivo*. Toxic free radicals are often formed as intermediates during reduction. Although some of these reactions, or more accurately the initial sequence of the reactions, occur under aerobic conditions *in vitro*, anaerobic conditions are generally required for the complete reduction of xenobiotics. Those reactions that go to completion *in vivo* are either reductions of carbonyl groups or are catalyzed by the intestinal microflora. Reduction that occurs anaerobically is of much less toxicological concern due to the decreased formation of toxic oxygen-free radicals.

Cytochrome P450-Dependent Reactions

Under aerobic and anaerobic conditions, several reduction reactions can be catalyzed by the intact P450 monooxygenase system or only by its flavoprotein component, NADPH-P450 reductase.

In addition to being oxidatively metabolized, many polyhalogenated alkanes are converted by a P450-dependent, one-electron reduction pathway to a free radical intermediate and inorganic halide. The best studied example of this reaction is the reduction of carbon tetrachloride (CCl_4) to chloroform ($CHCl_3$), which occurs *in vitro* under aerobic or anaerobic conditions and *in vivo*. The trichloromethyl radical formed (CCl_3) is believed to be a major contributor to CCl_4-mediated hepatotoxicity. Halothane, trichlorofluoromethane, hexachloroethane, pentachloroethane, and DDT are other halogenated compounds that are substrates for this P450-dependent reductive pathway.

Several other classes of xenobiotics are also efficiently reduced by the P450 monooxygenase system

under anaerobic conditions. These include tertiary amine *N*-oxides (converted to tertiary amines), hydroxylamines (primary amines), and hydrazo derivatives (primary amines).

Flavoprotein-Dependent Reactions

The first step of the NADPH-dependent reduction of aromatic nitro and azo compounds by hepatic microsomes is catalyzed by NADPH-P450 reductase and results in the formation of a free radical. In the presence of oxygen these radicals are rapidly reoxidized to the parent aromatic nitro or azo compound, concomitant with the generation of the superoxide anion radical. This futile cycling explains the toxicity of compounds, such as paraquat (**Figure 6**) or nitrofurantoin, which generate toxic superoxide under conditions in which little or no metabolism of the compound is detected. NADPH-P450 reductase is widely distributed in mammals and, consequently, these potentially toxic reactions occur in different tissues and subcellular organelles. Easily reduced compounds are readily reduced by NADPH-P450 reductase. Compounds that are more difficult to reduce, such as carbon tetrachloride, require the intact P450 monooxygenase system as a source of electrons for reduction.

Carbonyl Reductases

As mentioned previously, both alcohol and aldehyde dehydrogenases can function as reductases in the presence of NAD^+. In addition, there are a number of other carbonyl reductases that are $NADP^+$-dependent. Aldehyde reductases and carbonyl reductases are localized in the cytosol of cells, have a broad substrate specificity, have low molecular weights, and are widely distributed in extrahepatic tissues. In general, aldehyde reductases reduce only aldehydes, whereas carbonyl reductases reduce both aldehydes and ketones. Reduction of ketones can be an important metabolic pathway *in vivo*.

Hydrolysis Reactions

When certain xenobiotics, including esters and amides, are administered to animals they are hydrolyzed. Hydrolysis reactions are important for the sequential metabolism of chemicals converted to epoxides by the P450 system. These reactions are classified as phase I because they free up functional groups (e.g., $-COOH$, $-NH_2$, $-OH$, $-SH$, and $-SO_3H$) that are important sites for conjugation (phase II) reactions.

Epoxide Hydrolase

Epoxide hydrolases catalyze the hydration of epoxides to *trans*-dihydrodiols and are very important enzymes in toxication–detoxication processes. Unsaturated aliphatic and aromatic hydrocarbons are converted to epoxides (alkene and arene oxides, respectively) by P450 monooxygenase activity. Some of these electrophilic epoxides react covalently with macromolecules, such as proteins, RNA, and DNA, resulting ultimately in acute or chronic toxicity, including necrosis, mutagenesis, carcinogenesis, and teratogenesis. In most cases, the diols produced by epoxide hydrolase are much less toxic than the epoxide substrate. With some polycyclic aromatic hydrocarbons, however, the diols are precursors for potent carcinogenic and mutagenic products. For example,

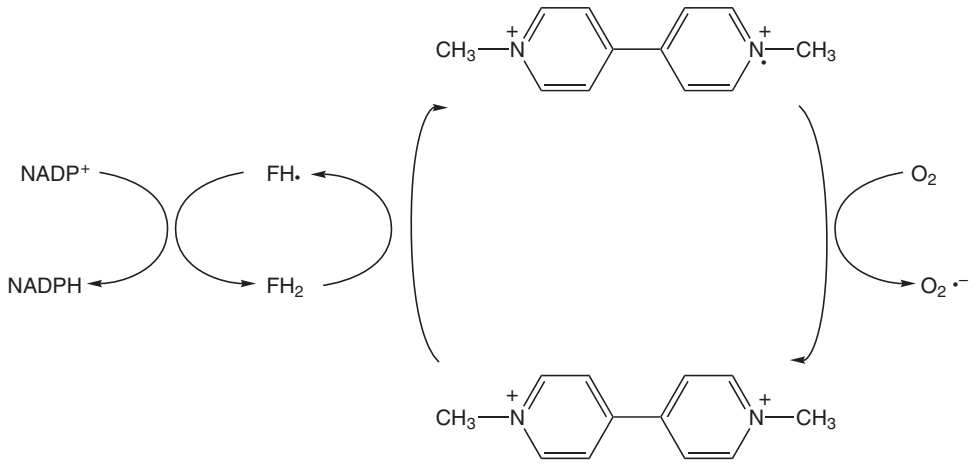


Figure 6 Futile cycle due to reaction of paraquat cation radical with molecular oxygen to generate superoxide radicals, with subsequent regeneration of paraquat. Cycle will operate as long as NADPH required as co-factor for P450 reductase is present.

benzo(*a*)pyrene 7,8-dihydrodiol, formed enzymatically from benzo(*a*)pyrene 7,8-oxide (**Figure 7**), is converted to the highly toxic benzo(*a*)pyrene 7,8-dihydrodiol-9,10-oxide by the P450 system or by cooxidation by prostaglandin H synthase.

There are two distinct types of epoxide hydrolases, both widely distributed in mammalian tissues. One type is localized primarily in the endoplasmic reticulum, the second in the cytosol. The microsomal and cytosolic enzymes have different properties, including substrate selectivities. Several inducers of xenobiotic metabolizing enzymes, including phenobarbital, planar PCB congeners, and *trans*-stilbene oxide, selectively increase (induce) microsomal, but not cytosolic, epoxide hydrolase activity.

Carboxylesterases/Amidases

The term carboxylesterase refers to a wide variety of enzymes with both esterase and amidase activity. They cleave carboxylesters, carboxylamides, and carboxylthioesters, producing a carboxylic acid and an alcohol or phenol (**Figure 8**), amine, or mercaptan, respectively. There are many different esterases, some of which are important for the hydrolysis and detoxication of toxic organophosphate esters. In general, esterases are present in almost all mammalian tissues, occur as multiple isozymes, and are concentrated in the liver. The esterase activity present in plasma is normally due to the release of these enzymes from liver.

Ester or amide cleavage can result in detoxication or metabolic activation, depending on the biological and chemical properties of the acids, alcohols, or amines released during hydrolysis. For example, hydroxamic acid hydrolysis has been implicated in the formation of proximate mutagens. The functional groups that become available for reaction during hydrolysis normally undergo phase II metabolism, as discussed below.

Conjugation Reactions

Most phase II reactions markedly increase the water solubility of xenobiotics and facilitate excretion of the chemical. Exceptions are acetylation and methylation reactions.

UDP-Glucuronosyltransferases

The most common phase II reaction is the synthesis of glucuronic acid derivatives (β-D-glucuronides) of lipophilic xenobiotics and endobiotics. Alcohols, phenols, carboxylic acids, mercaptans, primary and secondary aliphatic amines, and carbamates are converted to their β-glucuronide derivatives by UDP-glucuronosyltransferases (UDP-GT). Sixteen distinct human isozymes of UDP-GT have been identified, nine of which are encoded by a single gene. In common with the P450 monooxygenase system, UDP-GT is a microsomal enzyme, is present at highest concentrations in the liver, is expressed in many extrahepatic

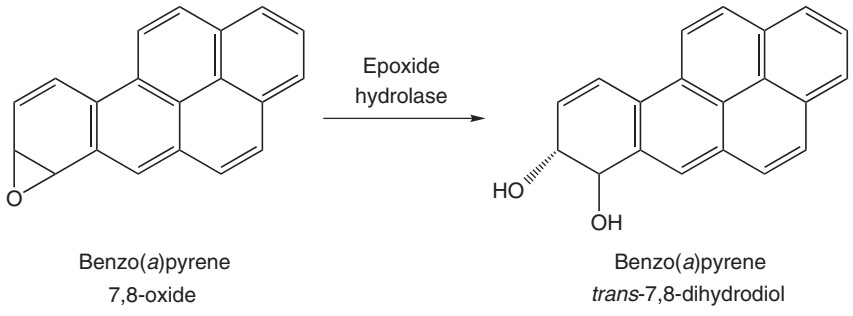


Figure 7 Conversion of benzo(*a*)pyrene 7,8-oxide to benzo(*a*)pyrene *trans*-7,8-dihydrodiol by microsomal epoxide hydrolase.

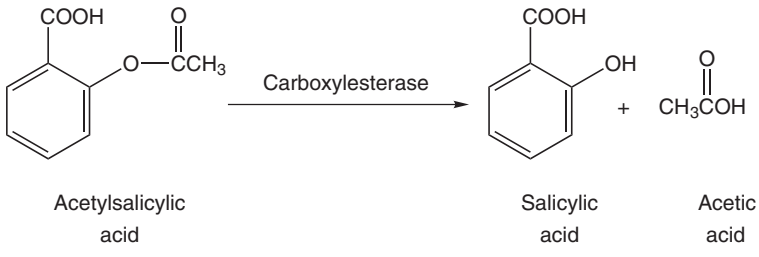


Figure 8 Hydrolysis of acetylsalicylic acid (aspirin) to acetic acid and salicylic acid (a phenolic acid) by carboxylesterase activity.

tissues, and is induced by exposure to different classes of compounds known to modulate P450, including phenobarbital, polycyclic aromatic hydrocarbons, planar PCB congeners, and dioxins.

UDP-GT catalyzes the translocation of glucuronic acid to a substrate from the cosubstrate UDP-α-D-glucuronic acid (UDPGA) as shown in **Figure 9**. The resulting glucuronide conjugates are excreted largely in the bile and can be hydrolyzed to their aglycone by β-glucuronidase of the intestinal microflora. The deconjugated chemical (i.e., the aglycone) can be reabsorbed and the cycle repeated. This process is called enterohepatic circulation and accounts for the prolonged excretion of some xenobiotics that are readily glucuronidated.

Certain β-glucuronides are electrophilic in nature and may also function in toxication processes. Covalent binding of the aglycone portions of several carboxylic acid (ester) glucuronides is known to occur to nucleophilic sites on serum albumin via transacylation reactions, for example.

Sulfotransferases

Another very common phase II reaction for phenols is conjugation with sulfate to form sulfate esters (**Figure 10**). Other substrates for this pathway include alcohols, primary and secondary amines, hydroxylamines, and sulfhydryl compounds such as thiophenols. These reactions are catalyzed by a family of cytosolic enzymes, the sulfotransferases, which require 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as the cofactor.

The sulfotransferases have been divided into several groups as a result of substrate specificity determinations with purified enzymes and molecular biology studies; aryl sulfotransferases are active toward phenols, hydroxylamines, tyrosine esters, and catecholamines; alcohol sulfotransferases are active toward primary and secondary steroid alcohols; and amine sulfotransferases are active toward arylamines.

A few sulfate esters are chemically reactive and alkylate nucleophilic sites on macromolecules. This electrophilic characteristic implicates these conjugates as ultimate chemical toxicants.

Phenols, quantitatively important P450-derived metabolites of aromatic hydrocarbons, are substrates for both UDP-GT and sulfotransferases. Generally, glucuronide metabolites predominate after administration of a phenol or phenol precursor to mammals because sulfate formation is a high-affinity, low-capacity (due to sulfate depletion) system, whereas glucuronidation is a low-affinity, high-capacity system.

Glutathione *S*-Transferases

The glutathione (L-γ-glutamyl-L-cysteinylglycine; GSH) *S*-transferases (GSTs) are a multigene family of dimeric

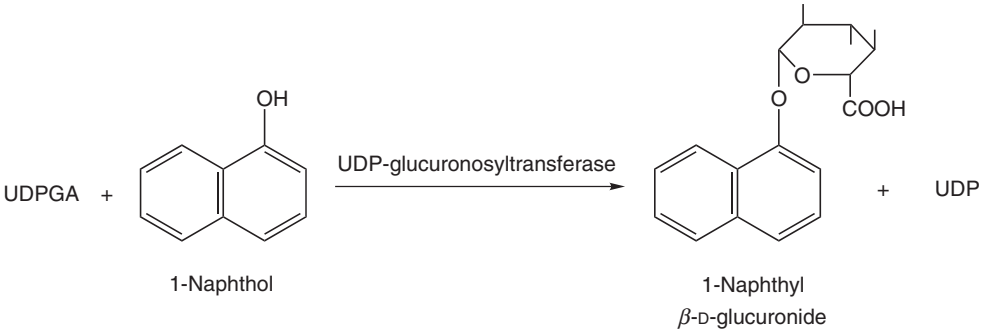


Figure 9 Conversion of 1-naphthol to its corresponding β-D-glucuronide.

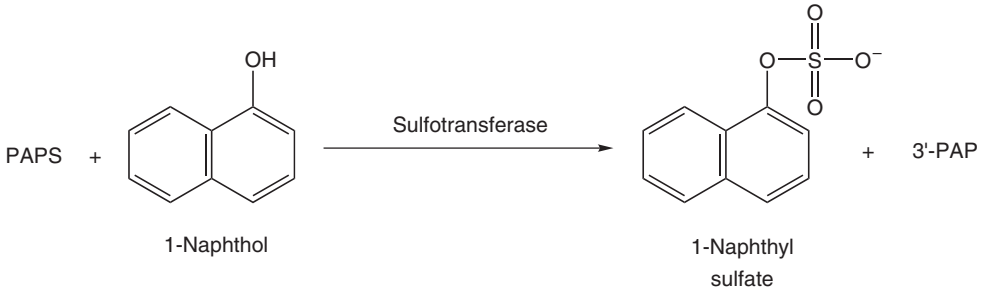


Figure 10 Conversion of 1-naphthol to 1-naphthyl sulfate by sulfotransferases.

proteins found at relatively high concentrations in the cytosolic fraction of mammalian liver, as well as in a wide variety of extrahepatic tissues. Some GST isozymes are also localized in microsomes and within the mitochondrial matrix of the liver, at much lower concentrations than the cytosolic enzymes. A wide variety of potentially toxic, electrophilic compounds (**Figure 11**) are converted to *S*-substituted GSH adducts by this family of enzymes. These include aromatic compounds containing good leaving groups (halogen, sulfate, sulfonate, phosphate, and nitro). Halogens are readily displaced from aromatic compounds as long as they are activated by the presence of electron-withdrawing groups (e.g., nitro). Strained three-membered rings, such as alkene and arene oxides, and four-membered lactones are readily cleaved by GSTs. The major factor in the transferase-catalyzed reaction of these substrates with GSH is the electrophilicity of the carbon atom where the thiol attacks. Since electrophilic chemicals are frequently very toxic the importance of the GSTs in detoxication cannot be overstated.

GSTs also catalyze a number of reactions in which an *S*-substituted GSH adduct is not formed or in which this adduct is oxidized glutathione. Examples of these reactions include the release of nitrate from nitrate esters and the release of cyanide from thiocyanates. Some GSTs also have glutathione peroxidase activity.

Although catalysis by GSTs is almost always associated with detoxication, a few substrates (e.g., the ethylene dihalides) are bioactivated to more toxic products by this pathway. Recent studies have also shown that glutathione conjugates are selectively accumulated in epithelial cells of the kidney where they are hydrolyzed. Those releasing metabolites that can undergo oxidation–reduction cycling result in cell-specific renal toxicity.

Mercapturic Acid Biosynthesis

A large variety of compounds, mostly xenobiotics, are excreted in urine as *S*-substituted *N*-acetylcysteines, also called mercapturic acids (**Figure 12**). The initial enzymatic reaction in their formation is catalyzed by the GSH *S*-transferases, as described previously. Subsequently, the glutamic acid residue is removed by γ-glutamyltranspeptidase, an enzyme with very high activity in the kidney. Next, the glycine moiety is removed by dipeptidases, which have cysteinylglycinase activity. The resulting *S*-substituted cysteine is converted to the corresponding mercapturic acid by *N*-acetyltransferase activity (see below).

Although mercapturic acids are normally the major thioether products of lipophilic xenobiotics found in urine of mammals, small amounts of the corresponding *S*-cysteine conjugates are also frequently excreted. All four thioether products formed during mercapturic acid biosynthesis are routinely excreted in bile.

Cysteine Conjugate β-Lyase/ Thiomethylation

In addition to being acetylated to mercapturic acids, some *S*-substituted cysteine conjugates are also hydrolyzed. The key enzyme in this reaction

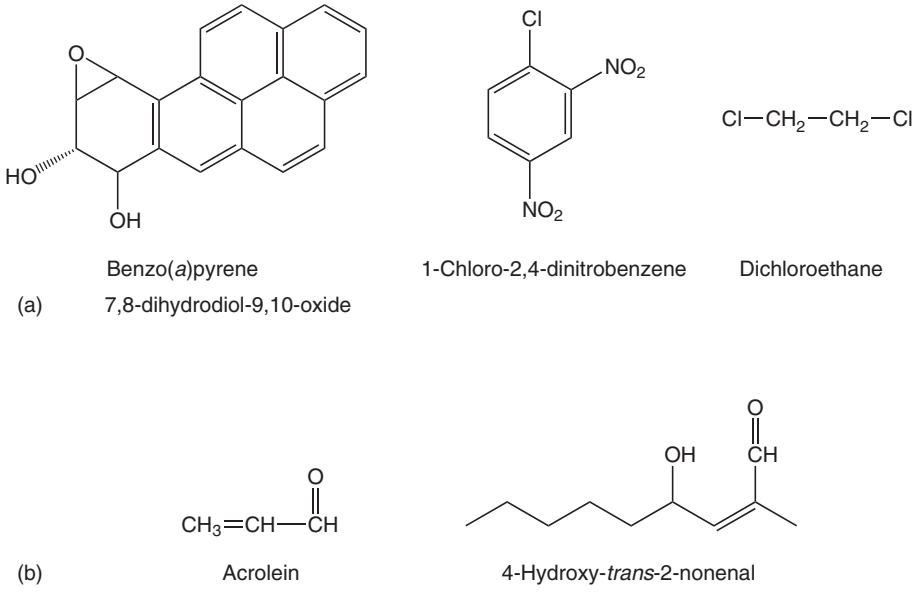


Figure 11 Structures of some common substrates of the glutathione *S*-transferases.

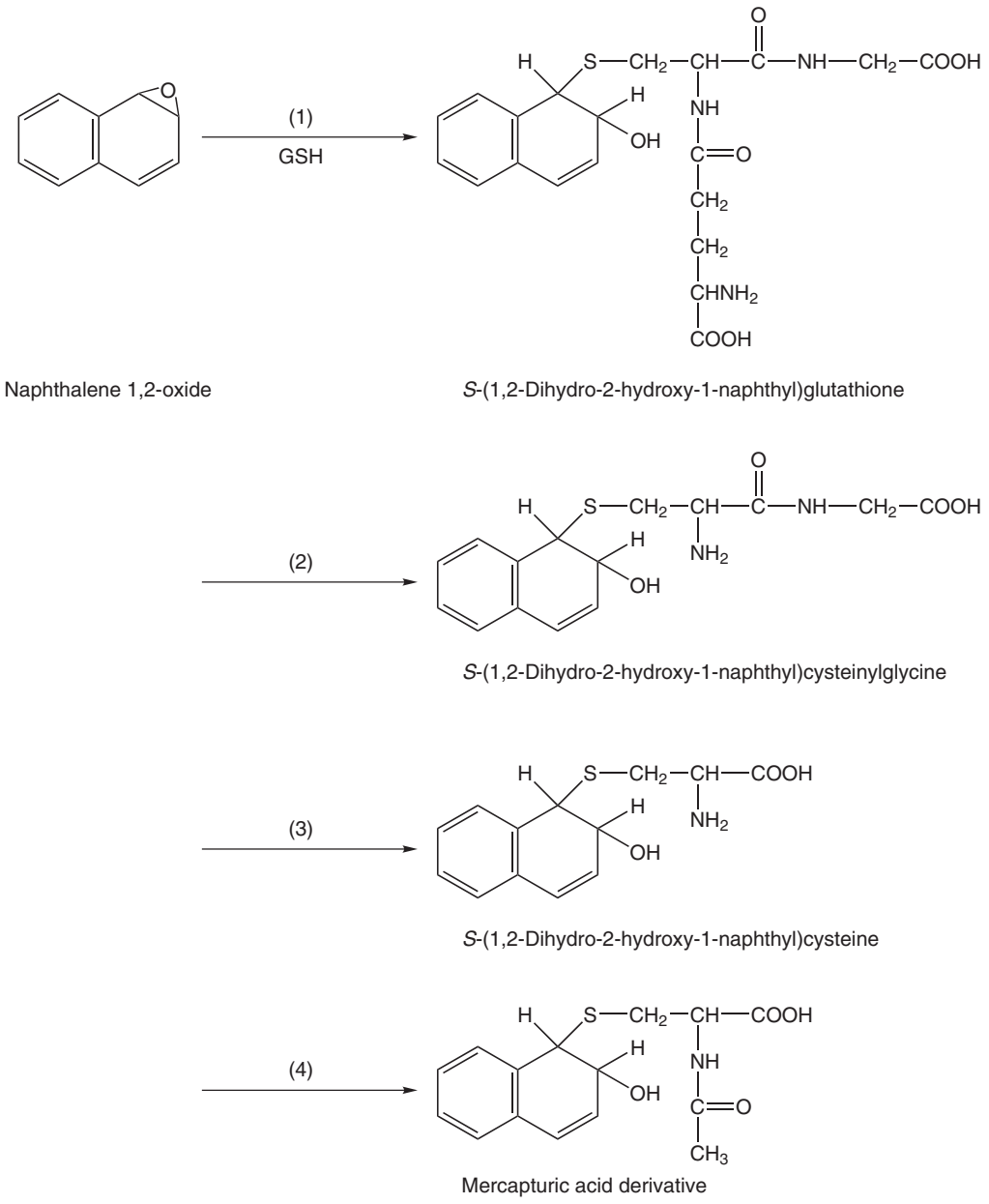


Figure 12 Mercapturic acid biosynthesis from a naphthalene 1,2-oxide. Only one of the isomers resulting from reaction of GSH with the arene oxide is shown. (1) glutathione *S*-transferase, (2) γ-glutamyltranspeptidase, (3) cysteinylglycinase activity (dipeptidases), and (4) *N*-acetyltransferase.

sequence is cysteine conjugate β-lyase, which cleaves the cysteine adduct to a free thiol, ammonia, and pyruvate (**Figure 13**).

This enzyme is present in the cytosolic fraction of rat liver and kidney and also in the microflora of the gut. Because thiols may be toxic and are more lipophilic than their cysteine conjugate precursors, β-lyase is generally a toxication pathway.

Thiols formed by mammalian or bacterial β-lyase *in vivo* are substrates for *S*-methyltransferase (**Figure 14**), an enzyme widely distributed in mammalian tissues.

This pathway accounts for the thiomethyl metabolites formed from several classes of xenobiotics. Thiomethyl metabolites can be further oxidized by the microsomal flavin-containing monooxygenases to their corresponding sulfoxide and sulfone derivatives.

Acyl-CoA:Amino Acid *N*-Acyltransferases

Several types of xenobiotic carboxylic acids (aromatic, heteroaromatic, arylacetic, and aryloxyacetic) are conjugated with a variety of endogenous amino

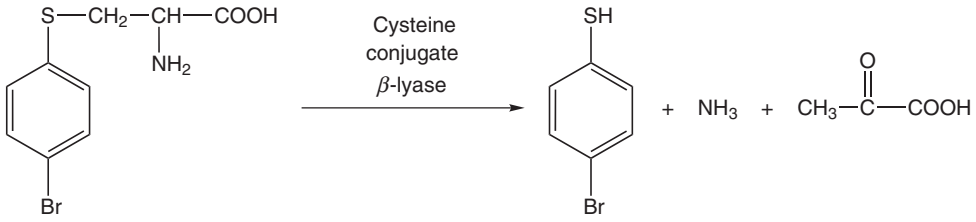


Figure 13 Hydrolysis of *S*-4-bromophenyl-L-cysteine by cysteine conjugate β-lyase.

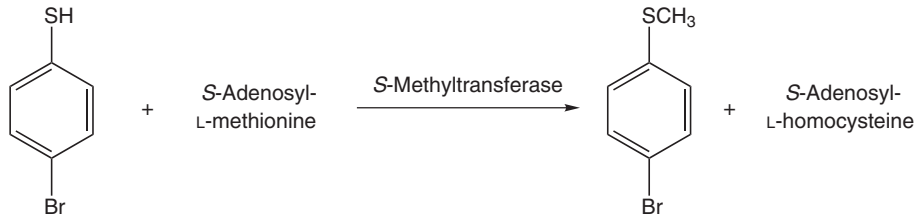


Figure 14 *S*-Methylation of 4-bromothiophenol by *S*-methyltransferase.

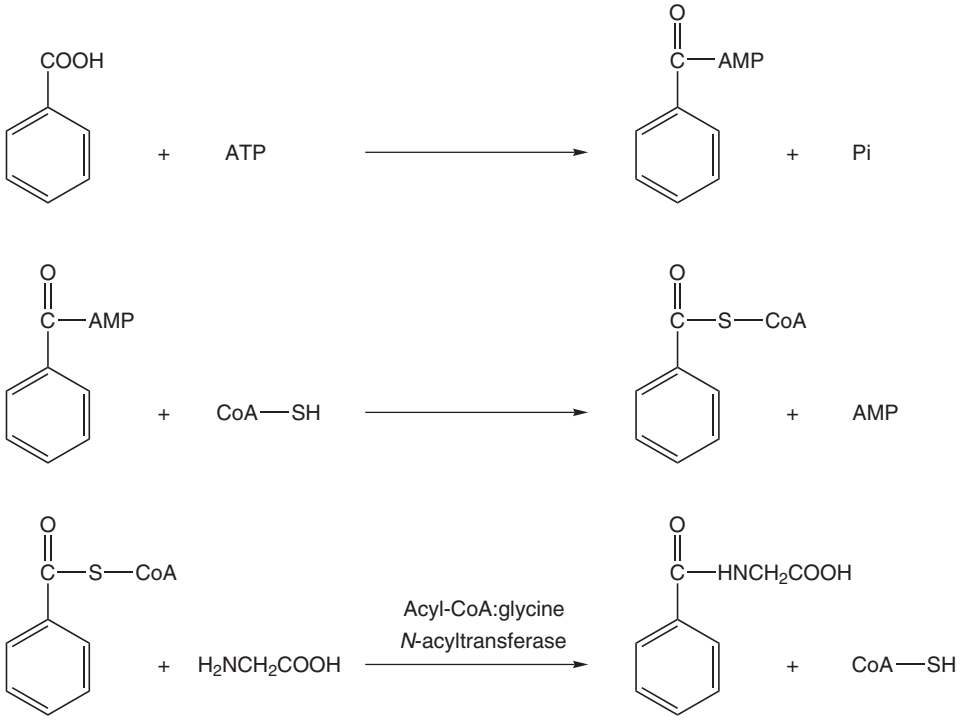


Figure 15 Metabolism of benzoic acid via its acetyl CoA derivative to hippuric acid (benzoylglycine).

acids, including glycine, glutamine, or taurine, prior to excretion in mammals. An amide (peptide) bond is formed between the carboxylic acid group and the α-amino group of the amino acid during conjugation. The reactions involved in the conversion of a carboxylic acid (e.g., benzoic acid) to its glycine derivative (hippuric acid) are illustrated in **Figure 15**.

Conversion of the carboxylic acid to its CoA ester derivative is the rate-limiting step. The enzyme that catalyzes the final reaction, acyl-CoA:amino acid *N*-acyltransferase, is localized in the mitochondria of the kidney and liver. The amino acid substrate selectivity, which varies from species to species, resides in the specific *N*-acyltransferase that

catalyzes this reaction. In most mammalian species conjugation with glycine predominates.

N-Acetyltransferases

Acetylation of xenobiotic primary amine groups is a common metabolic pathway, whereas acetylation of xenobiotic hydroxyl and sulfhydryl groups is not. Primary aliphatic and aromatic amines, sulfonamides, hydrazines, and hydrazides are readily *N*-acetylated *in vivo*, and the reaction is catalyzed by various acetyl CoA:*N*-acetyltransferases, commonly called *N*-acetyltransferases, as shown in **Figure 16**.

This family of enzymes is cytosolic and is widely distributed in a variety of mammalian tissues. There are also enzymes that hydrolyze *N*-substituted acetamides (i.e., amidases, as described previously) and the extent to which free versus acetylated amines are present *in vivo* depends on the relative rates of the acetylation and deacetylation reactions, on the physical and chemical properties of the two products, and whether or not the amine is metabolized by competing pathways. Some acetylated hydroxamic acids are chemically reactive and appear to be ultimate carcinogens.

N- and *O*-Methyltransferases

S-adenosyl-L-methionine (SAM)-dependent methylation was briefly discussed under Thiomethylation (see **Figure 14**). Other functional groups that are methylated by this mechanism include aliphatic and aromatic amines, *N*-heterocyclics, monophenols, and polyphenols. The most important enzymes involved in these methylation reactions with xenobiotics are catechol *O*-methyltransferase, histamine *N*-methyltransferase, and indolethylamine *N*-methyltransferase – each catalyzes the transfer of a methyl group from SAM to phenolic or amine substrates (*O*- and *N*-methyltransferases, respectively). Methylation is not a quantitatively important metabolic pathway for xenobiotics, but it is an important pathway in the intermediary metabolism of both *N*- and *O*-containing catechol and amine endobiotics.

Regulation of Biotransformation

The biotransformation and elimination of numerous potentially toxic xenobiotic compounds requires the concerted function of phase I and phase II enzymes. As such, exposure to elevated concentrations of xenobiotics can lead to the coordinate induction of genes encoding these enzymes. This inducibility is mediated by ligand-activated transcription factors that serve as sensors of intracellular xenobiotic concentration. Upon binding with xenobiotic compounds, these receptors interact with the regulatory region of target genes and increase the rate of gene transcription. Ultimately, this leads to an increase in the amount of phase I and phase II enzymes and the rate of biotransformation of the xenobiotic substrate. This process is self-limiting as the induction in metabolism ultimately leads to a decrease in the intracellular concentration of xenobic and thus, induction of the target gene. Examples of ligand-activated transcription factors that activate biotransformation include: the aryl hydrocarbon receptor that is activated by polycyclic aromatic hydrocarbons such as the procarcinogen benzo(*a*)pyrene; the pregnane X receptor and the constitutive androstane receptor that are activated by a large group of structurally diverse xenobiotic and endobiotic compounds; and, the peroxisome proliferator-activated receptor-alpha that is activated by a number of herbicides, industrial solvents, and plasticizers.

Summary

A number of enzyme systems have evolved in animals and plants which effectively convert lipophilic xenobiotics to more polar compounds that are efficiently excreted. Phase I enzymes, responsible for oxidation, reduction, and/or hydrolysis, are integrated with phase II or conjugation enzymes for reactions of both types and are normally required for the formation of products polar enough to be readily excreted. The intracellular level of these enzymes, and thus, the capacity for biotransformation, increases in a coordinate fashion in response to exposure to xenobiotic compounds. This response is

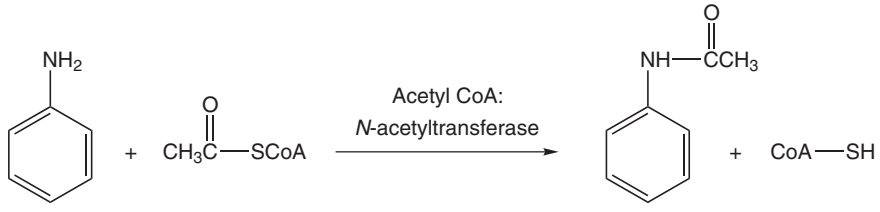


Figure 16 Acetylation of aniline by acetyl CoA:*N*-acetyltransferase activity.

achieved through changes in gene expression that are mediated by a number of ligand-activated transcription factors that serve as intracellular sensors of xenobiotic concentration. While the primary role of biotransformation is the elimination of potentially toxic xenobiotics, toxic metabolites can also be formed, primarily but not exclusively, during oxidation. When the concentration of these reactive metabolites exceeds the capacity of detoxication systems, acute (necrosis) or chronic (mutagenesis, carcinogenesis, and teratogenesis) toxicity can occur. Thus, anything that results in the reduced biotransformation of a toxic xenobiotic to an inactive metabolite, or alternatively, that increases the conversion of a relatively harmless xenobiotic to a reactive metabolite(s) increases the probability that a toxic response will occur.

See also: Carboxylesterases; Glutathione; Kidney; Liver; Pharmacokinetics/Toxicokinetics.

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Bis-Chloromethyl Ether *See* Chloromethyl Ether, Bis-.

Bismuth

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst and Shirley B Radding, volume 1, p. 174, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-69-9
- Chemical/Pharmaceutical/Other Class: Metal
- Chemical Formula: Bi^{3+}

Uses

Several bismuth compounds have been used medicinally. Some are used for gastrointestinal distress (pepto-bismol contains bismuth subsalicylate), others are used as salves and, in rare cases, for treatment of parasites. In the past, bismuth has also been used to treat syphilis and malaria.

Commercially, bismuth is also used in the manufacture of permanent magnets, semiconductors, and thermoelectric materials; as a catalyst in making acrylonitrile; and as an additive to improve the machinability of steels and other metals.

Background Information

Like water, the solid form is less dense than the liquid.

Exposure Routes and Pathways

The primary exposure pathway for bismuth is from medicinal preparations that are administered orally or intramuscularly. For the general population the total daily intake via food is ~5–20 μg, with much smaller amounts contributed by air and water. The cosmetic use of bismuth compounds still continues to be fairly widespread.

Toxicokinetics

Bismuth compounds are considered to be poorly to moderately absorbed following inhalation, topical application or ingestion. Gastrointestinal absorption depends on the water solubility of bismuth salts. Citrate enhances intestinal absorption. Absorbed bismuth is distributed throughout the soft tissues and bone. The biological half-life for whole-body retention is ~ 5 days but intranuclear inclusions containing bismuth seem to remain for years in the kidney of patients treated with bismuth compounds. Peak plasma bismuth concentrations were noted within one hour of consuming colloidal bismuth subcitrate, with none being detected by 4 h. Bismuth can accumulate, however, with repeated colloidal bismuth subcitrate exposures. Bismuth binds to plasma proteins and concentrates in the kidneys, the liver (to a lesser extent), and the skin. Bismuth can displace bound lead, thus increasing the concentration of lead in the circulatory system. The urine is the major route of excretion. For some bismuth compounds, elimination may be equal between urine and feces.

Mechanism of Toxicity

The mechanism by which bismuth produces toxicity has not been identified. Interaction with thiol compounds has been proposed as a primary mechanism.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral rat LD_{50} for bismuth metal is $5\,g\,kg^{-1}$. Insoluble salts, for example, bismuth nitrate and bismuth trioxide, also have reported oral LD_{50} values in rats of $4–5\,g\,kg^{-1}$.

Human

Oral human LD_{Lo} is equal to $221\,mg\,kg^{-1}$. Adverse acute reactions to bismuth include acute renal failure following ingestion of excessive concentrations. Bismuth can cause nausea, vomiting, and abdominal pain within hours of exposure. Muscle cramps and weakness, blurred vision, and hyperreflexia may be exhibited. Liver transaminase activities may be elevated.

Chronic Toxicity (or Exposure)

Animal

In animals, bismuth interferes with the metabolism of copper and zinc, induces metallothionein, and can alter heme biosynthesis in the liver and kidney. Bismuth has not been found to be carcinogenic in animal models. Bismuth subnitrate can decrease Leydig cell density and plasma testosterone levels in rats.

Human

High-level exposure causes renal failure with degeneration and necrosis of the epithelium of the renal proximal tubules, fatty changes and necrosis of the liver, reversible dysfunction of the nervous system, skin eruptions, gingivitis and pigmentation of the gums and intestine. Effects in humans also include reversible neurotoxic and sometimes fatal encephalopathy and bone weakness. Symptoms of bismuth poisoning include fever, weakness, pain similar to rheumatism, and diarrhea. Certain people display a rash. Bismuth salts may cause contact sensitivity. The bone and brain may also be targets for toxicity.

In Vitro Toxicity Data

Bismuth subsalicylate (pepto-bismol) was negative in the Ames assay at concentrations up to 0.67 mg per plate.

Clinical Management

There does not appear to be an antidote of choice for bismuth toxicity in humans. Gastric lavage can be used within 1 h of exposure. Replace fluids and electrolytes. Monitor renal and liver function for several days and treat failure conventionally. The newer chelating agents, meso-2,3-dimercaptosuccinic acid and D,L-2,3-dimercapto-propane-l-sufonic acid, are being investigated experimentally as antidotes for bismuth toxicity, and the latter has been shown to be effective. In mice, D-penicillamine has proven effective.

Environmental Fate

Bismuth may potentially lead to contamination through leaching of shotgun pellets.

Ecotoxicology

Little information is available on the ecotoxicity of bismuth or bismuth compounds.

Exposure Standards and Guidelines

The permissible exposure limit (PEL), threshold limit value (TLV), and the recommended exposure limit for bismuth metal have not been established. The PEL for bismuth trioxide is $15\,mg\,m^{-3}$. The PEL for

bismuth subsalicylate is 15 mg mg^{-3}, and the TLV is 10 mg m^{-3}.

See also: Metallothionein; Metals.

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Relevant Websites

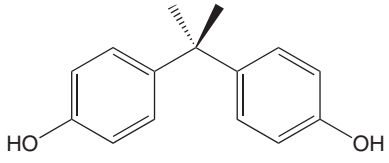
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Bismuth.

http://www.intox.org – International Programme on Chemical Safety.

Bisphenol A

Alan L Blankenship and Katie Coady

- Chemical Abstracts Service Registry Number: CAS 80-05-7
- Synonyms: 4,4′-Isopropylidenediphenol; 4,4′-Dihydroxy diphenyldimethylmethane; *p*,*p*′-Dihydroxydiphenyldimethylmethane; 4,4′-BPA; Bis (4-hydroxyphenyl)dimethylmethane; Bisphenol; DIAN; 2,2-Bis(4-hydroxyphenyl)propane; Bis(4-hydroxyphenylpropane; 4,4′-Bis-phenol a; *p*,*p*′-Dihydroxydiphenylpropane; 2,2-(4,4-dihydroxydiphenyl)propane; 4,4′-Dihydroxdiphenylpropane; 4,4′-Dihydroxydiphenyl-2,2-propane; 4,4′-Dihydroxy-2,2-diphenylpropane; Dimethylmethylene-*p*,*p*′-diphenol; *β*-di-*p*-Hydroxyphenylpropane; Dimethyl bis(*p*-hydroxyphenyl)methane; Diphenylolpropane; 2,2-di(4-Phenylol)propane; *p*,*p*′-Isopropylidenebisphenol; 4,4′-Dimethylmethylenediphenol; Phenol, 4,4′-(1-methylethylidene)bis-; 2,2-Bis(4,4′-hydroxyphenyl)propane
- Chemical/Pharmaceutical/Other Class: Phenolic
- Chemical Structure:



Uses

The estimated worldwide production of Bisphenol A (BPA) was 2.8 million tons in 2002. Approximately 90% of all BPA is used as an intermediate in the production of epoxy resins and polycarbonate plastics. Epoxy resins are used as food-contact surface coatings for cans, metal jar lids, coatings and finishes, automobile parts, adhesives, aerospace applications, and as a coating for polyvinyl chloride (PVC) water pipe walls. Polycarbonate plastics are hard plastics used to make numerous products, such as eyeglass lenses, water bottles, and consumer electronics. Some, but not all, dental sealants contain BPA. Additionally, BPA is a component of some specialty applications, such as flame-retardants, and as an antioxidant and stabilizer in the production of PVC and other plastics.

Background Information

In 1905, the synthesis of BPA, via the combination of acetone and phenol, was first reported by Thomas Zincke of the University of Marburg, Germany. One method of production is by the condensation of 2 mol of phenol with 1 mol of acetone while bubbling hydrogen chloride through the mixture. In 1953, the polycarbonate plastic manufacturing process was described using BPA as the starting material. Commercial production of polycarbonates began in 1957 in the United States and in 1958 in Europe. Because of the widespread use of BPA in polycarbonate plastics and epoxy resins, the manufacturing of BPA is expected to continue to increase in the future.

Exposure Routes and Pathways

The most probable routes of human exposure to BPA are inhalation and dermal contact of workers involved in the manufacture, use, transport or packaging of this compound. Potential exposure to BPA can also be expected through oral intake, since BPA is

largely used in resins and in food-can linings, from where it has the potential to leach into foods. Potential oral exposure to BPA is also possible through its use as a sealant in dentistry. However, studies have shown that the potential for exposure through such pathways is very low. The primary sources of environmental release of BPA are expected to be effluents and emissions from facilities which manufacture epoxy, polycarbonate, and polysulfone resins.

Toxicokinetics

Several studies have demonstrated the rapid clearance of BPA from blood following oral administration to adult rats. The principal metabolite of BPA in the rat is BPA-monoglucuronide (BPA-glucuronide). There appears to be route and dose-dependent differences in the pharmacokinetics of BPA. BPA administered by the oral route has reduced bioavailability and greater metabolism when compared with the subcutaneous route of exposure. This finding is consistent with the role of the liver in the first-pass metabolism of BPA through the oral exposure route. In order to evaluate the ontogeny of glucuronyl transferases (GT), the enzyme responsible for glucuronidation of BPA, a study was designed in which ^{14}C-BPA was administered via gavage at 1 or 10 mg kg^{-1} body weight (bw) to rats at postnatal day (PND) 4, PND 7, PND 21, or to 11-week-old adult rats (10 mg kg^{-1} dose only). Age dependency for the elimination of BPA-glucuronide was observed with more rapid elimination of BPA-glucuronide from the plasma of neonates ($t_{1/2}$: 4.4–9.8 h) when compared with adult animals ($t_{1/2}$: 10.8–22.5 h), likely due to reduced microflora β-glucuronidase activity in neonates and thus, an absence of enterohepatic recirculation. Nearly complete metabolism of BPA to BPA-glucuronide (94–100% of the plasma radioactivity) was observed at a dose of 1 mg kg^{-1}. Unlike the parent BPA, BPA-glucuronide is not a ligand for the estrogen receptor and it does not induce estrogenic activity in MCF-7 cells.

Studies indicate that BPA does not accumulate in body fat or sex organs of either male or female test animals, but is excreted in both rats and humans via the urine and feces. When administered as a single dose by gavage (800 mg kg^{-1} bw) to male rats, 28% of the ^{14}C-labeled BPA was excreted in the urine (primarily as glucosamide) and 56% in the feces (20% as free BPA, 20% as a hydroxylated BPA, and the rest as an unidentified conjugate). No ^{14}C-labeled residues were detected in animals killed after 8 days. In another rat study conducted with a lower dose (10 mg BPA kg^{-1} bw), 81.3% and 16.0% of the administered dose was excreted in feces and urine, respectively, in males, whereas 71.7% and 24.1% of the administered dose was excreted in feces and urine, respectively, in females. In BPA-dosed human volunteers, BPA was cleared from human blood and urine with a half-life of less than 6 h and the applied dose was completely recovered in the urine in the glucuronide form.

Mechanism of Toxicity

Investigations with the MCF-7 human breast cancer cell line showed that BPA binds to the estrogen receptor with a relative potency that is ~3–4 orders of magnitude less than that of 17-β-estradiol. BPA elicits estrogenic effects (e.g., increased cell proliferation) at concentrations at or above 2 μg l^{-1}. In addition to acting as a weak estrogen mimic, BPA also competitively inhibits estrogen from binding to the estrogen receptor. Studies have also indicated that BPA can act as an anti-androgen, blocking the androgen receptor-mediated effects of dihydrotestosterone in biological systems.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of BPA is relatively low. At 1–3 h after ingestion of high doses of BPA, animals exhibited atony and profuse diarrhea. Published LD_{50} values for laboratory mammals include 4150 mg kg^{-1} bw (male F344 rat, oral), 3300 mg kg^{-1} bw(female F344 rat, oral), 5280 mg kg^{-1} bw (male B6C3F1 mouse, oral), 4100 mg kg^{-1} bw (female B6C3F1 mouse, oral), 150 mg kg^{-1} bw (mouse, intraperitoneal), and 2230 mg kg^{-1} bw (rabbit, oral), and 4000 mg kg^{-1} (guinea pig, oral).

Human

One clinical report describes photoallergic contact dermatitis to BPA, with subsequent persistent light reactivity, in a group of eight outdoor workers.

Chronic Toxicity (or Exposure)

Animal

There are considerable data on the subchronic and chronic toxicity of BPA in laboratory animals. For decades, BPA has been shown to produce weak but consistent responses in uterotrophic assays. The focus of these investigations has typically been evaluation of the potential reproductive and developmental effects of BPA, due to its ability to modulate estrogen

receptor-mediated responses. Many end points are not consistently observed across studies. Some of this variability may result from differences in the conditions, design, and other test-specific variables of the toxicity tests. For example, since phytoestrogens are abundant in most laboratory animal feeds (such as found in soy and alfalfa) and are known to modulate estrogen receptor-mediated responses, phytoestrogens may be confounding factors as a result of the feed selection.

In a developmental toxicity test, CD rats were exposed to 0, 160, 320, or 640 mg BPA kg^{-1} day^{-1} and CD-1 mice were exposed to 0, 500, 750, 1000, or 1250 mg BPA kg^{-1} day^{-1} by daily dosing via gastric intubation on gestational days 6–15. Timed-pregnant dams were sacrificed 1 day prior to parturition. The uterine contents and fetuses were examined. In mice, some maternal mortality and an increase in relative maternal liver weight was observed at all BPA doses, reaching 18% at the high dose. At the highest dose (1250 mg BPA kg^{-1} day^{-1}), there was also a significant increase in the percentage of resorptions per litter, and reductions in gravid uterine weight and average fetal body weight. In rats, there were significant reductions in maternal weight gain during gestation, weight gain corrected for gravid uterine weight, and weight gain during treatment at all BPA doses. However, there were no observed effects of BPA on gravid uterine weight, average fetal body weight per litter, the percentage of resorptions per litter, or percentage fetuses malformed per litter. In summary, BPA treatment at maternally toxic dose levels during organogenesis produced fetal toxicity in mice but not in rats and did not alter fetal morphologic development in either species.

In a two-generation reproductive toxicity test conducted through the National Toxicology Program, CD-1 mice were exposed to 0%, 0.25%, 0.5%, and 1.0% BPA in feed to produce estimated daily intakes of 0, 437, 875, and 1750 mg BPA kg^{-1} day^{-1}. There was a 5–9% decrease in the number of litters/pair at the two highest doses and the number of live pups/litter was reduced by 20% at the 0.5% BPA dose and by 48% at the highest dose. The second generation did not appear more sensitive than the first to the reproductive toxicity of BPA.

There is some controversy over the possibility of low dose effects of BPA. Researchers who have published results suggesting that there is a low dose effect of BPA assert that the dose–response relationship is 'nonmonotonic', which means that health effects may only be observed at low doses while much higher doses result in no effects. However, these studies are weakened by lack of reproducibility and statistical robustness. Furthermore, there is considerable evidence to support a classical dose response for the effects of BPA. For example, based on pharmacokinetic studies, low doses of ^{14}C-BPA (via oral exposure) result in nearly complete metabolism of BPA to BPA-glucuronide (94–100% of the plasma radioactivity) at a dose of 1 mg kg^{-1}. In addition, there is a general lack of effects of low doses of BPA as noted above in chronic, multigenerational studies that focus on sensitive reproductive and developmental end points. For example, in a three-generation study, male and female Sprague–Dawley rats were fed a diet containing BPA at approximate dietary intakes of 0, 0.001, 0.02, 0.3, 5, 50, or 500 mg kg^{-1} bw day^{-1}. Exposures were continued until adulthood of the third-generation offspring. Analysis of the data for all of these end points for the parental and three offspring generations revealed no evidence of a low-dose effect of BPA for any of the reproductive and developmental end points including parental growth rate, food intake, reproductive performance, sperm production and motility, gross and histopathology, organ weights, litter size, pup survival and growth, and anogenital distance. This study clearly demonstrated the absence of low-dose effects of BPA.

In toxicity tests to evaluate the potential carcinogenicity of BPA, male and female F344 rats were exposed to 0, 1000, or 2000 mg BPA kg^{-1} (in feed) for 103 weeks and male and female B6C3F1 mice were exposed to 0, 5000, or 10 000 mg BPA kg^{-1} (in feed) for 103 weeks. Under the conditions of this bioassay, there was no evidence that BPA was carcinogenic for F344 rats or B6C3F1 mice of either sex.

Human

There are insufficient data to characterize chronic toxicity or exposure in humans.

In Vitro Toxicity Data

BPA, at levels greater than 2 μg l^{-1}, was found to be estrogenic in cultured human mammary cancer cells (MCF-7). Some of the reported effects of BPA in MCF-7 cells include induction of progesterone receptors in MCF-7 cells at a potency of 5000 times less than 17-β-estradiol, increased rate of cell proliferation, and competition with estradiol for estrogen receptor binding sites.

Environmental Fate

The primary sources of BPA to the environment are likely effluents and emissions from facilities that either manufacture or utilize BPA in large quantities.

If released to acclimated water, biodegradation would be the dominant fate process (half-life 2.5–4 days). BPA may adsorb extensively to suspended solids and sediments (K_{oc} values range from 314 to 1524), and it may photolyze in the presence of sunlight. BPA is not expected to bioaccumulate significantly in aquatic organisms (BCF 5–68), volatilize, or undergo chemical hydrolysis.

Ecotoxicology

Due to the potential for release to aquatic environments, considerable work has been done on evaluating the aquatic toxicity of BPA. Acute toxicity (96 h LC_{50} values) for freshwater organisms ranged from 4.6 mg l^{-1} for the fathead minnow (*Pimephales promelas*), to 9.4 mg l^{-1} for the Atlantic silverside (*Menidia menidia*).

BPA is considered an endocrine disruptor chemical and, in chronic studies, induces production of vitellogenin in male fathead minnows (*P. promelas*) at concentrations of 640 and 1280 µg l^{-1} after 43 days and 160 µg l^{-1} after 71 days. Induction of vitellogenin is a process normally occurring only in female fish in response to estrogenic hormones during the reproductive cycle.

Overall, chronic toxicity values of BPA for freshwater organisms ranged from 160 µg l^{-1} for the fathead minnow (*P. promelas*; based on egg hatchability) to 11 000 µg l^{-1} for rainbow trout (*Oncorhynchus mykiss*; based on growth), Typically, chronic effects on survival, growth, and reproductive end points only occur at concentrations of BPA greater than 160 µg l^{-1}. Published no-observed-effect concentrations in aquatic organisms range from 16 to 3640 µg l^{-1}. Taken together with concentrations of BPA in typical surface water samples that are less than 1 µg l^{-1}, potential risks to aquatic organisms are very low.

Exposure Standards and Guidelines

The Draft EPA water quality criterion for BPA is 5.9 µg l^{-1}. The State of Florida's drinking water guideline is 350 µg l^{-1}.

See also: Acetone; Phenol; Vinyl Chloride.

Further Reading

Tyl RW, Myers CB, Marr MC, *et al.* (2002) Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague–Dawley rats. *Toxicological Sciences* 68: 121–146.

Black Widow Spider *See* Spider, Black Widow.

Bleach

Julie Weber

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- Representative Chemical: Sodium hypochlorite
- Chemical Abstracts Service Registry Number: CAS 7681-52-9 (sodium hypochlorite)
- Synonyms: Household laundry bleach (Purex®, Clorox®, and Dazzle®); Commercial laundry bleach; Caustic soda bleach; Dakin's solution; Modified Dakin's solution; Sodium hypochlorite pentahydrate; Surgical chlorinated soda solution
- Chemical/Pharmaceutical/Other Class: Hypochlorites and related agents
- Chemical Formula: NaHClO

Uses

Sodium hypochlorite is used in household laundry bleach, disinfectant and cleaning products, toilet sanitizers, deodorizers, for water purification, and as antiseptics. Regular household laundry bleaches are ~5.25% sodium hypochlorite in water with an adjusted pH of 10.8–11.4. 'Ultra' formulations are slightly more concentrated and contain 6–8% sodium hypochlorite. Commercial laundry bleaches contain 15% sodium hypochlorite at a pH slightly over 11.

Exposure Routes and Pathways

Ingestion is the most common route of exposure to sodium hypochlorite. Other modes of exposure are inhalation, dermal, ocular, and inadvertent injection.

Mechanism of Toxicity

The toxicity of hypochlorite arises from its corrosive activity on skin and mucous membranes. Corrosive burns may occur immediately upon exposure to concentrated bleach products. Most of this corrosiveness stems from the oxidizing potency of the hypochlorite itself, a capacity that is measured in terms of 'available chlorine'. The alkalinity of some preparations may contribute substantially to the tissue injury and mucosal erosion. Sodium hypochlorite when combined with an acid or ammonia may produce chlorine or chloramine gas, respectively. An inhalation exposure to these gases may result in irritation to mucous membranes and the respiratory tract, which may manifest itself as a chemically induced pneumonitis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Emesis is likely to be spontaneous. Clinical signs may include salivation, emesis, abdominal pain and tenderness, hematemesis, and bleached hair. Rats given 5–15 ml kg^{-1} of an alkaline (pH 12.0) solution containing 4.5% sodium hypochlorite died within 1–3 h from severe local damage to the esophagus and stomach.

Human

The resulting symptoms from an exposure to sodium hypochlorite and related compounds may range from mildly irritating to corrosive depending on the volume/amount of the exposure, duration of contact, and pH and viscosity of the product. Small accidental ingestion of household bleach, containing 4–6% sodium hypochlorite, usually causes nothing more serious than orogastric irritation characterized by nausea, spontaneous emesis, sore throat, and abdominal pain. Very large ingestions, usually intentional, have caused fatal hypernatremia and hyperchloremic acidosis, and significant gastric injury. Sodium hypochlorite solutions stronger than 10% or powders may result in corrosive burns of the mouth, hypopharynx, and stomach. Prolonged dermal contact can result in irritation or burn. Inadvertent injection into the surrounding tissue during dentistry use of Dakin's solution (0.5–5% sodium hypochlorite) has resulted in severe acute pain and burning sensation accompanied by immediate edema of the surrounding area. Delayed effects can include tissue necrosis, paresthesia, and secondary infection. Household bleach has been advocated as a disinfectant for syringes and needles of IV drug users. There are few reports on the effects of inadvertent or intentional IV injection. Case reports of symptoms have included erythema at the injection site, vomiting, chest pain, bradycardia, and hypotension. Ocular exposure may result in irritation, lacrimation with a burning discomfort. Superficial disturbance of the corneal epithelium may occur, which recovers completely within 2 days. Eyelid edema has been reported, but is more common after exposure to chloramine gas. Inhalation of liberated chlorine or chloramine gas may cause respiratory tract irritation, cough, substernal chest discomfort and tightness, hoarseness, dyspnea, and wheezing. Chemical pneumonitis, acute respiratory distress syndrome, and hypoxia have developed in severe prolonged exposures.

Chronic Toxicity (or Exposure)

Animal

Bleach should not be considered a carcinogen in experimental animals. Exposure of the esophagus of rabbits and dogs to typical household bleach resulted only in minor lesions. Rats fed water with high bleach concentrations demonstrated decreased weight gain, but no other untoward signs or symptoms.

Human

Most data indicate that low-dose hypochlorite solutions (e.g., those seen in typical municipal drinking water) do not directly contribute to the development of cancer.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Treatment is generally symptomatic and supportive. Gastrointestinal evacuation procedures are generally unnecessary. If the patient is alert and able to swallow, milk or water should be immediately offered, stopping if vomiting occurs during administration. Administration of an acidic substance to neutralize sodium hypochlorite is contraindicated.

For inhalation exposure, the patient should be removed from fumes into fresh air. Respirations should be established along with the creation of an artificial airway if necessary. If cough or difficulty in breathing develops and is not relieved by the fresh air, the patient should be evaluated for respiratory irritation, bronchitis, or pneumonitis in a health care facility.

For ocular exposures to sodium hypochlorite and related agents, contact lenses should be removed if present. The eye(s) should be immediately irrigated with tap water or normal saline for at least 15 min. If ocular irrigation is delayed, the potential for injury is

greater and the patient may have to be evaluated in a health care facility.

For dermal exposures, contaminated clothing should be immediately removed and the exposed skin should be flooded with water. The skin should be gently washed with soap and water.

See also: Chlorine; Coniine.

Further Reading

Ehrich DG, Brian JD Jr, and Walker WA (1993) Sodium hypochlorite accident: Inadvertent injection into the maxillary sinus. *Journal of Endodontics* 19(4): 180–182.

Jakobsson SW, Rajs J, Jonsson JA, *et al.* (1991) Poisoning with sodium hypochlorite solution: Report of a fatal case, supplemented with an experimental and clinico-epidemiological study. *American Journal of Forensic Medicine and Pathology* 12: 320–327.

Blister Agents/Vesicants

Harry Salem and Frederick R Sidell*

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Background

Blister agents, also known as vesicants, are cytotoxic alkylating compounds. They are exemplified by chemicals collectively known as 'mustard' or 'mustard gas' (military designator: H). Other blister agents are sulfur mustard (HD), nitrogen mustard (HN), lewisite (L; an arsenic-containing vesicant), and phosgene oxime (CX; a halogenated oxime that is very different in properties and toxicity from the other agents). Mustard vapor injury is a particular threat in hot climates. In addition, humidity or moisture in a hot environment enhances damage to the skin.

Examples of vesicant or blister agents with military designators in parentheses:

1. mustard or mustard gas (H);
2. sulfur mustard (HD), characterized by delayed action;
3. sulfur mustard with Agent T (HT) – the latter is bis-2-(2)chloroethylthioethyl ether, similar to HD in structure;
4. nitrogen mustard (HN);
5. lewisite (L), similar to sulfur mustard in action, except that immediate effects occur within minutes;
6. mixture of mustard and lewisite (HL) – the combination of sulfur mustard (37%) with lewisite (63%) gives it a garlic odor;
7. phenyldichloroarsine (PD) – like lewisite, it is an organic dichloroarsine; and
8. phosgene oxime (CX), a pulmonary toxin with vesicant effects.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Mechanism of Toxicity

The action on cell components results in inhibition of cellular division (mitosis) with decreased tissue respiration that leads to cell death. It produces eye, airway, and skin and mucous membrane injury that can be fatal. Systemic effects with extensive exposures include bone marrow inhibition with a drop in the white blood cell count and gastrointestinal tract damage.

Mustard Gas (H)

Mustard gas was first used in chemical warfare during World War I in 1917 and more recently during the Iran–Iraq War (1984–88). The term mustard gas refers to several chemicals. Most commonly, it means sulfur mustard (HD), which is reviewed below. The word gas used in the context of mustard gas is not accurate since mustard gas is not a true gas, but rather a liquid. Mustard gas is stored as a liquid, and is not likely to change into a gas immediately if released at ordinary temperatures. As a liquid it is colorless and odorless when pure, but brown with a slight garlic smell when mixed with other chemicals. It dissolves easily in fats and petroleum products. It dissolves slowly in water, where it turns rapidly into less toxic chemicals. Therefore, drinking, cooking, bathing, and swimming in mustard gas-contaminated water are activities unlikely to lead to significant exposure. However, the chemicals produced in water may cause skin or eye irritation, rather than the characteristic blisters. Should mustard gas be released, it will stay in the air or on the ground for ~30–50 h with the full potential for toxic effects.

Mustard gas is a greater threat in hot and humid climates as a blister agent. Should unprotected exposure occur, mustard gas enters the body quickly either through the breathing of vapors or through skin contact with liquid or vapors. It can pass through clothing to get onto and through the skin. Subsequently, it enters the circulation and causes systemic effects at higher doses. Mustard gas and the

other chemicals it can change into in the body, leave the body through the urine within a few weeks. Initially, there is no pain, and exposure from the development of symptoms may not be apparent until the next day. At the point of contact, mustard gas can cause skin blisters to occur within several days. The extent of the blisters is determined by the amount and area of exposure, and this will determine the course of the immediate illness from mustard gas. Those areas which are exposed, and which are sweaty, are the most affected. Exposure to the eyes and the natural tears will increase susceptibility to tearing and blinking. Cough and bronchitis can result if vapors are inhaled. Large exposures can cause death in the near term.

The long-term consequences from mustard gas exposure, particularly at low doses, are unknown. However, a one-time high-dose exposure can result in chronic and recurrent lung and eye problems. Mustard gas is also a known carcinogen, and can cause lung cancer later in life. The ability to cause birth defects in the children of exposed adults is not presently known; however, it has the potential to be teratogenic.

Sulfur Mustard (HD)

Sulfur mustard is the distilled or purified form of mustard gas. Its properties and toxicities are similar to those of mustard gas. If spilled, sulfur mustard evaporates into air where it decomposes. It can persist within soil with its blister-forming activity intact for many years, particularly in colder climates. Because of its relative insolubility, it generally does not contaminate groundwater.

Unprotected exposure leads to symptoms that are delayed and occur within hours. Erythema and blistering occur 2–24 h after exposure to skin. With exposure to eyes, tearing, itching, burning, accompanied by a gritty feeling in the eyes, can occur within 4–24 h. With more significant exposure to the eyes, there may be conjunctivitis with eyelid swelling in 3–6 h. Severe eye exposure leads to marked eyelid swelling, corneal opacity, and eye pain, all occurring in 1–2 h. Airway toxicity can occur in 12–24 h, and this is marked by rhinorrhea, sneezing, coughing, nosebleeds, and hoarseness. Severe inhalation injury is marked by productive cough and shortness of breath. There can be variable gastrointestinal symptoms with ingestion.

Sulfur Mustard with Agent T (HT)

The toxicities and properties of HT are similar to those of mustard gas or sulfur mustard.

Nitrogen Mustard (HN)

Nitrogen mustard is composed of three similar compounds (HN-1, HN-2, and HN-3). The color of the liquid can range from pale yellow to dark, and the smell can be either odorless or like that of herring fish. Nitrogen mustard can persist in soil. HN-1 is less persistent, with one-fifth the blistering potency of sulfur mustard. HN-3 is more persistent and is equal to sulfur mustard in blistering potential. When nitrogen mustard is vaporized in air, it degrades in a matter of hours. Its properties and toxicity are similar to those of the agents mentioned above, and it is a strong blister agent. Eye irritation and skin erythema occur sooner upon exposure to nitrogen mustard than with sulfur mustard. Eye lesions also appear to be more severe with nitrogen mustard. It is known to cause cancer years later.

Lewisite (L)

Lewisite (L) contains arsenic and is a potent blister agent. Chlorovinyldichloroarsine is another name for lewisite, and like phenyldichloroarsine (PO), ethyldichloroarsine, and methyldichloroarsine, it is an organic dichloroarsine. When purified, lewisite and the organic dichloroarsines are colorless, odorless oily liquids, but when produced with impurities, they have a fruity or geranium-like odor. As liquids, they can penetrate rubber and most fabrics, and are more dangerous as liquids than as vapor.

Little is known about lewisite's stability in the environment, but it can react with water in a manner whereby its volatility and most of its blistering potency are lost. As a potent blister agent, it has irritant effects on the eyes and respiratory system, and has similar toxicities to the other blister agents mentioned above (except that it exhibits less bone marrow suppression). Similar to its dichloroarsine cousins and phosgene oxime, but unlike the mustard vesicants, it can cause pain at the time of initial contact. There is often no erythema around the vesicles as with other mustard agents.

Mustard and Lewisite Mixture (HL)

Mixing lewisite (L) with sulfur mustard (HO) at the concentrations of 63% L and 37% HL produces a liquid with a low freezing point that provides a more effective weapon in colder climates at higher altitudes. It has a garlic odor and is effectively insoluble in water. It persists on the ground from 1 to 2 days under average weather conditions when splashed, and can remain 1 week or more under very cold conditions before dissipating. Along with its blistering properties, it is also cytotoxic to the hematopoietic or blood-forming cells in the bone marrow. Other

mixtures like mustard and phenyldichloroarsine act in a similar manner.

Phenyldichloroarsine (PO) and Other Dichloroarsines

Phenyldichlorarsine, ethyldichloroarsine, and methyldichloroarsine have similar properties and toxicities as lewisite. They may be mixed with sulfur mustard similarly as can be done with lewisite and mustard mixtures, and this can confuse the diagnosis between either an arsenical or a mustard injury.

Phosgene Oxime (CX)

Phosgene oxime or dichlorformoxime is a nettle gas or urticant. These act as irritants to the skin and mucous membrane and, like arsenicals but unlike mustards, cause pain on immediate skin contact. Severe pain is noted. Very low doses cause lacrimation. It is a liquid or colorless solid and has a disagreeable odor. Unlike mustards and arsenicals, it is readily soluble in water.

Diagnosis

Diagnosis of a blister agent injury, without obvious overt contamination, requires a high level of suspicion when eye, skin, and respiratory signs and symptoms become evident. The first effects of blister agent exposure are eye and airway irritation. Conjunctivitis can occur after 1 h at a concentration of a blister agent that is barely perceptible by odor. Mild exposure results in tearing and the sensation of eye grit in 4–12 h. Severe eye lesions may occur within 2 h on heavy exposure. Lewisite and the dichloroarsines can cause gray scarring of the cornea at the point of contact. Severe lacrimation can occur with low doses of phosgene oxime.

Skin damage may not be immediately evident because the first effects may be painless until deeper skin layers are involved and blisters appear. However, the diagnosis of a chemical skin injury is readily made when the fluid-filled skin blisters appear and are recognized. There is a 1–12 h (or more) latent period, during which skin burning and itching may occur. Erythema or skin redness appears on exposed skin after 2–48 h. In darker-skinned individuals, sulfur mustard lesions may turn coal black in the face, neck, axilla, groin, and genitalia areas. Most American survivors from World War I had scrotal and perianal burns, because of increased moisture and ambient temperature in these areas. This redness (or darkness) is followed by coalescing blisters on a red base. At this stage, any vesicant on contaminated patients may still pose a hazard to other individuals coming in contact with them, so care needs to be taken in decontamination. Lewisite (L) and phosgene oxime (CX) differ in that pain may be immediately noted on contact, but areas of erythema may recede without blister formation. Lewisite and the dichloroarsines cause a more opaque blister fluid than the mustards. They also lead to deeper injury to the connective tissue, which can include muscle and vasculature, with more inflammation. Phosgene oxime causes immediate pain and skin necrosis at the site of contact. In 30 s, the contact area becomes blanched and is surrounded by a ring of erythema. A wheal then occurs in 30 min, and this area turns brown within 1 day. An eschar forms and sloughs off within 1–2 weeks.

Healing and resorption of uninfected blisters occur in 1–3 weeks for all vesicants. Broken blisters must be protected to minimize chances of infection and subsequent scarring of denuded skin. There is no useful medical test to determine if there has been mustard gas exposure.

Respiratory symptoms can include fever, dyspnea, ronchi with moist rales. Chest X-rays can reveal pulmonary edema. Changes consistent with chemical pneumonitis may appear after the first 24 h. Lewisite and the other organic dichloroarsines do not cause a significant respiratory injury from vapor concentrations found in the field. Skin pain usually occurs on immediate cutaneous contact, and that is a signal to wear a mask to prevent further respiratory and eye injury.

Treatment

Treatment follows decontamination of the patient, after donning protective gear. The various agents may vary in their ability to generate local and systemic pathology; however, the general treatment principles remain the same for all vesicants except for the availability of British Antilewisite (BAL) for dichloroarsine exposure.

Mild eye lesions require little treatment other than flushing with water immediately. Slow running water is applied as one tilts the head from side to side, pulling the eyelids apart. Steroid and antibiotic ointment can be applied to the eye.

Sterile petroleum jelly between the eyelids can provide lubrication, as would boric acid 5%. With eyelid edema, which occurs with more severe injuries, the eyelids may be gently opened to provide reassurance to the patient that one is not blind. Pain can be controlled by oral or parenteral narcotics. Photophobia can be eased by placing the patient in a darkened room, and by providing sunglasses or eyeshades. Do not cover the eyes with bandages. Atropine sulfate ointment should be instilled in each eye

to obtain good mydriasis in all cases where there are corneal erosions, iritis, cyclitis, or marked photophobia or miosis. Blepharospasm, or eyelid spasm, is treated with atropine sulfate solution 1% applied 3 times a day. To prevent infection, a few drops of sodium sulfacetamide 15% should be instilled every 4 h. Another antibacterial ophthalmic preparation may be substituted. The eye must not be bandaged and the lids must be kept separated. The patient should be seen by an ophthalmologist as soon as possible.

Treatment of skin lesions also follows decontamination and removal of clothes. Decontamination should be completed within 15 min after exposure to minimize any systemic effects. Contaminated hair should be shaved off. The decontaminating solutions should be washed off within 3–4 min to prevent additional skin injury. Sodium hypochlorite (5%) or liquid household bleach can be used. If erythema is already present, soap and water are preferred. Blisters should be left intact, but if broken, should be debrided to prevent secondary infection. Cleansing with tap water or saline and the application of dressings is done when needed. Silver sulfadiazine or mafenide acetate can be applied and the wounds treated as burn wounds. Infected skin wounds require antibiotics as appropriate.

In cases of lewisite skin injury, dimercaprol (BAL) ointment should be used on contaminated skin where blisters have not yet formed. Sometimes BAL itself causes irritation with stinging and itching with wheal formation, but this should resolve 1 h after application. Frequent BAL ointment application does cause a mild dermatitis, so it cannot be used as a protective barrier on skin not contaminated by dichloroarsines. Because of the deeper injury with dichloroarsines as with lewisite, wounds may heal more slowly and skin grafting may be required in the future.

Systemic treatment with parenteral antilewisite is considered when there is (1) greater than 5% area of skin contamination (1 ft^2) which results in immediate skin blanching or erythema within 30 min after exposure or (2) a burn the size of the palm (1% of skin area) which was not decontaminated within the first 15 min after exposure. There are two types of parenteral BAL therapies which can be used. One involves applying BAL ointment liberally onto the skin (after removing any other protective ointment first), and allowing that area to remain covered. The other parenteral method is to give an intramuscular injection of 10% BAL in oil into the buttocks (without injecting into a blood vessel). The dose given of 10% BAL in oil is 0.5 ml per 25 lb body weight, up to a maximum of 4.0 ml for those individuals who weigh 200 lb or over. Intramuscular injection of BAL in oil (10%) should be repeated every 4 h for a total of four doses at alternate sites on the buttocks. In severe cases, the frequency can be shortened between the first and second doses by 2 h. For severe cases, one injection can be given per day for 3–4 days. One should be aware of symptoms which occur with BAL injections, which may last 30 min but do not indicate that therapy should be stopped. These symptoms include tightening of the throat, chest pressure, lip burning, lacrimation, eye redness, mouth dryness, aching muscles, abdominal pain, tenderness and increased muscle tone at the injection site, anxiety, nausea, vomiting, and transient increased blood pressure.

Inhalation of vapors from mustard or arsenical vesicants can result in laryngeal and tracheobronchial mucosal injury. Mild injury with hoarseness and sore throat requires either no treatment or mist inhalation. Moderate exposures result in hyperemia and necrosis of the bronchial epithelium, and require hospitalization to prevent secondary infection. Antibiotics are used in an appropriate manner. Pneumonia was the usual cause of death from mustard agents during the preantibiotic World War I era. Severe injuries cause tracheobronchial tree casts from pseudomembrane formation. Hypoxia can occur, but subsequent bronchitis and pneumonia from infection were the chief causes of pulmonary-related deaths in World War I (2% mortality). Pulmonary resuscitation is required if breathing stops. Mustard–lewisite (HL) mixtures can cause pleural effusion in severe cases. Blister agents at extensive exposure can have systemic toxicity that affects not only the lungs, but also the bone marrow, lymph nodes, spleen, and endocrine systems. In these cases, complete blood counts with monitoring of granulocytes, red cells, and platelets should be performed routinely. If granulocyte depletion occurs, isolation and antibiotic prophylaxis may be necessary. Many past fatalities were due to the combination of pneumonia and bone marrow failure. Anemia and thrombocytopenia should be treated as the situation dictates. If local effects remain mild, systemic effects are not likely to be significant. In severe cases of lewisite or dichloroarsenine respiratory injury, dyspnea with frothy sputum indicating pulmonary edema indicated that intramuscular BAL is necessary.

If ingestion of blistering agent occurs, do not induce vomiting. Milk can be given to drink to mitigate damage. Giving 0.4–0.8 mg atropine subcutaneously can help in reducing systemic or local gastrointestinal activity. Morphine can be given intravenously for intestinal pain with close monitoring for shock. Fluid resuscitation for vomiting and diarrhea will require intravenous saline. Sedatives may be necessary.

Prognosis

With eye injury, temporary blindness occurs, but permanent blindness is rare with vapor exposure. The patient should receive this reassurance except for the severest eye injury. Blindness is more likely to occur when liquid mustard is directly splashed into the eye. With mild eye injury, recovery occurs in 1–2 weeks. More severe involvement with corneal erosions as detected by fluorescein staining can take 2–3 months of hospital care before recovery occurs. Corneal involvement beyond erosions with opacification and ulceration (less than 0.1% of mustard casualties in World War I) takes several months for recovery, and then late relapses can still occur. In these cases blindness may ensue. Eye injuries are more severe with nitrogen mustard than with sulfur mustard. The iris is frequently discolored and atrophied with nitrogen mustard exposure.

With mild blister formation, healing occurs with little scarring, but it may take months to heal while remaining painful during this time. When secondary infection occurs or in more extensive blistering, scarring can be more severe. Itching may persist after healing. Hypopigmentation or hyperpigmentation can occur as with any healing process. Deeper burns with lewisite and the dichlorarsines have similar outcomes as second- or third-degree thermal burns. Repeated exposures over time to mustards or arsenicals such as dichloroarsines can cause sensitization. Delayed healing beyond 2 months occurs with skin lesions caused by phosgene oxime.

A single low-dose exposure to mustard vapor with laryngeal and tracheobronchial mucosal effects may not lead to significant injury once healed. A cough may persist 1 month or longer. Hoarseness usually lasts only 1–2 weeks. However, repeated or chronic low-dose exposure can lead to progressive pulmonary fibrosis, chronic bronchitis, and bronchiectasis.

See also: Lewisite; Mustard Gas; Nitrogen Mustard; Phosgene Oxime.

Relevant Websites

http://www.bt.cdc.gov – (US) Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – (US) National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Blood

Gary R Krieger and Scott D Philips

Introduction

Hematology is the study of pathophysiology of the cellular elements and coagulation proteins in the blood. Physicians who specialize in this field are referred to as hematologists. Hematology has several new tools available to detect abnormalities in numbers or dysfunctions. Hematologists diagnose and treat both benign and malignant blood disorders. Primary hematologic diseases are uncommon, while secondary hematologic conditions occur frequently. Thus the inquiring physician must extensively question the patients for all forms of medications, herbals, folk-remedies, occupations and family history to name a few areas that require an in-depth history.

The formed elements of the blood – red blood cells (RBCs or erythrocytes), white blood cells (myeloids), immunocytes (T and B cells), platelets (thrombocytes), and their diseases – have traditionally been the main focus of hematology. However, with the advent of increasingly sophisticated molecular investigatory tools, such as recombinant DNA technology, the appreciation of the scope and inherent complexity of the blood-forming organ has dramatically increased. The bone marrow and its formed elements can be considered as a complex organ with a total mass that is over twice as large as the liver. The cells produced by this organ provide several critical functions such as the transport of oxygen (RBCs), hemostasis (platelets), and host resistance (immunocytes and white blood cells). Generally, each step in the intricate sequence required to produce the formed elements is vulnerable to adverse effects from a wide variety of chemicals and drugs. This entry will present a basic overview of normal bone marrow function, followed by a discussion of some of the abnormal physiologic effects that can be produced by exposure to various common drugs and chemicals.

Bone Marrow Structure and Function

In the normal adult, the marrow is found in the central hollow segment of bones. Hematopoiesis, or the production of the formed blood elements, occurs in the bone marrow. However, in the adult, it is largely restricted to scattered clusters of hemopoietic cells in the proximal epiphyses of the long bones, skull, vertebrae, pelvis, ribs, and sternum. The hematopoietic

picture in adults is quite different from that seen in either prenatal or childhood time periods. Within the first 1–5 prenatal months, the liver and spleen act as the hematopoietic organs. By the fifth prenatal month, the marrow achieves sufficient maturity to assume the dominant role in hematopoiesis. During childhood, there are high demands on the bone marrow system to produce large quantities of the formed elements; however, with increasing chronological maturity, there is less demand on the bone marrow system and the total output of the bone marrow significantly declines.

In addition to hematopoietic cells, there are other separate and distinct cells that support and augment marrow activities. Among these cells are fibroblasts, fat cells, and reticuloendothelial and endosteal cells. In aggregate, these cells are known as the bone marrow stroma. Occasionally, the term hematopoietic microenvironment is also employed to differentiate these cells and supporting structure from the stem and progenitor cells. These cells are the focus of the next section, which presents an overview of the basic physiology of the blood-forming elements.

Hematopoiesis

In the average adult, between 200 and 400 billion blood cells are destroyed and replaced each day. This enormous turnover implies that new cells are constantly formed rather than simply released from a central storage area that contains all the cells necessary for an individual's lifetime. Hemopoiesis is the key concept that has been used to explain how the body can provide a lifetime worth of formed blood elements. Hemopoiesis is a process of cell amplification and differentiation in which a few stem cells give rise to increasingly more developed or differentiated progenitor cells, which in turn give rise to the formed blood elements. The earliest cell is known as the pluripotent stem cell or PSC. PSCs are uniquely responsible for the production of the formed elements throughout the lifetime of a human. Relatively few PSCs are required since, as these cells undergo mitosis or cell division, one replacement stem cell and one committed or daughter cell are produced. This daughter cell subsequently develops and proliferates into the various formed elements. Hence, the PSCs are considered to be self-renewing because of their ability to reproduce themselves. **Figure 1** presents the overall organization and development of the bone marrow cells. This structure is quite hierarchical and resembles a company organization chart with a single chief executive officer presiding over separate divisions, which in turn develop other specialized departments or functions. Not surprisingly, each step in the organization requires both a series of growth factors and interactions with the hematopoietic microenvironment to promote and control the development of each cell type. The stimulatory or growth factors are known as poietins or colony stimulating factors (CSFs). CSFs can either be lineage-specific, i.e., they act on specific cell lines, or direct acting on multipotential progenitors and stem cells. Examples of lineage-specific CSFs include (1) erythropoietin, which stimulates production of erythrocytes or RBCs; and (2) interleukin-7, which induces the growth of B- and T-lymphocyte progenitors. Direct-acting CSFs include interleukins 2–6, which act on a variety of cell lines.

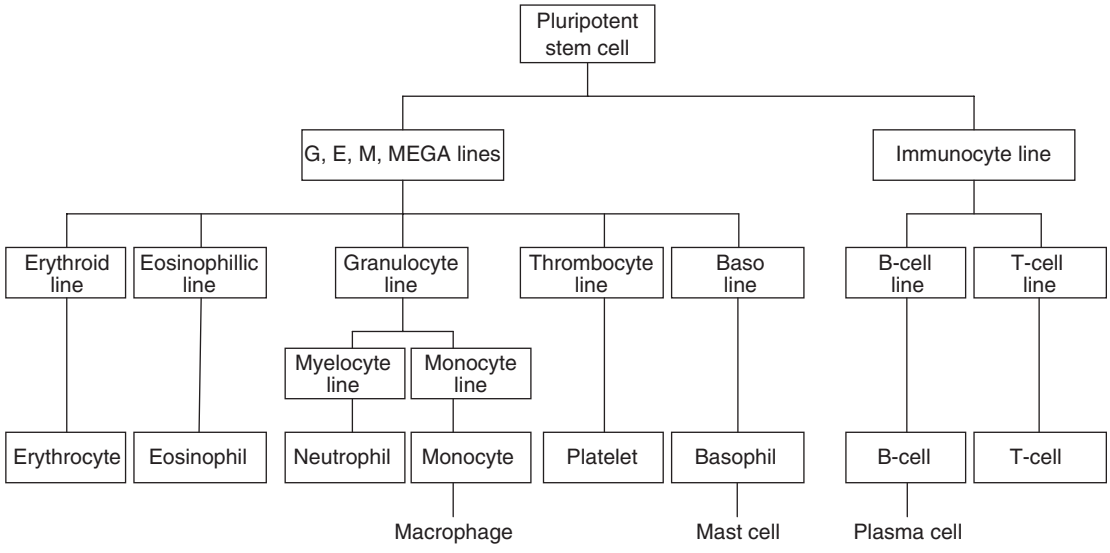

Figure 1 Bone marrow cell organization. G, Granulocyte; E, Erythrocyte; M, Monocyte; and MEGA, Megakaryocyte.

Formed Elements – Erythrocytes, Myeloids, and Thrombocytes

Erythrocytes

The RBC is a biconcave disk with a diameter of ~8 μm and a lifespan in the circulation of ~120 days. Due to its unique shape, the RBC is twice as thick at the edges (2.4 μm) as at its center. The explanation for this specialized geometry is not fully known; however, this shape tends to minimize intracellular diffusional distance and allows for easier passage through small blood vessels. The critical function of the RBC is transportation and delivery of oxygen to peripheral tissues. Approximately 30% of the wet weight of the RBC is composed of hemoglobin, the essential protein which is integral to the oxygen/carbon dioxide transport and delivery system. Hemoglobin is also capable of transporting nitric oxide (NO). NO is a unique gas that can affect the ability of blood vessels to expand or contract in addition to having a role in learning and memory.

The mature RBC is formed through a series of cell divisions that progressively increase the amount of hemoglobin in the cytoplasm. Following the last division, a special cell known as a reticulocyte is formed. The reticulocyte stays in the bone marrow for 2 or 3 days before being released into the general circulation, where over a period of 24 h it undergoes a series of transformations that results in the appearance of a mature RBC. The reticulocyte is easy to identify in laboratory tests and the reticulocyte count or index is an important parameter that can provide information about marrow function. The reticulocyte index is equal to the reticulocyte percentage multiplied by the ratio of the patient's hematocrit (packed cell volume) to a normal hematocrit.

Hemoglobin

Hemoglobin, in the normal adult, is a protein whose main function is to transport oxygen from the lungs to tissues and to transport carbon dioxide from tissues to the lung. The hemoglobin molecule contains four separate folded peptide chains, which form a hydrophobic or water 'repelling' pocket around a heme group. The heme group is composed of a central iron atom complexed to four nitrogen atoms. Oxygen is capable of reversibly binding to the heme unit in a process known as oxygenation. The interactions among the subunits in a hemoglobin molecule are known as cooperativity. There are well-described regulators of the affinity of hemoglobin for oxygen that provide a control mechanism. The S-shaped graph of this oxyhemoglobin relationship is known as the oxyhemoglobin dissociation curve and represents the relationship between the partial pressure of oxygen (Po_2) in mm of mercury (Hg) and the oxygen content per 100 ml of blood (**Figure 2**).

The shape of this relationship is very important since it can be moved to the right, i.e., decreased affinity of hemoglobin for oxygen producing oxygen unloading, or to the left, i.e., increased affinity. These changes are produced by a variety of intracellular cofactors: hydrogen ion (pH), carbon dioxide, and the RBC enzyme 2,3-biphosphoglycerate (BPG). Molecules of 2,3-BPG bind to hemoglobin and decrease the affinity of the molecule for oxygen. This causes enhanced oxygen release, or unloading, and is frequently seen in situations in which the body responds to conditions of low oxygen supply. There are a wide variety of potential diseases and toxic exposures that can impact oxygenation and cooperativity and these will be discussed in subsequent sections.

Anemia

There are many other events that can produce a significant reduction in the RBC mass and a subsequent decrease in the oxygen-carrying capacity of the blood. Normally, the blood volume is maintained at a relatively constant level; hence, any process or event that causes a reduction in either RBCs or hemoglobin produces a condition known as an anemia. Anemias can also shift the oxyhemoglobin dissociation curve

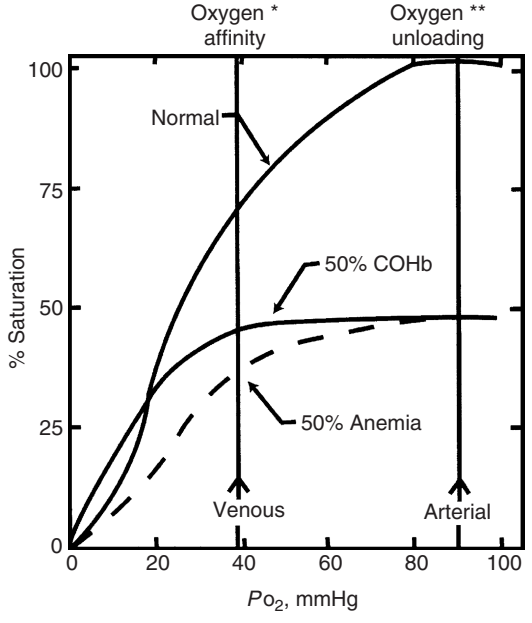

Figure 2 Oxyhemoglobin dissociation curve. *Modifiers of oxygen affinity – increase in plasma pH, decrease in temperature, decrease in 2,3-BPG. **Modifiers of oxygen unloading – decrease in plasma pH, increase in temperature, increase in 2,3-BPG.

as the body attempts to compensate for reduced oxygen-carrying capacity. In general, the etiology of anemias falls into three general categories: (1) acute or chronic blood loss from any source, (2) underproduction associated with a decreased reticulocyte count, and (3) hemolysis or destruction of RBCs associated with an increased reticulocyte count. There are a variety of laboratory tests that are useful for the evaluation of anemia; however, three of the most critical are the measurement of RBC size and shape (known as RBC indices), examination of the peripheral blood smear, and bone marrow examination. Each of these tests reveals information that can provide clues which lead to the etiology of the anemia. In later sections, some examples of toxins (e.g., carbon monoxide, hydrogen sulfide, and hydrogen cyanide) which cause anemias by altering the binding affinity for hemoglobin and oxygen will be presented.

In general, the amount of oxygen delivered to a given organ or tissue is directly related to three variables: (1) blood flow or cardiac output, (2) hemoglobin concentration, and (3) the difference in oxygen content (saturation) between arterial and venous blood. For example, the cardiac output can significantly increase in order to maintain adequate oxygenation of vital organs such as the brain and kidney at the expense of the smooth muscle. Similarly, erythropoiesis can be stimulated by erythropoietin so that the overall hemoglobin levels increase. Finally, as illustrated in **Figure 2**, oxygen unloading or delivery can be augmented by a right shift in the oxygen dissociation curve facilitated by the RBC enzyme 2,3-BPG.

The converse to anemia is known as polycythemia or erythrocytosis, which is an increase above normal in the circulating quantity of RBCs. Not surprisingly, this increase in total RBCs is usually associated with a corresponding increase in hemoglobin. There are numerous causes of polycythemia such as response to high altitude, pulmonary disease, steroids (both androgenic and glucocorticoid), stress, and smoking.

Myeloids

The myeloids or leukocytes are a highly complex and sophisticated group of cells that are primarily involved in host resistance and inflammatory response against both foreign organisms and material (e.g., chemicals and toxins). For simplicity, the leukocytes can be divided into two major groups: (1) immunocytes and (2) phagocytes. The general organizational structure and normal values are shown in **Figure 1**. These cells are thought to arise from a common PSC in the bone marrow; hence, any toxin that affects the PSC will have a potentially disastrous impact on the body's ability to respond to challenges from an external agent or foreign substance.

The immunocytes are all involved in specific types of immune response that are generally divided into two types: (1) cell-mediated, i.e., specifically sensitized T cells (derived from the thymus) which are associated with graft rejection, resistance to certain viruses, bacteria, fungi, and protozoa, and delayed-type hypersensitivity; and (2) humoral-mediated, i.e., B cells (bursa equivalent) which produce specific antibodies after the body is exposed to a specific antigen.

The phagocytes are so named because their major function is to engulf or ingest foreign organisms or material. The phagocytes include the three granulocytes known as neutrophils (54–62%), eosinophils (1–3%), and basophils (<1%) and the monocytes (3–7%). Monocytes circulate in the blood for several days until they migrate into the reticuloendothelial tissues (liver, spleen, and bone marrow), where they are known as macrophages. Macrophages are not only involved in inflammatory responses but also have a major role in the destruction and removal of old RBCs and other plasma proteins, including hemoglobin.

The phagocytes act by engulfing the foreign material/agent and produce a respiratory burst. The respiratory burst involves the production of hydrogen peroxide and other highly reactive chemicals that attack the ingested material. An inflammatory response is quite commonly produced in this situation. Glucocorticoids (steroids such as prednisone) tend to decrease the numbers of granulocytes that will be involved in an inflammatory reaction. This effect accounts for beneficial impact of these drugs when an anti-inflammatory result is desired; however, there is also an increased susceptibility to infections that has been well documented.

There are several key terms and definitions that are given to absolute decreases or increases in the numbers of leukocytes. A fall in the total granulocyte count below $3000\,mm^{-3}$ is known as granulocytopenia. Granulocytopenia is commonly associated with chemically induced bone marrow damage; however, ionizing radiation and a myriad of drugs can also produce this effect. Finally, a particularly severe form of bone marrow failure is known as aplastic anemia. Aplastic anemia is diagnosed when at least two different marrow cell lines are severely depressed as demonstrated by (1) granulocytes $<500\,mm^{-3}$, (2) platelets $<20\,000\,mm^{-3}$, (3) reticulocyte count <1%, or (4) a bone marrow biopsy demonstrating <25% cellularity.

Granulocytosis is the opposite phenomenon of decreased cellularity and refers to elevated counts over $10\,000\,mm^{-3}$. Stress, drugs, and some bacterial

toxins can produce short-term granulocytosis; however, chemical exposure is not typically associated with mild, elevated counts. Leukemias are associated with counts over 30 000 mm^{-3} and have been associated with certain chemical exposures. This association will be presented in further detail in a subsequent section.

Thrombocytes

Platelets are produced by the fragmentation of megakaryocytes, the largest cell type in the bone marrow. Approximately one-third of the platelets are taken up by the spleen, while the other two-thirds freely circulate for 7–10 days until they are taken up by phagocytic cells. A normal platelet count is between 150 000 and 450 000 mm^{-3}. The normal platelet count is quite variable and can be affected by an individual's nutritional state or, in females, by the menstrual cycle.

Platelets are the rapid reaction troops in the situation of accidental blood loss associated with damaged blood vessels that expose collagen fibers. Normally, platelets are nonsticky; however, they rapidly and easily adhere or aggregate to exposed collagen fibers where they undergo a series of reactions that results in the formation of a thick mass known as a platelet plug. This plug acts to quickly stop bleeding; however, it must usually be reinforced by help from the clotting system so that vascular integrity is maintained. Platelet reactions are highly sensitive and vulnerable to substances that interfere with the aggregation reaction. For example, aspirin acts in a unique fashion to inhibit the aggregation reaction and has become a useful drug in the prevention of heart attacks and strokes caused by small platelet plugs.

Any disorder or agent that injures the stem cells or prevents their proliferation can drastically affect the absolute platelet count. The minimal platelet count necessary for initial hemostasis is $\sim$50 000 mm^{-3}. If the platelet count falls below 20 000 mm^{-3}, a condition known as thrombocytopenia exists and the affected organism is extremely vulnerable to spontaneous bleeding episodes. Usually, thrombocytopenia due to marrow failure is also associated with reduced leukocyte and red blood cell production since chemicals or disorders that affect the megakaryocytes also impact other stem cells. This is typically determined by examining a peripheral smear of the blood or by a hematologist's bone marrow aspiration.

The opposite phenomenon, elevated platelet count or thrombocytosis, is diagnosed when counts are greater than 400 000 mm^{-3}. There are many causes of thrombocytosis, including primary (e.g., essential thrombocytosis (ET)) and secondary (e.g., response to inflammation, acute bleeding, iron deficiency, or cancers). In ET, there are colonies of megakaryocytes in the absence of any known stimulus.

Toxic Agents and Responses

Carbon Monoxide

Carbon monoxide (CO) is an odorless, tasteless, and colorless gas that is rapidly absorbed by the lungs and attaches to hemoglobin with an affinity that is 250 times greater than oxygen. Due to this extreme differential, as CO concentrations increase, the number of available sites on the hemoglobin molecule for oxygen decreases. Normally, this reaction would cause oxygen to be more freely released so that adequate tissue oxygenation can be maintained. This would typically produce a right shift of the oxyhemoglobin dissociation curve; however, with increasing exposure to CO and formation of carboxyhemoglobin (COHgb), there is a change in the oxyhemoglobin complex which produces a left shift in the oxygen dissociation curve (**Figure 2**). The overall effect is decreased tissue oxygenation, anaerobic metabolism, and lactic acid formation.

Exposure to CO results in a wide variety of potential adverse effects, particularly in individuals who have pre-existing cardiac or lung disease. Infants, the elderly, and the developing fetus are particularly vulnerable since they have less capacity to tolerate cardiovascular compromise. An additional problem is the delayed neurological and neuropsychiatric effects that have been documented after some significant exposures. The incidence of delayed neurotoxicity is between 2% and 30%.

CO poisoning is usually diagnosed by measuring the presence of (COHgb) in blood. Nonsmokers have COHgb levels of $<1\%$, whereas smokers have levels of 5–10%. Unfortunately, the measured COHgb level does not always correlate with clinical findings and symptoms; therefore, the clinician should always have a high index of suspicion and aggressively evaluate and treat exposed patients. Treatment consists of removal from the source and administration of 100% oxygen and any other basic life-support measures required. In certain circumstances, i.e., COHgb levels over 25%, the use of hyperbaric oxygen is indicated.

Hydrogen Cyanide and Hydrogen Sulfide

Both hydrogen cyanide (HCN) and hydrogen sulfide (H_2S) are metabolic poisons that act in relatively similar mechanistic ways. At the cellular level, the major energy source is adenosine triphosphate (ATP).

ATP is primarily produced through a process known as oxidative phosphorylation, which involves the transfer of electrons to substances known as cytochromes. The cytochrome system can be viewed as a 'bucket brigade' that moves critical electrons in an orderly fashion so that cellular respiration is maintained. As electrons are transferred, energy is released and used to generate ATP and water. Oxygen is the final electron acceptor in the cytochrome system and can be severely affected by metabolic toxins like HCN and H_2S. These toxins ultimately act by blocking electron transfer to molecular oxygen. This blockade produces a rise in peripheral tissue partial pressure of oxygen and a decrease in the unloading gradient for oxyhemoglobin. The net effect is the production of both high levels of oxyhemoglobin in venous return blood and significant levels of lactic acid. At high exposure concentrations, cardiopulmonary compromise is rapidly produced and death ensues.

The treatment of either HCN or H_2S toxicity is based on the use of chemicals that interrupt the binding of these materials to the cytochrome oxidase system. Sodium nitrate and amyl nitrate are both used as antidotes. These substances act by overwhelming the RBC with oxidant stress and producing a somewhat less toxic material known as methemoglobin (MetHgb). MetHgb serves as a source of circulating ferric iron (Fe^{3+}), which preferentially competes for binding by cyanide or sulfide and causes the cyanide or sulfide to dissociate from the cytochrome system and move into blood in a form complexed to methemoglobin in RBCs. This less toxic material is further detoxified by the use of another drug, sodium thiosulfate, which further enhances the conversion of cyanide to the less toxic thiocyanate. The situation with H_2S is somewhat more complex since the second step use of sodium thiosulfate is not typically recommended; however, vigorous use of 100% oxygen therapy is appropriate for treating exposure to both HCN and H_2S.

Methemoglobin

At the molecular level, the transport of oxygen in the body is highly dependent on the maintenance of intracellular Hgb in a chemical condition known as the reduced state, or Fe^{2+}. When hemoglobin is oxidized, the Fe^{3+} state, it is known as MetHgb and is unable to bind oxygen. A small amount, $<1\%$, of MetHgb is always found in normal RBCs. MetHgb can be chemically reduced by an enzyme system so that the body maintains adequate levels of Fe^{2+}. If MetHgb exceeds 10% of the total hemoglobin, then clinically observable changes such as dusky complexion can be detected in the affected individual. As MetHgb levels reach 35%, symptoms such as headache, fatigue, and shortness of breath are common. MetHgb levels over 80% are usually fatal.

There are many causes of MetHgb, including both hereditary and acquired. Drugs and toxins, such as nitrates, nitrites, nitroglycerine, aniline dyes, and sulfonamides, are associated with the production of MetHgb in certain situations. Toxic levels of MetHgb can be treated with a compound known as methylene blue, which acts to rapidly reduce the level of circulating MetHgb.

Leukemia

The leukemias are a diverse group of hematologic malignancies that arise from the malignant transformation of hematopoietic cells. These cells develop in the bone marrow and lymphoid tissue and ultimately interfere with normal cell development and immunity. Leukemias are generally divided into two groups, myeloid and lymphoid. In addition, leukemias can be further subdivided by their natural history into acute or chronic forms. The leukemias represent 3% of all malignancies and $\sim$24 000 new cases a year develop in the United States.

The etiology of leukemia in most cases is unknown, although a combination of genetic and environmental factors is probably important. The most important environmental factors are drugs, radiation, and chemical exposures to a few selected substances. The most common form of leukemia associated with either chemicals or drugs is the acute nonlymphatic leukemias (ANLL), which are also referred to as acute myeloid leukemias (AML). In ANLL, large numbers of immature hematopoietic cells develop and replace the normal cells. These abnormal cells are released into the circulation and can easily be seen on peripheral blood smears. Since these cells are quite immature, the blood does not contain adequate numbers of normally functioning mature RBCs, leukocytes, and thrombocytes. AML is an aggressive and rapidly fatal disease unless appropriate therapy is begun.

The role of chemical exposure and development of ANLL has been quite controversial. This controversy is partially due to the problems associated with accurately and appropriately classifying the various leukemias. Since the mid-1980s, the nomenclature of the ANLL subtypes was established by the French–American–British Cooperative group also known as FAB. Older studies in the literature that do not use this classification scheme present a serious problem since there was a tendency to lump different

categories together in order to achieve sufficient statistical power for epidemiological analysis. Nevertheless, there does appear to be sufficient evidence to link ANLL with certain exposures to benzene.

The association between benzene exposure and leukemia has been made since the late nineteenth century; however, the dose–response relationship and mechanistic explanation have been quite contentious. The most reliable evidence associating chronic benzene exposure with AML was presented in a retrospective NIOSH study of rubber hydrochloride workers in Akron, OH, from 1940 to 1949. Unfortunately, the mechanism of how benzene exposure leads to the development of AML is not known. The two most frequently discussed potential mechanisms of toxicity involve either a point mutation or a chromosomal deletion. The latter is considered more likely since neither benzene nor its metabolites are mutagenic or teratogenic.

See also: Benzene; Carbon Monoxide; Cardiovascular System; Distribution; Hydrogen Sulfide; Immune System; Kidney; Liver.

Further Reading

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Blue-Green Algae *See* Algae.

Boric Acid

Michael Wahl

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- Chemical Abstracts Service Registry Number: CAS 10043-35-3
- Synonyms: Boracic acid; Orthoboric acid; Borofax; Three elephant; NCI-C36417
- Chemical Formula: H_3BO_3

Uses

Boric acid is used as a fireproofing agent for wood, as a preservative, and as an antiseptic. It is used in the manufacture of glass, pottery, enamels, glazes, cosmetics, cements, porcelain, leather, carpets, hats, soaps, artificial gems, and in tanning, printing, dyeing, painting, and photography. It is a constituent of nickling baths and electric condensers, and it is used for impregnating wicks and hardening steel. In laboratory procedures, boric acid is used in the preparation of buffer solutions.

Boric acid is also used as a fungicide and as an insecticide powder. Domestic use may include its application as an insecticide for crawling insects such as roaches. In medicine, it has been used as a disinfectant and is a constituent of baby powders, antiseptics, diaper rash ointments, eye washes, gargles, and a variety of other consumer products for its mild antiseptic property.

Background Information

Boric acid exists in natural deposits as a mineral, sassolite. It is also found in hot mineral water sources. The minerals are extracted with sulfuric acid and crystalline boric acid is separated.

Exposure Routes and Pathways

Accidental ingestion and subcutaneous routes are the primary exposure pathways. The maximum workplace concentration is $10\,mg\,m^{-3}$. The maximum concentration in water used in fisheries is $0.1\,mg\,l^{-1}$.

Toxicokinetics

Water emulsifying and hydrophobic ointments containing boric acid liberate only small amounts within 24 h compared with a near total liberation from a

jelly. Boric acid is readily absorbed from the gastrointestinal tract, mucous membranes, and abraded skin. Boric acid is excreted unchanged in urine with ~50% excreted in the first 12 h and the remainder excreted over a period of a few days. The half-life of boric acid given orally is estimated to be 21 h. The fatal dose of boric acid is estimated to be ~20 g in an adult and ~5 or 6 g in an infant.

Mechanism of Toxicity

The exact mechanism of toxicity is not known. Boric acid can inhibit production of adenosine triphosphate, a cellular form of energy.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals have demonstrated similar toxic effects to those seen in humans.

Human

Acute boric acid poisoning is extremely rare. Symptoms in extremely large doses will be similar to those seen in chronic overexposure (see below).

Chronic Toxicity (or Exposure)

Animal

Dogs and rats were able to tolerate boric acid doses of up to 350 ppm for 2 years. Larger doses of boric acid (1750 ppm) over a period of time have been shown to cause testicular damage and sterility in rats and dogs.

Human

Toxicity may occur after ingestion, injection, application to damaged skin (e.g., abrasion, burns, or diaper rash), lavage, or enema. Severe systemic toxicity is most likely to occur from repeated dermal application to damaged skin; this has been reported mainly in the treatment of diaper rash in young children. Symptoms include nausea, vomiting, bloody diarrhea, severe colic, and abdominal pain. There may be restlessness, delirium, headache, tremors, and generalized convulsions usually followed by weakness and coma. There is fever and tachypnea followed by Cheyne–Stokes-type respirations and respiratory arrest.

Changes on the skin include an erythematous skin eruption, with papules or vesicles appearing between the fingers and on the back of the hands initially and eventually becoming generalized enough to give a 'boiled lobster' appearance. The skin lesions may undergo bullous formation, desquamation, excoriation, and sloughing. Hypothermia often occurs.

Renal injury can occur, usually in the form of renal tubular necrosis, and can be demonstrated by the presence of oliguria, albuminuria, and eventually anuria. Signs of meningeal irritation, oliguria, and circulatory collapse may be followed by death within 5 days. Infants and young children are more susceptible to boric acid intoxication. Low levels of boric acid ingestion may lead to dry skin and mucous membranes, followed by the appearance of a red tongue, patchy alopecia, cracked lips, and conjunctivitis. Infertility among men is possible.

No major toxicological distinctions between boric acid and its salts are recognized in human beings.

In Vitro Toxicity Data

No mutagenic effects have been seen in *Salmonella typhii* strains TA98 and TA100 via the preincubation method.

Clinical Management

There is no specific antidote. Supportive care should be instituted for all patients with history of serious boric acid exposure. Substantial recent ingestions may benefit from administration of activated charcoal. Fluid and electrolyte balance, correction of acid/base disturbance, and control of seizures are essential to therapy. Hemodialysis has been successfully used to treat acute boric acid poisoning. Sodium bicarbonate may be used for any metabolic acidosis.

See also: Cosmetics and Personal Care Products; Federal Insecticide, Fungicide, and Rodenticide Act, US; Nickel and Nickel Compounds.

Further Reading

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Boron

William S Utley

- Chemical Abstracts Service Registry Number: CAS 7440-42-8

Uses

Boron is used as a reinforcing material for composites. It is used in the nuclear industry as a neutron absorber. Boron is used to harden metals and used as an oxygen scavenger for copper and other metals. Amorphous boron is used in pyrotechnic flares to produce a green color. Used as a catalyst in olefin polymerization and alcohol dehydration. The principal consumption pattern in the United States for boron is for the production of glass products with minor usage in the production of soaps and detergents.

Exposure Routes and Pathways

Ingestion and inhalation are the primary routes of exposure. Boron can be found in dusts, water, and in fruits and vegetables. Dermal absorption will not be a factor unless the dermal barrier is compromised.

Toxicokinetics

Absorption

Boron is well absorbed via the gastrointestinal tract. Systemic toxicity is more likely to result from multiple exposures rather than from single acute exposures.

Distribution

Boron is distributed fairly rapidly (30 min to 3 h) to all tissues of the body.

Elimination

The apparent half-life of elimination is 5–10 h. The primary route of elimination is via the kidneys.

Mechanism of Toxicity

Boron is concentrated in the kidneys during excretion, making the kidneys a prime target organ for boron toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Gastrointestinal and pulmonary disorders have been reported in lambs grazing in pastures containing high boron content in the soil. High exposures to boron (1000–2000 ppm) for 90 days have caused oligospermia and testicular atrophy in rodents. Exposure to boron in pregnant rats has led to central nervous system abnormalities in offspring. It is interesting to note that the dog appears to be twice as sensitive to the toxic effects of boron as rodents. Animal studies also indicate that high levels of boron may affect the testis.

Human

Single acute exposures to boron are well tolerated. Reversible irritation to the respiratory tract and mucosal membranes may be seen initially but these are expected to resolve themselves. Chronic exposures can lead to anorexia, weight loss, vomiting, mild diarrhea, erythematus rash, alopecia, convulsions, anemia, and kidney damage. Both vomitus and feces will be blue-green.

Clinical Management

General life support should be maintained. Symptoms should be treated and decontamination undertaken if necessary. Emesis may be indicated in instances where the patient has recently ingested a significant quantity.

Environmental Fate

Boron in the form of various oxides is removed from the atmosphere by precipitation and deposition fairly rapidly (a half-life measured in days). Whether via atmospheric deposition, precipitation, or weathering of boron-containing rocks, boron can be expected to migrate to the water column where it will hydrolysis to its weak acid form. Once in the water column the only significant factor that will affect its fate is possible adsorption to soils and sediment. The adsorption process is not well predicted, and will need to be determined for each sediment type being considered.

Exposure Standards and Guidelines

The oral reference dose for boron is 0.09 mg kg^{-1} day^{-1} and is based on testicular atrophy and

spermatogenic effect. An ambient water quality criterion of 750 ppb boron in water has been suggested based on long-term irrigation of sensitive crops. The Federal drinking water guideline for boron is 600 ppb.

Miscellaneous

Powdered boron has pyrotechnical properties and can spontaneously ignite in the air.

Further Reading

World Health Organization/International Programme on Chemical Safety (1998) Environmental Health Criteria 204. Boron, pp. 1–10.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Boron.

Botulinum Toxin

Fermin Barrueto Jr.

- Chemical Abstracts Service Registry Number: CAS 93384-43-1
- Synonyms: *Clostridium botulinum*; Foodborne (classic) botulism; Infant botulism; Wound botulism; Unclassified botulism
- Chemical/Pharmaceutical/Other Class: Foodborne toxins

Uses

The use of botulinum toxin has been increasing in cosmetic dermatology, muscle rigidity/spasticity syndromes, hyperhidrosis, some types of chronic pain syndromes, and headaches. It has the unfortunate distinction of being the most potent toxin that exists and is a weapon of mass destruction.

Exposure Routes and Pathways

Ingestion is the primary exposure pathway for botulism. Wound botulism occurs when the bacterium encounters devitalized human tissue, synthesizes toxin, and thus causes disease. Intestinal (adult and infant types) botulism involves ingestion of spores or the live bacterium and, due to impaired intrinsic defenses, the gastrointestinal tract becomes colonized with *Clostridium botulinum*. In infant type, the mucosal surface of the intestines is susceptible to colonization due to multiple factors including decreased acidity of the stomach and lack of bile of acids, which are natural barriers. In adult-type intestinal botulism, patients have had surgical vagotomy/Billroth procedures and/or medical treatment for peptic ulcer disease, making them susceptible to colonization of the toxin producing bacterium. Foodborne botulism usually results from exposure to canned foods that are inadequately sterilized during cooking and canning. Occasional larger outbreaks occur following ingestion of contaminated food at restaurants or from commercial sources. A variety of preserved foods have been implicated, including string beans, corn, garlic, seafood, pork, and beef.

Infant botulism is the most common form of the illness. This occurs in children less than 1 year old before the gastric mucosa becomes an acidic environment. Most cases occur from 1 week to 11 months of age, with a peak incidence at 2–4 months of age. Breast-feeding, feeding of honey or corn syrup, decreased frequency of bowel movements, and living in a rural area have been implicated as sources of *C. botulinum* spores that cause infant botulism. For this reason it has been recommended to avoid feeding honey to any child 12 months of age or younger. Types A or B botulinum toxin, and rarely type F, have been responsible for all infant cases. Inhalation through aerosolization, though rare, is thought to be the method in which botulinum toxin would be dispersed in a biological attack.

Mechanism of Toxicity

Despite the ubiquitous nature of botulinum spores, the incidence of disease is low. For optimal growth, *C. botulinum* requires a low acidic environment (generally pH >4.5), a temperature of at least 10°C, an anaerobic environment, and a lack of competition from other bacteria. While most toxins are destroyed by boiling, only pressure cooking to 240°F will ensure destruction of spores.

Three classified types of botulism (foodborne, intestinal-infant type, and wound) result from infection

with *C. botulinum* organisms whereas, in a biological attack, aerosolization of the neurotoxin itself would be used. *C. botulinum* is a strictly anaerobic, spore-forming, gram-positive rod that elaborates a potent exotoxin. Botulinum toxin is a protein that consists of a heavy chain and a light chain, bridged by a disulfide bond. The light chain, a zinc-dependent endopeptidase, is pharmacologically active and responsible for both the therapeutic and toxic effects. The heavy chain facilitates endocytosis of the toxin into the cell.

There are seven distinct antigenic types of botulinum toxin, assigned letters A–G. Guided by the heavy chain, the toxin enters a neuron in the peripheral nervous system. The light chain of the toxin binds the SNARE proteins (Synaptobrevin, SNAP-25, Syntaxin) that normally facilitate exocytosis of acetylcholine-containing vesicles. The endopeptidase site on the light chain then cleaves a portion of the SNARE proteins rendering the complex inactive, thereby blocking acetylcholine release. By weight, botulinum toxin is the most potent natural poison in the world where 7 pg of toxin is sufficient to kill a 70 kg adult if administered intravenously. While there are seven immunologically distinct toxins (A–G), the majority of poisonings in humans are caused by three toxins: A, B, and occasionally E.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cattle seem to be particularly sensitive to botulinum toxin. The estimated LD_{50} for lactating cows is $0.388\,ng\,kg^{-1}$, $\sim$13 times more sensitive to botulinum toxin type C than are mice.

Human

Initially, the toxin affects the bulbar musculature and patients typically present with any combination of signs and symptoms such as diploplia, dysphagia, dysarthria, and ptosis. Although the dose of the toxin may affect the rate of progression of the paralysis, all patients will develop multiple cranial nerve palsies with progression to a symmetrical descending flaccid paralysis with loss of deep tendon reflexes. Despite the neurologic findings, patients should maintain a normal mental status until the paralysis affects the muscles of respiration and results in respiratory failure and hypoxia. Once affected, a muscle remains paralyzed for several weeks, which is the time required for resynthesis of the SNARE proteins that were destroyed by the botulinum toxin.

Chronic Toxicity (or Exposure)

Human

Botulinum toxin type A is approved for use in humans for the treatment of strabismus, blepharospasm associated with dystonia, head position and neck pain associated with cervical dystonia (a movement disorder characterized by involuntary muscle contractions), as well as for the temporary improvement in the appearance of moderate to severe glabellar lines in adult men and women 65 years or younger. Clinical trials have noted few adverse effects associated with use of botulinum toxin type A for these conditions.

In Vitro Toxicity Data

Recent studies have demonstrated that secretory vesicle proteins, synaptotagmins I and II, mediate the entry of botulinum toxin type B into PC12 cells and function as protein receptors for the toxin. These findings were not demonstrated with botulinum toxins A or E.

Clinical Management

Proper supportive care and administration of antitoxin are the mainstays of current therapy. Patients who present with respiratory failure will need full ventilatory support; however, there will be a subgroup of patients who present early without obvious signs of respiratory muscle paralysis. The negative inspiratory force, pulse oximetry, and gag reflex of these patients should be evaluated serially to determine the degree of respiratory muscle weakness and likelihood of impending respiratory failure.

The current antitoxin is a trivalent equine-derived antibody that is only available from the Centers for Disease Control through local and state health departments. It contains types A, B, and E, which are the most common natural foodborne causes of botulism. Since the antitoxin prevents the progression of paralysis but will not reverse existing paralysis, its administration should not be delayed pending definitive laboratory confirmation of the diagnosis. This is critical as patients who progress to respiratory failure and become mechanically ventilated will be exposed to all the risks of the intensive care unit setting, including barotrauma, pneumonia, and sepsis, for several weeks to months.

The antitoxin itself carries some risk during administration since it is an equine-derived whole antibody. It can cause anaphylaxis, uriticaria, serum sickness, and other hypersensitivity reactions.

See also: Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents.

Further Reading

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Bovine Spongiform Encephalopathy (Mad Cow Disease)

Todd Canedy

Background

Bovine spongiform encephalopathy (BSE) is a chronic, degenerative disease affecting the central nervous system of cattle. BSE was first reported in the United Kingdom in November 1986. BSE belongs to a family of diseases known as transmissible spongiform encephalopathies, or TSEs. Though the source of the transmissible agent is yet to be fully characterized, there are common characteristics of all TSEs including a prolonged incubation period of months to years, a progressive debilitating neurological illness that is always fatal, pathological changes appear to be limited to the central nervous system and include vacuolation and astrocytosis, and the transmissible agent elicits no detectable specific immune response in the host. Furthermore, detergent treated extracts of brain tissue from animals or humans affected by these diseases reveal the presence of scrapie associated fibrils under electron microscopic examination.

Due to the lack of detectable specific immune responses, development of a preclinical live animal diagnostic test has been unsuccessful. There is no treatment for BSE, and affected cattle will die, usually within 2 weeks to 6 months from the onset of clinical symptoms.

Signs of Infection

The clinical signs of BSE begin to appear after a prolonged incubation period from 2 to 8 years. Symptoms include a change in temperament, such as nervousness or aggression, abnormal body posture, incoordination, difficulty in rising, decreased milk production, and/or the loss of body condition despite continued appetite.

Causes of Infection

The causative agent of BSE, like other TSEs, is yet to be fully characterized. Several theories currently under study include:

1. An unconventional virus.
2. A prion or abnormal partially proteinase K-resistant protein, devoid of nucleic acid, capable of causing normal prion protein in the host to change form into an abnormal protein.
3. A virino or 'incomplete' virus composed of naked nucleic acid protected by a host protein.

The BSE agent is smaller than most viral particles and is highly resistant to heat, ultraviolet light, ionizing radiation, and common disinfectants that normally render viruses and bacteria inactive. It causes no detectable immune or inflammatory response in the host, and has not been observed microscopically.

Detection of BSE

There is no test to detect the disease in a live animal. There are two methods currently available for the postmortem diagnosis of BSE:

1. A microscopic examination of the brain tissue to identify characteristic changes.
2. Use of immunohistochemistry, immunoblotting, and enzyme-linked immunosorbent assay to detect the partially proteinase resistant form of the prion (PrP^{res}) protein.

Transmission of BSE in Cattle

There is no evidence supporting the causal spread of BSE among cattle, that is, through contact with infected animals. Infection is thought to occur through feed containing meat-and-bone meal derived from infected animals. This feeding method has historically had widespread use, but is currently highly restricted in the United States. There is an occurrence of BSE in otherwise unexposed offspring of BSE-affected cattle. However, the study did not ascertain whether the symptoms were the result of genetic factors or true transmission from mother to offspring. The study concluded that the epidemic under study could not have been sustained through maternal transmission alone.

Extent of Epidemic

As of June, 2003, there have been no cases of BSE reported in the United States, and only one known case has been reported in Canada, from a cow imported from Europe. The United Kingdom, where the disease was first identified, claims some 95% of all BSE cases. Worldwide, more than 185 000 cattle have tested positive for BSE since the disease was first diagnosed. Other countries affected include Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Israel, Italy, Japan, Luxembourg, Liechtenstein, the Netherlands, Northern Ireland, Poland, Portugal, Slovakia, Slovenia, Spain, and Switzerland.

BSE in Humans

BSE is not transmissible to humans. However, there appears to be a strong connection between BSE and a variation of Creutzfeldt–Jakob disease, known as variant Creutzfeldt–Jakob disease (vCJD), another disease grouped with other TSEs. Evidence to date indicates that there has never been a case of vCJD transmission from person to person, but rather it is thought to spread from the consumption of cattle products contaminated with BSE. BSE and vCJD share many characteristics, to the point of being nearly indistinguishable from each other. Clinical studies have shown that mice inoculated with BSE showed the same pattern of incubation time, clinical signs, and brain lesions as mice inoculated with tissues from patients with vCJD. This provides evidence that BSE and vCJD are of the same 'strain'. Furthermore, these two diseases were not similar to other TSEs such as sporadic CJD and known scrapies strains.

See also: Food and Drug Administration, US; Neurotoxicity.

Relevant Websites

http://www.organicconsumers.org – US Continues to Violate World Health Organization Guidelines for BSE. January 23, 2004, Michael Greger, MD, for the Organic Consumers Association.

http://www.cdc.gov – CDC. Update 2002: Bovine Spongiform Encephalopathy and Variant Creutzfeldt–Jakob Disease. National Center for Infectious Diseases.

http://www.who.int – Bovine Spongiform Encephalopathy (BSE) (From the World Health Organization).

http://www.fda.gov – Bovine Spongiform Encephalopathy (BSE) (From the US Food and Drug Administration).

www.aphis.usda.gov – Animal and Plant Health Inspection Service.

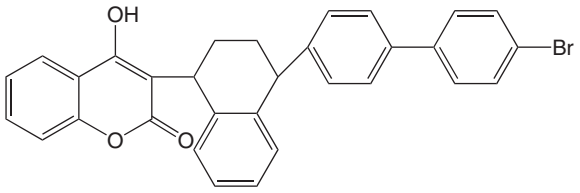
Brodifacoum

Henry A Spiller

This article is a revision of the previous print edition article by Michael J Hodgman, volume 1, pp. 185–187, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 56073-10-0
- Synonyms: PP 581; WBA 8119; 3-[3-(4-Bromo [1,1-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2*H*-1-benzopyran-2-one; Talon G; Ratac; Havac
- Chemical/Pharmaceutical/Other Class: A long-acting 4-hydroxycoumarin derivative; one of the superwarfarins
- Chemical Formula: $C_{31}H_{23}BrO_3$

- Chemical Structure:



Uses

Brodifacoum is used as a rodenticide (commonly 0.005% by weight).

Exposure Routes and Pathways

The most common route of exposure is oral. Transcutaneous and inhalation exposures have been implicated in workers involved in the manufacture of brodifacoum and pesticide operators.

Toxicokinetics

The metabolic fate of brodifacoum in humans is not well understood. Brodifacoum is much more lipid soluble than warfarin, resulting in a larger volume of distribution. There is extensive hepatic sequestration and prolonged high liver concentrations in the rat. Brodifacoum may also undergo enterohepatic recirculation in the rat. Based on the limited data available, the elimination half-life of brodifacoum in humans ranges from 16 to 36 days. There is considerable species variation. The apparent elimination half-life in dogs is 120 days. Inducers of the cytochrome P450 system have been reported to reduce the half-life of brodifacoum in animals.

Mechanism of Toxicity

Brodifacoum, like other hydroxycoumarins, interferes with the production of vitamin K-dependent coagulation factors. Vitamin K is a cofactor for the carboxylation of specific glutamic acid groups in coagulation factors II (prothrombin), VII, IX, and X. During this step, vitamin K is oxidized to vitamin K 2,3-epoxide. The regeneration of vitamin K by vitamin K 2,3-epoxide reductase is prevented by brodifacoum. As a result, dysfunctional decarboxycoagulation factors are produced and coagulation is impaired. Brodifacoum is over 100 times more potent than warfarin on a molar basis in rats.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity has been described in dogs, cats, horses, cows, pigs, sheep, goats, rats, mice, rabbits, voles, possums, Australian marsupials, chickens, ducks, and hedgehogs. Toxicity is expected in mammals, marsupial, and avian species. Owls died of hemorrhaging after feeding on rats killed with brodifacoum. Signs of poisoning occur after a latent period of 12 h to several days and may include: bruising easily with occasional nose or gum bleeds; blood in stools or urine; excessive bleeding from minor cuts or abrasions; labored breathing; pale mouth and cold gums; anorexia; and general weakness. You may also see lethargy, weakness, and lack of muscular coordination. Prolonged bleeding may occur from any small wounds and extensive bruising and subcutaneous hemorrhage.

Human

Depletion of preformed, circulating coagulation factors must occur before any anticoagulant effects are apparent. Typically, there is a delay of 24–36 h following ingestion before any effect is evident by measurement of the prothrombin time (PT). Significant toxicity from brodifacoum may be the result of large, one-time intentional ingestions. However, generally, repeated exposures over time are more likely to produce clinical toxicity. Single, small accidental ingestions in children are usually benign. Bleeding may occur virtually anywhere although cutaneous, mucosal, urinary, and gastrointestinal bleeding would be expected to be most common. Fatal intracerebral hemorrhage has been reported. Poisoning due to brodifacoum has led to prolonged periods of anticoagulation, often weeks and in some cases up to 6 months or longer. The clinical effect of brodifacoum is best monitored by following the PT and International Normalized Ratio (INR). Serum brodifacoum levels can be measured to confirm exposure, although there are no data to correlate serum levels and extent of toxicity. Factor activity can be assayed. An elevated serum ratio of vitamin K epoxide to vitamin K is further evidence of the presence of vitamin K reductase inhibition.

Chronic Toxicity (or Exposure)

Animal

Repeated exposures over time can lead to prolonged anticoagulation and appears to require a lower total

dose than acute exposure. Clinical effects are similar to those seen in acute exposures.

Human

Repeated exposures over time can lead to prolonged anticoagulation and appears to require a lower total dose than acute exposure. Clinical effects are similar to acute exposure.

In Vitro Toxicity Data

Ames *Salmonella* tests for genotoxicity have not demonstrated mutagenic effects. However, use of brodifacoum concentrations of $50\,g\,ml^{-1}$ in cultures of human lymphocytes did show mitotic activity, but no chromosomal aberrations.

Clinical Management

Animal

Treatment in animals is as for humans. The dose of vitamin K recommended for dogs and cats is 2.5–$5.0\,mg\,kg^{-1}\,day^{-1}$ for up to 4 weeks with monitoring of coagulation parameters.

Human

For acute, single-dose ingestions, activated charcoal may be administered. Induced emesis should be avoided in the anticoagulated individual. In large acute ingestions the PT should be determined at 24–48 h postingestion to assess the potential for toxicity. In the patient with clinical evidence of significant anticoagulation, extreme caution should be exercised with any invasive procedure. The airway should be protected if compromised by bleeding or hematoma formation. Volume resuscitation should be provided as indicated by clinical status. With active, uncontrolled, or life-threatening hemorrhage, fresh frozen plasma will provide preformed coagulation factors. Vitamin K_1 (phytonadion) is a specific antidote for brodifacoum toxicity. Pharmacologic doses of vitamin K allow the production of functional coagulation factors despite the presence of brodifacoum. The dose and route depend on the clinical setting. For rapid reversal, 5–25 mg should be administered intravenously no faster than $1\,mg\,mm^{-1}$. In children, doses of $0.6\,mg\,kg^{-1}$ have been recommended in warfarin poisoning, and larger doses may be necessary with brodifacoum. Clinical effects may be seen within hours. The response and duration of a single dose is variable and depends on the severity of the intoxication. Repeat doses will be necessary. In the less emergent setting, vitamin K may be given subcutaneously or orally. The doses needed to maintain adequate coagulation status may be quite large; in some cases, doses of $100\,mg\,day^{-1}$ or more orally have been reported, although typical doses are in the range of $25\,mg\,day^{-1}$. Titrate the daily dose by monitoring response to changes in the PT and INR. Oral vitamin K therapy may be necessary for weeks to months. Serial monitoring of the PT should be used to help guide therapy. Factor activity analysis may also be of use in assessing the adequacy of therapy. (*Note*: Anaphylaxis has been reported with intravenous vitamin K. Vitamin K_3 (menadione) is not an effective therapy.) Phenobarbital, $100–180\,mg\,day^{-1}$, has been administered to adults in an attempt to induce liver microsomal enzymes and hasten metabolism of brodifacoum, but its efficacy has not been proven. Administration of phenobarbital to an adult poisoned with chlorophacinone (another long-acting hydroxcoumarin derivative) resulted in a decrease in the apparent elimination half-life from 22.8 to 5.9 days.

Environmental Fate

In an aerobic soil, the half-life of brodifacoum is 14 days. If released into water, brodifacoum is expected to adsorb to suspended solids and sediment. Volatilization from water surfaces is not expected to be an important fate. The potential for bioconcentration in aquatic organisms is high. Brodifacoum is stable to hydrolysis in the environment. Brodifacoum is degraded by UV light when in solution.

See also: Coumarins; Warfarin.

Further Reading

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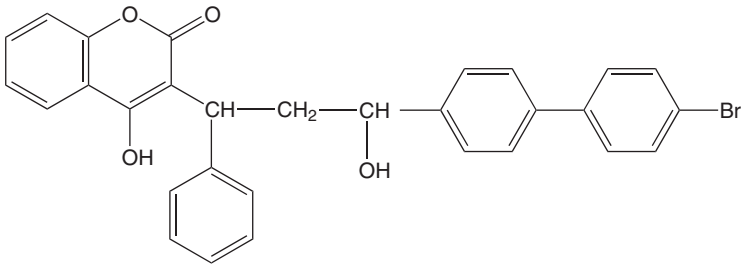
Bromadiolone

K S Rao

- Chemical Abstracts Service Registry Number: CAS 28772-56-7
- Synonyms: 3-[3-(4′-Bromo[1,1′-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2*H*-1-benzopyran-2-one
- Chemical/Pharmaceutical/Other Class: Rodenticide
- Chemical Formula: $C_{30}H_{23}BrO_4$
- Chemical Structure:



Uses

Bromadiolone is a rodent control agent for rats and mice in and around buildings, inside transport vehicles, and inside sewers. It is formulated as meal bait, paraffinized pellets, rat and mouse bait ready-to-use place packs, and paraffin blocks (all formulations contain 0.005% active ingredient). Baits and bait packs are placed at 15 ft intervals for rats and 8 ft intervals for mice. The maximum rates of application are 16 ounces of bait for 15 ft intervals for controlling commensal rats and 2 ounces of bait for 8 ft intervals for house mice. According to labels, all baits are to be placed out of the reach of children, pets, domestic animals, and nontarget wildlife; or tamper-resistant bait stations may be used.

Exposure Routes and Pathways

Bromadiolone is a nonfood use pesticide. Therefore, it is unlikely that there will be any exposure through food sources or residues in ground or surface water. At this time, some products containing bromadiolone are intended primarily for homeowner use, and others are intended primarily for occupational use.

The Environmental Protection Agency (EPA) has determined that there is a potential exposure to applicators or other handlers during typical use patterns associated with bromadiolone. Specifically, the Agency is concerned about potential dermal and inhalation exposures of handlers during the loading and application of bromadiolone at bait stations.

Based on the use patterns, it is possible that applicators may experience dermal exposure during bait station loading. Some inhalation exposure may be associated with meal baits or pellets.

The EPA has determined that there is a potential for exposure to consumers and others following applications of bromadiolone, particularly in residences. The EPA has concerns about possible post-application exposures if (1) baits are not placed out of reach of children or are not placed in tamper-resistant bait stations, as specified in the labeling; (2) baits are available to homeowners in packages that are not tamper resistant and could be accessible to children; or (3) baits are brightly colored or packaged in a way in which they could be appealing to children or mistaken by children as food or candy.

Toxicokinetics

Bromadiolone is absorbed rapidly and effectively by the oral route but less effectively by the dermal route. Oral administration of bromadiolone results in substantial retention of the chemical in the liver for an extended period of time. The half-life for the decline of liver bromadiolone concentration was calculated to be 63 days.

Mechanism of Toxicity

Bromadiolone toxicity is a function of its anticoagulant properties. In an antidotal treatment study, groups of male Crl:CD rats (10 per dose) were exposed to bromadiolone baited pellets (0.005% a.i.) for 24, 48, or 78 h. The estimated mean total bromadiolone

doses were 5.69, 9.76, and 15.63 mg kg^{-1} for the 24, 48, and 72 h groups, respectively. At the end of the exposure period, the first five surviving rats of each group were given vitamin K_1 at 5 mg kg^{-1}. Initially, a loading dose was given subcutaneously and, subsequently, vitamin K_1 was administered daily by gavage for 13 days. The survivors were sacrificed at 8–10 days after discontinuing the vitamin K_1 treatment. The animals in each exposure group which did not receive vitamin K_1 died. The deaths frequently occurred within 3–4 days of the beginning of the study. The clinical and gross pathology findings indicated hemorrhage-related toxicity in all test-article treated animals. The death rates in vitamin K_1-treated animals were 1/5, 2/5, and 5/5 in the 24, 48, and 72 h exposure groups, respectively. With vitamin K_1 treatment, the clinical findings (hemorrhagic-related toxicity) were resolved by the fifth day of the antidote treatment, and the decrease in body weight observed during the bromadiolone treatment was also restored in the surviving animals. At the second week of the study, the prothrombin times of the vitamin K_1-treated animals were essentially comparable to those of the control group. However, for the 48 h exposure group, the prothrombin time was slightly decreased relative to that of the control group. The results demonstrate that vitamin K_1 treatment, as employed in this study, can restore the clotting process of an animal that is exposed to bromadiolone below an estimated total dose of 15.63 mg kg^{-1} body weight during a 72 h period. However, the antidotal treatment may not completely prevent death (i.e., all the rats in the 72 h exposure groups died despite vitamin K_1 treatment) when rats are exposed to bromadiolone even at the lowest exposure dose (5.69 mg kg^{-1}) in this study.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral LD_{50} of bromadiolone is 10 mg kg^{-1}. It is slightly irritating to eyes and is not a skin sensitizer. It does not induce acute delayed neurotoxicity in hens.

Human

No case reports of toxicity have been directly attributed to bromadiolone. However, based on its mechanism of action as an anticoagulant, it is expected that excessive human exposure of bromadiolone is likely to produce epistaxis, bleeding of gums, pallor, and sometimes petechial hemorrhage leading to hematomas around the joints and on the buttocks, and ultimately blood in urine and feces. Exposures to very high concentrations of bromadiolone would be expected to cause paralysis due to cerebral hemorrhage, leading to hemorrhagic shock and death.

Chronic Toxicity (or Exposure)

Animal

Subchronic oral administration of bromadiolone to dogs caused signs of loose, bloody stools following 15 μg kg^{-1} dosing. Five days following 100 μg kg^{-1} dosing, animals also showed signs of hypothermia, respiratory difficulties, pale mucosa, drowsiness, atonia, bloody urine, hematomas, and external hemorrhage. Both mid- and high-dose dogs had increased prothrombin time and hematuria. Histological examination showed that in high-dose groups, four out of four male or female dogs had hemorrhage, congestion, and/or edema of the spleen, kidneys, lungs, urinary bladder, small intestine, liver, thyroid, and skin. Based upon the clinical and hematological findings, the lowest-observed-effect level (LOEL) for subchronic toxicity of bromadiolone is 15 μg kg^{-1}; the no-observed-effect level (NOEL) is 10 μg kg^{-1}. No developmental toxicity was observed in rats and rabbits. In addition, bromadiolone did not induce any mutagenic effects.

Clinical Management

The principal diagnostic test for excessive exposure of bromadiolone is markedly reduced prothromibin activity, and therapy is directed at correcting this by the administration of vitamin K_1.

Environmental Fate

Bromadiolone is readily metabolized in aerobic soil ($t = 14$ days) and is generally immobile except in soils low in organic matter and clay, such as sand. Bromadiolone was stable to hydrolysis in pH 5, 7, and 9 buffer solutions. Although the parent compound is not persistent and is essentially immobile except in soils low in organic matter and clay, two of the major degradates identified in the aerobic soil metabolism study are persistent. Bromadiolone can leach in soils low in organic matter and clay; leaching was observed in a soil column (silt loam) with 0.5% organic matter and 3.2% clay. Since bromadiolone is applied as a food bait (pellets, place packs, or paraffinized blocks), leaching is expected to be minimal. Bioaccumulation factors of 160× and 1658× were

obtained for edible and nonedible tissues in bluegill sunfish, respectively.

Ecotoxicology

Bromadiolone is highly toxic to birds with an LC_{50} of 37 ppm in Northern Bobwhite. The results of the 96 h bluegill sunfish and rainbow trout acute toxicity studies indicate that bromadiolone is moderately toxic to fish with an LC_{50} of 3 ppm. Bromadiolone is considered moderately to highly toxic to freshwater invertebrates on an acute basis with an EC_{50} in the range of 0.1–10 ppm. The EPA believes that there is a high risk of secondary poisoning to mammals that feed on poisoned rodents in rural and suburban areas.

See also: Federal Insecticide, Fungicide, and Rodenticide Act, US; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Pesticides.

Further Reading

World Health Organization (1995) *Environmental Health Criteria No. 175, Anticoagulant Rodenticides*. Geneva: UNEP/ILO/WHO.

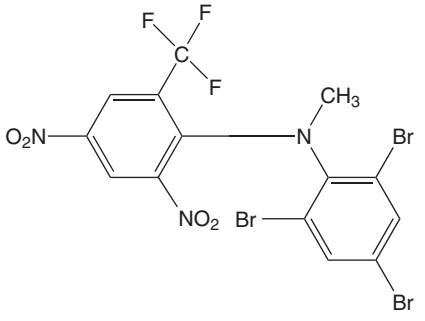
Relevant Website

http://www.epa.gov – US Environmental Protection Agency (1998) *Registration Eligibility Decision (RED): Rodenticide Cluster*. Washington, DC: Office of Prevention, Pesticides and Toxic Substances.

Bromethalin

Eric M Silberhorn

- Chemical Abstracts Service Registry Number: CAS 63333-35-7
- Synonyms: α,α,α-Trifluoro-*N*-methyl-4,6-dinitro-*N*-(2,4,6-tribromophenyl)-*o*-toluidine; *N*-Methyl-2,4-dinitro-*N*-(2,4,6-tribromophenyl)-6-trifluoromethyl)benzenamine
- Chemical/Pharmaceutical/Other Class: Acute rodenticide
- Chemical Formula: $C_{14}H_7Br_3F_3N_3O_4$
- Chemical Structure:



Uses

Single-dose rodenticide: This chemical is used in commercially available rodenticide baits (usually pellets or meal) for the control of rats and mice both indoors and outdoors. In the United States, federal regulations require that bromethalin rodenticide products be applied only in locations out of reach of children, pets, domestic animals, and nontarget wildlife, or in tamper-resistant bait stations. Bait stations must be resistant to destruction by dogs and by children under 6 years of age, and must be used in a manner that prevents children from reaching into bait compartments and obtaining bait.

Background Information

The concentration of bromethalin in rodent baits is 0.005% or 0.01%. It is effective against rodents that are resistant to anticoagulant rodenticides and does not induce bait shyness. Anorexia and neurological effects occur after an effective dose has been consumed.

Exposure Routes and Pathways

The primary exposure route to bromethalin is through accidental ingestion of commercially available rodenticide products that are used for rodent control. Children and pets are most likely to be accidentally exposed. Bait applicators may also be exposed through dermal contact.

Toxicokinetics

Information on toxicokinetics is largely derived from studies in the rat. After ingestion, bromethalin is rapidly absorbed with a peak plasma concentration occurring in ~4 h. The primary route of metabolism in the rat is through *N*-demethylation to desmethylbromethalin. Elimination is quite slow, with a plasma half-life for bromethalin of ~6 days.

Excretion occurs mainly in the bile, and enterohepatic circulation is suspected.

Mechanism of Toxicity

Bromethalin is an uncoupler of oxidative phosphorylation in mitochondria in cells of the central nervous system. Uncoupling leads to a decreased cellular ATP production and failure of the Na^+, K^+-ATPase pumps, which in turn leads to sodium retention and a loss of ability to maintain osmotic control. The outcome is a buildup of cerebrospinal fluid and vacuolization of myelin. The resulting edema and high intracranial pressures cause damage to nerve axons, inhibiting neural transmission, and leads to paralysis, convulsions, and, ultimately, death.

Acute and Short-Term Toxicity (or Exposure)

Animal

Symptoms of poisoning include ataxia, tremors, convulsions, prostration, and hind-limb paralysis. A single dose in excess of the LD_{50} will cause death within 8–12 h and is typically preceded by one to three episodes of clonic convulsions with death usually due to respiratory arrest. Oral LD_{50} values for pure bromethalin range from a low of $1.8\,mg\,kg^{-1}$ in the cat to $\sim 13\,mg\,kg^{-1}$ in rabbits. (*Note*: the LD_{50} values for rodenticide products containing bromethalin are 10 000 or more times higher on a milligram per kilogram basis because of the very small concentrations of bromethalin used in these products, typically 0.005% or 0.01%.) The guinea pig is highly tolerant of bromethalin with an oral LD_{50} in excess of $1000\,mg\,kg^{-1}$. The high LD_{50} is believed to be related to the fact that guinea pigs do not effect *N*-demethylation and therefore do not produce the highly toxic desmethylbromethalin metabolite (their oral LD_{50} for desmethylbromethalin is $7.5\,mg\,kg^{-1}$, which is similar to that for bromethalin in other species).

The dermal LD_{50} in rabbits is $2000\,mg\,kg^{-1}$. The inhalation LC_{50} for the rat is $0.024\,mg\,l^{-}$. Bromethalin causes slight irritation of the eye in rabbits but is not an irritant of the skin in this species, and does not cause dermal sensitization in the guinea pig. In addition, bromethalin does not cause acute delayed neurotoxicity in the hen.

Human

Reports of toxicity in humans have not been widely documented. The very low concentrations of bromethalin (0.005% or 0.01%) in rodenticide products help reduce the potential for toxicity due to accidental ingestion or direct contact. Children are potentially most at risk because of their small body size. Irritation of the eye or skin is not expected due to dermal contact based on studies in rodents. Expected signs and symptoms of oral exposure include headache, confusion, personality change, tremors, convulsive seizures, and respiratory distress.

Chronic Toxicity (or Exposure)

Animal

Multiple low (sublethal) doses of bromethalin yield hind leg weakness and loss of tactile sensation in rodents. Histopathology of the brain and spinal cord of these animals reveals spongy degeneration of the white matter (intramyelenic edema). The 90 day no-observed-effect level (NOEL) for dogs and rats is reported to be $0.025\,mg\,kg^{-1}\,day^{-1}$. The NOEL for developmental toxicity in both rats and rabbits is $0.5\,mg\,kg^{-1}\,day^{-1}$ when given by gavage during gestation. No compound-related external, visceral, or skeletal effects in treated fetuses were observed compared to controls on either a litter or fetal basis.

Human

Bromethalin is an acute rodenticide and most effects are expected to be short term in nature unless exposures continue over a long period. Symptoms of chronic exposure could include those associated with cerebral edema such as neuromuscular disorders.

In Vitro Toxicity Data

No data of this type are available for bromethalin.

Clinical Management

No specific antidote is available. If ingested, absorption should be limited by either emesis or gastric lavage. Sublethal symptoms, if present, would result in cerebral edema and should be treated accordingly through administrations of an osmotic diuretic and corticosteroid. Treatment regimens for dogs and cats exposed to bromethalin includes emesis (in nonsymptomatic animals only) and multiple doses of activated charcoal.

Environmental Fate

Bromethalin is stable to hydrolysis over the pH range of 5–9 when incubated in the dark for up to 30 days. Data from an anaerobic soil metabolism study indicate that bromethalin is relatively stable to

microbial/chemical degradation in the soil, with a calculated half-life for the parent compound of 178 days.

Ecotoxicology

Nontarget animals most at risk from exposure to rodenticides containing bromethalin include pets such as dogs and cats that may accidentally ingest baits used in and around homes. Assuming ~21 g of bait per pack and a bromethalin concentration of 0.01% in the bait, a typical 5 kg dog would need to consume five to six packages of bait to reach toxic levels, while a cat would need to consume only about one or two packages.

Bromethalin in its pure form is highly to very highly toxic to birds via oral ingestion (acute single-dose oral $LD_{50} = 4.6–11.0\,mg\,kg^{-1}$) and moderately to highly toxic via a 5 day dietary exposure (LC_{50} = 210–620 ppm in diet).

Bromethalin is classified as very highly toxic to aquatic organisms, although because this compound has an extremely low solubility in water, very little, if any, exposure of aquatic organisms is anticipated through use of rodenticides containing this compound. The acute 48 h EC_{50} for the water flea, *Daphnia magna,* is $\sim 2.0–5.1\,\mu g\,l^{-1}$ (ppb). Acute 96 h LC_{50} values for bluegill sunfish and rainbow trout are 598 and $38\,\mu g\,l^{-1}$, respectively.

Other Hazards

Rodenticide products containing bromethalin are not flammable or reactive as formulated.

Exposure Standards and Guidelines

There are no occupational exposure guidelines for this compound.

Miscellaneous

Use of rubber gloves is recommended when handling rodent baits containing this compound.

See also: Pesticides.

Further Reading

Dorman DC (2001) Bromethalin. In: Peterson ME and Talcott PA (eds.) *Small Animal Toxicology*, pp. 435–444. Philadelphia, PA: Sanders.

US Environmental Protection Agency (1998) Reregistration Eligibility Decision (RED) Rodenticide Cluster. EPA738-R-98-007. Office of Prevention, Pesticides and Toxic Substances, Washington, DC.

Van Lier RBL and Cherry LD (1988) The toxicity and mechanism of action of bromethalin: A new single feeding rodenticide. *Fundamentals of Applied Toxicology* 11: 664–672.

Bromine

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 7726-95-6
- Synonyms: Brom; Brome; Broom; Dibromine
- Chemical/Pharmaceutical/Other Class: Antiseptic

Uses

Bromine was formerly used medicinally as a topical antiseptic. It is used in gold extraction, in the bleaching of fibers and silk, in shrink-proofing of wool, in photography, and in the manufacture of bromine compounds, military gas, antiknock compound (ethylene bromide), and fire extinguishing fluid.

Background Information

Bromine is a naturally occurring element that can be found in many inorganic substances. Through industrial revolution, organic bromines in the environment at high concentrations have been introduced. These are not natural and can cause serious harm to human health and the environment. Humans can absorb organic bromines through the skin, with food, and by inhalation. Organic bromines are widely used as sprays to kill insects and other unwanted pests. They are not only poisonous to the animals that they are used against, but also to larger animals. In many cases they are poisonous to humans too. The most important health effects that can be caused by bromine-containing organic contaminants are malfunctioning of the nervous system and disturbances in genetic material. However, organic bromines can also cause damage to organs such as liver, kidneys, and lungs and can cause stomach and gastrointestinal

malfunctioning. These bromines can damage the nervous system and the thyroid gland. Some forms of organic bromines, such as ethylene bromide, can even cause cancer.

Exposure Routes and Pathways

Inhalation, ingestion, and eye and skin contact are the most common routes of exposure. Bromine may be absorbed through skin.

Toxicokinetics

Bromine has cumulative properties and is deposited in tissues as bromides, displacing other halogens.

Mechanism of Toxicity

Bromine causes toxicity as bromides by displacing other halogens from the body.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity in animals is similar to that in humans. The LC_{50} value in mice by inhalation exposure is 750 ppm/9 months.

Human

The respiratory system, eyes, and central nervous system are the points of attack. Bromine is extremely irritating to skin, eyes, and mucous membranes of the upper respiratory tract. Severe burns of the eye may result from liquid or concentrated vapor exposure. Liquid bromine splashed on skin may cause vesicles, blisters, and slow-healing ulcers. Inhalation of bromine is corrosive to the mucous membranes of the nasopharynx and upper respiratory tract, producing brownish discoloration of the tongue and buccal mucosa, a characteristic odor of breath, edema and spasm of the glottis, asthmatic bronchitis, and possibly pulmonary edema, which may be delayed until several hours after exposure. A measles-like rash may occur. Exposure to high concentrations of bromine may lead to death due to choking caused by edema of glottis and pulmonary edema. Exposure to low concentrations results in cough, copious mucus secretion, nose bleeding, respiratory difficulty, vertigo, and headache. Usually, these symptoms are followed by nausea, diarrhea, abdominal distress, hoarseness, and asthmatic-type respiratory difficulty.

Chronic Toxicity (or Exposure)

Animal

Pulmonary edema, pneumonia, diarrhea, and rashes may be delayed complications of severe exposures.

Human

Chronic exposure may cause acne-like skin lesions and neurotoxicity.

Clinical Management

Exposure should be terminated as soon as possible by removing the victim to fresh air. The eyes and mouth should be washed with copious amounts of water. A 15–20 min wash may be necessary. Skin should be washed with soap. Contaminated clothing and jewelry should be removed and isolated. Contact lenses should be removed from the eyes to avoid prolonged contact of the chemical with the area. When the chemical has been swallowed, large quantities of milk should be given; if milk is not available, water should be given. Emetics should not be given. If breathing has stopped, artificial respiration should be given. If breathing is difficult, oxygen should be given.

Environmental Fate

The average content of bromine in soils of grasslands, orchards, and upland crop fields were 10-fold higher than those recorded from overseas. The average content in these soils was higher than those recorded in forest soil of the basins. The average values reported in the leaves of plants were 12 ppm. The contents of iodine and bromine in the forest soil, plants, and rainwater were generally higher in coastal than in the inland areas.

Ecotoxicology

Organic bromines are often applied as disinfecting and protecting agents, due to their damaging effects on microorganisms. When they are applied in greenhouses and on farmland they can easily rinse off to surface water, which has very negative health effects on daphnia, fishes, lobsters, and algae. Organic bromines are also damaging to mammals, especially when they accumulate in the bodies of their preys. The most important effects on animals are nerve and DNA damage, which can enhance the chances of development of cancer. The uptake of organic bromine takes place through food, breathing, and the skin. Organic bromines are not very biodegradable; they are decomposed to inorganic bromines. At high doses these can damage the nervous system. It has occurred in the past

that organic bromines ended up in the food of cattle. Thousands of cows and pigs had to be killed in order to prevent contagion of humans. The cattle suffered from symptoms such as liver damage, loss of sight, depletion of growth, decrease of immunity, decreasing milk production, sterility, and malformed children.

Exposure Standards and Guidelines

The acceptable daily intake is $1\,mg\,kg^{-1}$ body weight and the threshold limit value is 0.1 ppm.

See also: Ecotoxicology; Peyote.

Further Reading

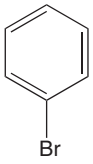
Shannon M (1998) Bromine and iodine compounds. In: Haddad L, Shannon M, and Winchester J (eds.) *Clinical Management of Poisoning and Drug Overdose*, 3rd edn., pp. 803–812. Philadelphia: Saunders.

Seiler HG, Sigel H, and Sigel A (1988) *Handbook on the Toxicity of Inorganic Compounds*, p. 150. New York: Dekker.

Bromobenzene

William S Utley

- Chemical Abstracts Service Registry Number: CAS 108-86-1
- Synonyms: Monobromobenzene; Phenyl bromide
- Chemical Formula: C_6H_5Br
- Chemical Structure:



Uses

Bromobenzene is used as an industrial solvent, in organic synthesis, and as an additive to motor oils.

Exposure Routes and Pathways

The primary routes of exposure are via inhalation, skin, and accidental ingestion.

Toxicokinetics

Bromobenzene is excreted as the free and sulfate or mercapturic conjugates of the catechol derivatives. Initially, bromobenzene may concentrate in the adipose tissues. Bromobenzene concentrations can be 300 times higher in the adipose tissues in the first 3 h of exposure. Bromobenzene is rapidly excreted in the urine; one report indicated that 85% of the bromobenzene may be excreted in the urine in the first 24 h.

Mechanism of Toxicity

Bromobenzene is believed to be relatively inert, requiring metabolic activation to express toxicity to the liver and the kidney. Liver toxicity is believed to result from activation of bromobenzene to a reactive epoxide by the cytochrome P450 system. The reactive epoxides are primarily detoxified by glutathione transferase and epoxide hydratase. The involvement of glutathione transferase explains in part the decrease in glutathione levels observed following exposure to bromobenzene. It is interesting to note that in animal experiments, sulfhydrol-containing compounds such as cysteine or methionine partially prevented bromobenzene-induced hepatic necrosis.

It has been suggested that the enterhepatobiliary cycle plays a role in the hepatic necrosis observed in bromobenzene toxicity. This is supported by the experimental findings that bromobenzene-induced hepatic necrosis can be prevented by the administration of cholestyramine.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral route of exposure in dogs to high concentrations leads to vomiting, diarrhea, and death. The major histopathological findings were centrilobular hepatic necrosis. The necrosis included the central hepatic veins and their respective tributaries. Bromobenzene was not found to be mutagenic in the Ames assay.

Human

Bromobenzene is known to be irritating to the skin and is suspected of being irritating to the eyes and

respiratory tract. The probable lethal dose is between one teaspoon and one ounce for a 154 lb person. Bromobenzene is directly irritating to the skin and can act as an anesthetic when inhaled in high concentrations.

Clinical Management

General life support should be maintained. Symptoms should be treated and decontamination undertaken if necessary. Treatment is generally symptomatic and supportive.

Environmental Fate

Bromobenzene poorly degrades and is expected to be persistent in the environment. The low water solubility and moderate K_{oc} (268) suggest that when released to the water, bromobenzene will tend to migrate to soils. Bromobenzene has a low to moderate chance of bioconcentrating (measured bioconcentration factors (BCFs) of 8.8–190). Bromobenzene exhibits enough of a vapor pressure (4.18 mmHg at 25°C) that it can be expected to volatilize from soils and surface water.

Ecotoxicology

Bromobenzene is moderately toxic to aquatic organisms (acute LC_{50} to fathead minnow of 5.6 mg l^{-1}).

Exposure Standards and Guidelines

Bromobenzene is regulated under section 111 of the Clean Air Act. Bromobenzene is a DOT and IMDGC Marine pollutant. European Union classifies bromobenzene as dangerous to the environment and a skin irritant: Xi, N: R-10, R-38, R-51/53.

Further Reading

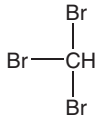
Bambal RB and Hanzlik RP (1995) *Chemical Research in Toxicology* 8(5): 729–735.

Heijen WH, Slitt AL, van Bladeren PJ, *et al.* (2004) Bromobenzene-induced hepatoxicity at the transcriptome level. *Toxicological Sciences* 79(2): 411–422.

Bromoform

William S Utley

- Chemical Abstracts Service Registry Number: CAS 75-25-2
- Synonym: Tribromomethane
- Chemical Formula: $CHBr_3$
- Chemical Structure:



Uses

Bromoform is used as a chemical intermediate in the synthesis of organic chemicals and pharmaceuticals. It is used as an ingredient in fire-resistant chemicals and as an industrial solvent in liquid-solvent extractions. Bromoform is used in polymer reactions and in the vulcanization process for rubber. Bromoform is also used for medicinal purposes as a sedative, antitussive, and antiseptic.

Exposure Routes and Pathways

Ingestion is the most common form of accidental and intentional exposure. This occurs primarily via drinking bromonated water. In the past, inhalation was a more common route of exposure during anesthesia. However, due to the associated toxicities, bromoform is no longer popular as an anesthetic. Inhalation can still be a significant route of exposure via volatilization of household or workplace water. Dermal absorption is possible but not likely to be a significant route of exposure in intact skin.

Toxicokinetics

Absorption

Bromoform is readily absorbed from the gastrointestinal tract following ingestion and from the lungs following inhalation. Significant absorption may occur through abraded skin or open wounds.

Biotransformation

Bromoform is metabolized in the liver by the mixed function oxidase system (cytochrome P450) to carbon monoxide and bromide. Inorganic bromide has

been observed in tissues and urine following administration of bromoform.

Distribution

Bromoform readily distributes through the tissues of the body.

Elimination

Bromoform and its metabolites are rapidly expelled out of the body through exhalation. It has been shown that the majority of bromoform (50–90%) is expelled in an 8 h period. As such, bromoform does not tend to build up in the body.

Mechanism of Toxicity

The wide spectrum of toxic effects elicited by bromoform suggests multiple mechanisms of actions. Bromoform is an irritant, capable of directly irritating mucosal membranes when directly exposed. Chronic exposure to bromoform may also cause the defatting of the skin leading to drying and cracking. Bromoform is capable of dissolving in phospholipid membranes giving it the ability to produce anesthetic effects when given in high concentrations.

The toxic effects on the liver and kidneys may be mediated by reactive intermediates produced by the hepatic P450 oxidative metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

Bromoform was noted to be more toxic to the liver and more irritating than chloroform when given via inhalation. Bromoform was noted to produce decreased liver functions and pathologic changes to both the liver and kidneys by both oral and inhalation routes in rodents. Single oral doses to rodents produced sedation, ataxia, piloerection, and prostration. Undiluted bromoform caused moderate irritation to the eyes of rabbits, which recovered in 1–2 days.

Bromoform tested positive for the induction of sex-linked recessive lethal mutations in *Drosophila* at a dose of 3000 ppm when administered to males by feeding.

Human

The odor has been described as 'chloroform-like' and the taste has been described as 'sweet'. Bromoform can be toxic by all routes of exposure. People appear to be able to detect bromoform at very low concentrations in liquid (0.3 ppm). Symptoms of acute exposure appear to be primarily one of sedation.

Chronic Toxicity (or Exposure)

Animal

In a 2 year gavage study conducted by the National Toxicology Program, rats and mice were given bromoform 5 days a week for 103 weeks. Dose-related lethargy and nonneoplastic changes to the liver (mixed cell foci and fatty changes) were noted. These observations were noted at the lowest dose tested 100 mg kg^{-1} (lowest-observed-adverse-effect level).

In Vitro Toxicology Data

Bromoform is mutagenic in the Ames assay.

Clinical Management

Medical surveillance may be indicated in persons with predisposing skin, liver, kidney, or respiratory conditions. General life-support should be maintained, symptoms treated and decontamination considered if necessary. Treatment is generally symptomatic and supportive. The patient should be monitored for delayed liver and kidney damage. If central nervous system depression occurs, EKG and vital signs should be monitored carefully. Patients who exhibit dermal hypersensitivity may require systemic or topical antihistamines or corticosteroids.

Environmental Fate

Bromoform poorly degrades and is expected to be persistent in the environment. The low K_{oc} (24) suggests bromoform will be highly mobile in soils. Bromoform has a low probability of bioconcentrating (measured bioconcentration factor (BCF) of 4). Bromobenzene exhibits enough of a vapor pressure (44.4 mmHg at 25°C) to be expected to volatilize from soils and surface water, where it will undergo photochemical oxidations with a half-life of 142 days.

Ecotoxicology

With the exception of the eastern oyster (48 h LC_{50} 1 mg l^{-1}), most aquatic species are relatively immune to the acute effects of bromoform expressing LC_{50} values in the range of 1600–29 000 mg l^{-1}.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit and the American Conference of Governmental Industrial Hygienists threshold limit value is 0.5 ppm (with a notation for skin absorption). As a waste, bromoform is considered a toxic waste (number U225). The US Environmental Protection Agency recommends that drinking water levels for bromoform should not be more than 0.7 ppm. Integrated Risk Information System (IRIS) has assigned an oral reference dose of $0.02\,mg\,kg^{-1}\,day^{-1}$ for bromoform.

Bromoform is regulated under Section 112 of the Clean Air Act and under Section 307 of the Clean Water Act. The CERCLA reportable quantity for a spill (RQ) is 100 pounds. Bromoform is reportable under EPCRA SARA 313. Transport is regulated under DOT and IMDG as a marine pollutant. Bromoform is regulated as a California Proposition 65 carcinogen with a no significant risk level of $64\,\mu g\,day^{-1}$. Bromoform is regulated in the European Union as toxic by inhalation, dangerous to the environment, and irritating to the skin and eyes (T, N: R-23, R-36/38, R-51/53). International Agency for Research on Cancer has classified bromoform as a class 3 carcinogen (not classifiable as a human carcinogen based on inadequate human data and limited animal data). IRIS has classified bromoform as a B2 (probable human carcinogen based on sufficient animal evidence).

See also: Chloroform.

Further Reading

International Toxicity Estimates for Risk (ITER) from Toxicological Excellence for Risk Assessment (available through the US National Library of Medicine's TOXNET system).

Relevant Websites

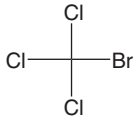
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Bromoform.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Bromoform.

Bromotrichloromethane

Midhun C Korrapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 75-62-7
- Synonyms: EINECS 200-886-0; Carbon bromotrichloride; Trichlorobromomethane
- Chemical/Pharmaceutical/Other Class: Not a member of any pharmaceutical class
- Chemical Formula: $CBrCl_3$
- Chemical Structure:



Uses

Although bromotrichloromethane is no longer produced in the United States, it is still used in organic syntheses of various compounds.

Background Information

Bromotrichloromethane is a colorless nonflammable liquid. It is sparingly soluble in water and readily evaporates when exposed to air.

Exposure Routes and Pathways

It is used in organic syntheses and may result in its release into the environment directly to the atmosphere. The most common exposure route to bromotrichloromethane is through inhalation or dermal contact in workplaces, where it is produced or used.

Toxicokinetics

Bromotrichloromethane is readily absorbed from the lungs and rapidly reaches equilibrium with levels in blood and expired air approximately proportional to the exposure concentration. At high concentrations, kinetic processes like metabolism or excretion may become saturated, limiting the rate of uptake. It is metabolized by conjugation with glutathione to yield

S-methylglutathione, *S*-methyl cysteine, and other sulfur-containing compounds.

Mechanism of Toxicity

With the loss of bromide ion, mediated by cytochrome P450 enzyme in the liver, the trichlorocarbon free radical is responsible for lipid peroxidation, which is the predominant mechanism of hepatotoxicity:

$$BrCCl_3 \xrightarrow{P450} Br\bullet + \bullet CCl_3$$

It is also known to cause cerebellar degeneration in rodents. It is cytotoxic to the sperm in the testes at the time of exposure. Renal tumors are induced in male mice due to depletion of glutathione, increased lipid peroxidation, and DNA lesions.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation of bromotrichloromethane by rats increased total lipids in liver and stimulated hepatic lipid peroxidation. After intragastric administration, liver steatosis was observed. Rats injected with 0.26 mmol bromotrichloromethane died after massive accumulation of neutral lipids and necrosis of the liver.

Human

Bromotrichloromethane causes irritation and reddening of eyes. Prolonged or repeated exposure may cause cataract and severe, permanent damage to the eyes. Bromotrichloromethane causes rash, blistering, and allergic reactions upon dermal contact; it may also cause nasal, gastrointestinal, and lung irritation.

Chronic Toxicity (or Exposure)

Animal

Bromotrichloromethane, when tested for its mutagenic activity, gave positive results for the Ames test.

Human

Rated D (not classifiable as human carcinogen) in Environmental Protection Agency's IRIS Database.

In Vitro Toxicity Data

In the *in vitro* test with FAF-cells of Chinese hamsters only bromochloromethane produced an increase of the sister chromatid exchange frequency.

Clinical Management

Administration of vitamin E and cadmium acetate were shown to be protective against bromotrichloromethane toxicity by free radical scavenging and chelating properties of vitamin E and cadmium acetate, respectively.

Environmental Fate

Terrestrial Fate

Bromotrichloromethane is expected to have low mobility in soil. The potential for volatilization of bromotrichloromethane from dry soil surfaces may exist based on a measured vapor pressure of 39 mmHg. Based upon the highly halogenated structure of bromotrichloromethane, biodegradation in soil is expected to be slow.

Aquatic Fate

Bromotrichloromethane is expected to adsorb to suspended solids and sediment in water. Volatilization from water surfaces is expected. The potential for bioconcentration in aquatic organisms is moderate. Based upon the highly halogenated structure of bromotrichloromethane, biodegradation in water is expected to be slow.

Atmospheric Fate

According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere, bromotrichloromethane, which has a measured vapor pressure of 39 mm Hg at 25°C, is expected to exist solely as a vapor in the ambient atmosphere. Based on bromotrichloromethane's structural similarity to bromotrifluoromethane, it is expected to slowly degrade in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for bromotrichloromethane's reaction in air is estimated to be greater than 44 years. Photolysis may occur based on bromotrichloromethane's structural similarity to other halogenated methane compounds but not at an environmentally relevant rate.

Ecotoxicology

Bioconcentration of bromotrichloromethane in aquatic organisms is moderate. Aquatic toxic effects to aquatic organisms are not reported as such.

Exposure Standards and Guidelines

According to Occupational Safety and Health Administration, the threshold limit value – time-weighted

average (TLV – TWA) limit for bromotrichloromethane is 8 h time-weighted average (TWA) 200 ppm. Excursions in worker exposure levels may exceed three times the TLV – TWA for no more than a total of 30 min during a work day, and under no circumstances should they exceed five times the TLV – TWA.

See also: Ames Test; Carcinogen Classification Schemes; Vitamin E.

Further Reading

Burk RF and Lane JM (1979) Ethane production and liver necrosis in rats after administration of drugs and other chemicals. *Toxicology and Applied Pharmacology* 50: 467.

Lowrey K (1981) Destruction of liver microsomal calcium pump activity by carbon tetrachloride and bromotrichloromethane. *Biochemical Pharmacology* 30: 135.

Brown Recluse Spider *See* Spider, Brown Recluse.

BSE *See* Bovine Spongiform Encephalopathy (Mad Cow Disease).

Buckthorn

Christopher P Holstege

This article is a revision of the previous print edition article by Lanita B Meyers, volume 1, pp. 190–191, © 1998, Elsevier Inc.

- SYNONYMS: Rhamnaceae family; *Rhamnus frangula*; Alder buckthorn; Frangulin; Trollidora; Coyotillo; Wild cherry; Purging buckthorn; Arrow wood; Berry alder; Black dogwood; Cascara; Senna, Hart's thorn; May thorn; Persian berry; Rhine berry; Common buckthorn

Uses

Buckthorn and its pharmaceutical derivatives (senna, cascara) are most commonly taken as laxatives. Other purported but unsubstantiated uses include treating skin disorders, ulcers, cardiovascular disease, and cancer.

Background Information

Buckthorn is a shrubby tree that grows 6–12 ft tall. The leaves (up to 6 cm) are simple, ovate elliptic, with serrate margins. Some of the branches end in short thorns. The flowers have four small petals and grow solitary or in clusters from the leaf axis. They are replaced later by a berry that is green at first, turns red, and then turns black at maturity. The berry contains up to four seeds. This ornamental shrub is often found as hedges throughout the eastern United States. The shrubs are also grown along gullies in the southwestern United States and northern and central Mexico. Other species of this plant are found throughout the northern temperate zones.

Exposure Routes and Pathways

The most common route of exposure is ingestion of any part of the plant. The seeds are the most poisonous part of the plant. Laxatives (senna, cascara) are derived from these plants and are taken either orally or rectally.

Toxicokinetics

Buckthorn contains anthraquinone glycosides that are poorly absorbed after ingestion. Anthraquinone glycosides undergo hydrolysis by colonic bacteria into senna and cascara. Bowel movements occur within 6–12 h of ingestion of senna and cascara laxatives. Fresh plant ingestions are associated with much more rapid symptom onset. After ingestion and hydrolysis, the anthraquinones are eliminated by the kidneys, in the feces, and in the bile. Senna and cascara are not significantly excreted in breast milk and subsequently do not cause toxicity in breastfeeding infants.

Mechanism of Toxicity

Anthraquinone glycosides exhibit their effect by increasing the tone of the smooth muscle wall in the

large intestine. A direct action is exhibited on the intestinal mucosa, increasing the colonic motility and colonic transit and inhibiting water and electrolyte secretion. These agents may also act directly on the intramural nerves of the colon and have stool-softening properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals that have ingested anthraquinone-containing plants are reported to have developed signs and symptoms similar to those of humans.

Human

Buckthorn poisonings are rare. Determination of a toxic dose is difficult due to various species containing different concentrations of anthraquinones. Mild intoxications may result in nausea, vomiting, diarrhea, abdominal cramps, and possible palpitations. Severe poisoning has been reported from use of the bark as an abortifacient. In severe cases, kidney damage, oliguria, proteinuria, gastrointestinal hemorrhage, seizures, dyspnea, and fluid depletion can result. Ingestion of seeds or fruit of the *K. humboltiana* species can also produce neurotoxic symptoms after a latent period of 1–4 weeks. A diffuse segmental demyelination has been reported, causing a rapidly ascending motor neuropathy with minimal sensory findings similar to Guillain–Barre syndrome. This may progress for a month or longer and can lead to respiratory failure.

Chronic Toxicity (or Exposure)

Human

Electrolyte abnormalities and dehydration may occur with chronic ingestion of these agents. Chronic ingestion of *K. humboltiana* has been reported to cause a progressive, symmetrical polyneuropathy that resulted in flaccid quadriplegia and respiratory insufficiency. A slow but progressive improvement to an almost complete functional recovery occurred with some persistent reflex deficits. Finger clubbing has been reported with abuse of senna and is reversible with discontinuation of the drug.

Clinical Management

Basic and advanced life-support measures should be utilized. Activated charcoal without a cathartic may be used in early decontamination. Most ingestions are self-limiting. Treatment after decontamination is symptomatic and supportive. Monitoring of fluids and electrolytes is recommended for symptomatic patients. If a significant ingestion of anthraquinones does occur, Borntrager's reaction may occur (red color is seen in alkaline urine and a yellow-brown color in acid urine). No other specific laboratory tests are available to assist in diagnosis and treatment.

See also: Charcoal; Gastrointestinal System; Plants, Poisonous.

Relevant Website

http://www.cbif.gc.ca – Canadian Poisonous Plants Information System; search for Alder Buckthorn.

Busulfan

Matthew Janes

- Chemical Abstracts Service Registry Number: CAS 55-98-1
- Synonyms: Preferred: 1,4-Butanediol dimethanesulfonate; Myleran®. Also 1,4-Bis- or di(methane-sulfonoxyl)butane; Tetramethylene ester of methanesulfonic acid; Busulphan; C.B.2041; Mablin; Myeloleukon; Mielevcin; Misulban; Myelosan; Mitosan; Sulfabutin; Tetramethylene bis(methanesulfonate); 1,4-Bis(methanesulfonoxy) butane; 1,4-Bis(methanesulfonyloxy)butane
- Chemical/Pharmaceutical/Other Class: Chemotherapeutic agent, bifunctional alkylating agent
- Chemical Formula: $CH_3SO_2O(CH_2)_4OSO_2CH_3$

Uses

Busulfan has been used extensively as a chemotherapeutic agent in the treatment of a variety of cancers. Busulfan is clinically successful for treating leukemia, solid tumors, and correcting hemoglobinopathies, and other inborn errors of the immune system. It is

currently used primarily in the treatment for myeloproliferative disorders such as chronic myelogenous leukemia (CML), polycythemia vera, and essential thrombocythemia. Busulfan is also an effective and sufficiently safe drug used as a conditioning regimen for autologous or allogeneic bone marrow transplantations (BMTs) in efforts to induce organ transplant tolerance.

Background Information

First investigations of busulfan's antitumor characteristics were noted in CMLs in 1953 by Galton. Active development of the compound was pursued by GlaxoSmithKline, launching Myleran in 1954 for the treatment of CML in the United States. Following its development in the United States, Myleran was recommended for approval in Europe in 1983. Busulfan has become a widely adopted therapy. The drug was developed as a substitute for total body irradiation preceding BMT. Preparative regimens were first studied in a rat myelocytic leukemia model and then moved into clinical trials. Doses of busulfan in combination with cyclophosphamide or other drugs have been shown to be effective as a preparative regimen for patients undergoing allogeneic or autologous BMT. There are reports of more than 500 trials involving more than 15 000 patients receiving BMTs in the literature. Complete response rates from various leukemia patients described in literature have exceeded 50% in busulfan-based regimens. Regimen-related mortality is described to be lower then antileukemia benefits in the aggregate for acute and chronic myeloid leukemia as well as acute lymphocytic leukemia.

Recent clinical studies have been focusing on a major drawback of high-dose busulfan regimens. Oral formulation is the major route of uptake for the drug. Erratic and unpredictable absorption of the compound from the gastrointestinal tract have resulted in large inter-/intrapatient variations in the busulfan plasma concentrations. Pharmacokinetic studies have verified great interpatient variations due to age, underlying diseases, and drug–drug interactions. Busulfan bioavailability shows great variation in children to adults. Investigators have reported hepatic veno-occlusive disease (VOD) as a common complication associated with this regimen. VOD occurs in over half of bone marrow transplant patients, and although approximately half of all cases resolve, the mortality rate can be over 90% in severe cases. Acute central nervous system toxicity (grand mal seizures), pulmonary complications, and VOD are associated with high systemic exposure expressed as area under the plasma concentration–time curve (AUC). The aim of current clinical investigations lies in the variable toxicodynamic effects of busulfan.

Exposure Routes and Pathways

Exposure to this compound therapeutically is by the oral route. Since variation in the AUC for oral busulfan results in substantial risk of over- or undertreatment with risk of toxicity or relapse, the use of an intravenous (IV) formulation has been studied. IV formulation reduces this variability by eliminating variability in absorption. Busulfan is a small, highly lipophilic molecule that easily passes the blood–brain barrier. The typical dosage level (tablet form) is 4–8 mg daily. The recommended intravenous dose given prior to bone marrow transplant is $0.8\,\mathrm{mg\,kg^{-1}}$ body weight given as a 2 h infusion every 6 h for 4 days.

Toxicokinetics

Toxicokinetic studies have shown that high-dose busulfan toxicities are age dependent, and directly correlated with AUC. Pharmaco-/toxicokinetic-guided dose adjustments are given to patients receiving IV formulations. Targeted AUC of 1250 µmol min ($\pm 20\%$) are monitored in an attempt to optimize the antitumor effect and minimize serious toxicity. AUC values are currently being studied in correlation with patient outcomes, including survival time, gastrointestinal toxicity, mucositis, hepatic toxicity, and acute graft-versus-host disease, to effectively define an optimal therapeutic window. Radioactively labeled busulfan studies have been carried out to quantitate toxicokinetics. Gas liquid chromatography analysis suggests that busulfan doses of 2–6 mg are well absorbed and the data can be extrapolated to a zero-order absorption, one compartment open model. The mean half-life of elimination was 2.57 h in humans. Patients receiving high-dose administration ($1\,\mathrm{mg\,kg^{-1}}$ orally every 6 h for 4 days) show variability in mean steady-state plasma concentrations after dosing, with unpredictable elimination half-life. Variable absorption kinetics suggests oral formulations are very difficult to effectively evaluate for clinical activity.

Mechanism of Toxicity

Busulfan is a bifunctional alkylating agent. It consists of two methanesulfonate groups attached at opposite ends of a four-carbon alkyl chain. Alkylating agents form covalent DNA interstrand cross-links that inhibit DNA synthesis. Toxicity of busulfan's alkylation of intercellular nucleophiles is associated with its

biological activity. The N-position of guanine and other DNA sites tend to be the main site of alkylation and the release of the methylsulfonate group. Its mechanism of action is still not fully understood.

Induced changes in biological parameters of the cell cycle have been assayed in various doses and exposure times, all indicating cytotoxic effects to rapidly proliferating tissues, in particular to the cells of the granulopoietic lineage of the bone marrow. In all studies busulfan toxicities occur in an AUC-dependent manner inducing apoptosis. *In vitro* studies show decrease in proliferation and colony formation and arrest of cell cycle in G2 phase. The development of apoptosis occurred secondarily to the interruption of other vital metabolic pathways that still remain to be characterized. Typical chemotherapeutic-induced apoptotic effects are observed in *in vitro* studies.

Acute and Short-Term Toxicity (or Exposure)

Busulfan is a potent cytotoxic drug. Early in development of the compound, *in vivo* experiments indicated that busulfan caused severe depression in the bone marrow. The most prevalent acute toxic effects associated with busulfan in animals are severe pancytopenia from bone marrow failure. Associated *in vivo* experiments show bone marrow aplasia, stromal cell damage, immunosuppression (impaired T-lymphocyte function), and pronounced adverse effects on reproductive glands, germ cells, and fertility in animals (lowest effective dose tested was $2\,mg\,kg^{-1}$).

Chronic Toxicity (or Exposure)

Busulfan is mutagenic in mice and, possibly, in humans. Chronic toxicity in animals is manifested by carcinogenic, teratogenic, mutagenic, and reproductive effects. Busulfan is known to be a human carcinogen based on sufficient evidence of carcinogenicity in humans. Patients receiving busulfan have developed leukemia as well as cytological and hematological abnormalities. Human studies show severe side effects associated with bone marrow failure resulting in severe pancytopenia.

In Vitro Toxicity Data

Busulfan has been shown to induce gene mutations and chromosomal damage in bacteria, fungi, plant species, *Drosophila*, and in animal cell lines in culture. Busulfan does not require S9 activation in *in vitro* toxicity assays.

Clinical Management

The difficulty in determining dose delivered with oral administration of high-dose busulfan in preparative regimens for hematopoietic stem cell transplantation results in lethal toxicity due to overdosing and increased potential for relapse with recurrent disease. Oral pharmacokinetic studies ineffectively determine proper AUC for reliable establishment for a proper therapeutic dose. Studies with IV formulations have demonstrated that all patients are evaluable. With the development of a limited sampling strategy to analyze proper AUC over intermittent time periods, improved patient risk profiles for busulfan have been implemented in clinical practice.

Environmental Fate

Busulfan is a white powder that is soluble in acetone. The powder is insoluble in water and readily hydrolyzes in water. Any environmental hazards that pose threats to the environment remain unknown. Busulfan is a pharmaceutical and is used in relatively small amounts; therefore, so far it has been of little regulatory concern to the US Environmental Protection Agency. The potential threat of busulfan exposure is limited to workers involved in its manufacture, to patients receiving this agent as a chemotherapeutic regime, and to hospital personnel administering the drug to patients.

Miscellaneous

The risks of taking the medicine must be weighed against the good it will do. Precautions should be taken if therapy is prescribed during pregnancy and breastfeeding. Children and older adults are particularly susceptible to side effects and require closer monitoring during therapy. Busulfan will temporarily lower the number of white blood cells in the blood, increasing the chances of getting an infection. It can also lower the number of platelets, which are necessary for proper blood clotting. A physician or drug index should be consulted for other common side effects, including allergies.

See also: Cancer Chemotherapeutic Agents; Immune System.

Further Reading

Bishop JB and Wassom JS (1986) Toxicological review of busulfan (Myleran). *Mutation Research* 168: 15–45.

Buggia I, Locatelli F, Regazzi MB, and Zecca M (1994) Busulfan. *The Annals of Pharmacotherapy* 28: 1055–1062.
Galton DGA (1953) Myleran in chronic myeloid leukemia: Results of the treatment. *Lancet* 1: 208–213.
Vaughan WP, Carey D, Perry S, Westfall AO, and Salzman DE (2002) A limited sampling strategy for pharmocokinetic therapy with intravenous busulfan. *Biology of Blood and Marrow Transplantation* 8: 619–624.

Relevant Website

http://www.rxmed.com – RxMed: Pharmaceutical Information – Myleran.

Butadiene, 1,3-

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 106-99-0
- Synonyms: Biethylene; Butadiene; Divinyl; Erythrene; Pyrrolylene; Vinylethylene
- Chemical Formula: C_4H_6
- Chemical/Pharmaceutical/Other Class: Hydrocarbon
- Chemical Structure: $H_2C=CH–CH=CH_2$

Uses

Butadiene is used as a chemical intermediate and as a polymer component in the synthetic rubber industry, the latter accounting for ~75% of the butadiene produced. Styrene–butadiene rubber, polybutadiene rubber, adiponitrile, styrene–butadiene latex, acrylonitrile–butadiene–styrene resins, and nitrile rubber are used in the manufacture of tires, nylon products, plastic bottles and food wraps, molded rubber goods, latex adhesives, carpet backing and pads, shoe soles, and medical devices.

Exposure Routes and Pathways

Butadiene is a gas under normal environmental conditions. In the workplace, the most significant route of exposure to butadiene is inhalation during its production and use. Potential exposures to butadiene are likely to be limited to the industrial setting as the residual butadiene monomer content in consumer products is low and unlikely to pose a significant health threat to the general public. Butadiene is also produced during the combustion of organic matter. Significant amounts of butadiene are released to the environment from both natural and anthropogenic sources such as forest fires, gasoline and diesel engine exhaust, and wood space heating. It is also a component of cigarette smoke.

Toxicokinetics

Butadiene appears to be readily absorbed through the respiratory tract. Dermal absorption is anticipated to be limited due to the volatility of liquid butadiene. Although limited, available data indicate that the uptake of butadiene at comparable exposure levels is greatest in the mouse (5- to 100-fold more than rat), with progressively lesser amounts by the rat and monkey (4- to 14-fold less than rat).

Metabolism of butadiene is qualitatively similar across species, although there are quantitative differences in the amounts of metabolites formed. Butadiene is rapidly metabolized via enzyme systems located in the liver, lung, nasal mucosa, and possibly bone marrow. Initially, butadiene is converted to the reactive metabolite 1,2-epoxy-3-butene (EB) by cytochrome P450 monooxygenase, an enzyme that also metabolizes EB to another reactive metabolite 1,2:3,4-diepoxybutane (DEB). EB and DEB, which are thought to cause the DNA damage necessary for the butadiene-induced tumorigenesis, are further metabolized (inactivated) by conjugation with glutathione via glutathione *S*-transferase and by hydrolysis via epoxide hydrolase. Glutathione conjugation predominates in mice followed by rats and then humans; conversely, hydrolysis via epoxide hydrolase predominates in humans followed by rats and then mice. The activation/inactivation profiles of these three enzyme systems are species specific. In comparably exposed rodents, tissue levels of EB and DEB in mice can be several fold and 100-fold higher, respectively, than those in rats.

Once absorbed, butadiene is rapidly distributed throughout the body. A tissue distribution study in rats indicates that the highest concentrations of butadiene are located in peripheral fat; lower concentrations are observed in the liver, brain, spleen, and kidney.

Based on a radiolabel study in mice, most of the absorbed butadiene is exhaled as the parent compound, with a lesser amount exhaled as CO_2. Smaller amounts of butadiene and/or its metabolites are detected in urine and feces, with most of the label being eliminated from the carcass within 65 h. This is

consistent with another study in rats and mice that showed that the bulk of their butadiene body burden (77–99%) is eliminated with a half-life of 2–10 h.

Mechanism of Toxicity

The mechanism by which butadiene exerts acute toxicity is unknown. The carcinogenic potential of butadiene in rodents is thought to reflect the metabolism of butadiene to DNA-reactive metabolites resulting in genetic alterations in protooncogenes and/or tumor suppressor genes. Mechanistic data suggest that the higher carcinogenic potency of butadiene in mice versus rats is primarily due to the higher body burden of DEB in mice. This is supported by the observations that carcinogenicity tests with EB were equivocal while DEB was carcinogenic in mice and rats when administered via dermal or subcutaneous exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute inhalation studies have shown that butadiene exhibits low toxicity in animals. Butadiene is a relatively weak central nervous system (CNS) depressant. Mice exposed to butadiene for 6–12 min exhibited excitement and narcosis (200 000 ppm), light narcosis (150 000 ppm), and no effects (100 000 ppm). Deep anesthesia was produced in rabbits exposed to 200 000–250 000 ppm butadiene for 8–10 min; death due to respiratory paralysis occurred within 25–35 min at 250 000 ppm. The LC_{50} in rats exposed to butadiene for 4 h is 128 000 ppm; the LC_{50} in mice exposed to butadiene for 4 h is 117 000 ppm. The acute oral LD_{50} values for butadiene in rats and mice are 5480 and 3210 mg kg^{-1}, respectively. Butadiene is mutagenic and clastogenic in rodents with mice being more sensitive to genetic damage than rats. Both EB and DEB are mutagenic and/or clastogenic *in vivo* with little consistent evidence of interspecies differences. These *in vivo* genotoxicity data suggest that the interspecies differences in butadiene-induced toxicity are related to quantitative differences in the formation of reactive metabolites.

Human

Exposures of industrial workers to butadiene concentrations of 2000–8000 ppm have been reported to cause eye, skin, and nasal irritation. High butadiene levels may cause CNS depression as evidenced by blurred vision, drowsiness, fatigue, bradycardia, and hypotension. The mildly aromatic odor of butadiene, which can be detected at $\sim$1 ppm, serves as a good warning aid. Dermal contact with liquid butadiene may produce frostbite due to cooling caused by the rapid evaporation of butadiene from the skin.

Chronic Toxicity (or Exposure)

Animal

Lifetime studies in rats and mice indicate that the inhalation of butadiene increases the incidence of tumors at various sites, with mice being significantly more susceptible to the tumorigenic effect of butadiene than rats. In mice, tumors were induced at lifetime exposures of 6.25–1250 ppm or in as little as 13 weeks at 625 ppm. In rats, tumors were observed primarily at 8000 ppm and typically only in organs where tumors develop spontaneously. A similar species difference was noted in noncarcinogenic effects on reproductive organs. In mice, ovarian atrophy was induced in a dose-dependent fashion at lifetime exposures $\geqslant$6.25 or at 1000 ppm after a 13 week exposure. Degenerative changes in the testes of mice were observed only after lifetime exposures to butadiene at $\geqslant$200 ppm. No effects on the reproductive organs were seen in rats receiving a lifetime exposure up to 8000 ppm butadiene. Based on limited available data, there is no conclusive evidence that butadiene is fetotoxic or teratogenic at concentrations below those toxic to the mother.

Human

It is not known if butadiene itself poses a carcinogenic risk in humans. A large well-conducted epidemiological study reported an increase in mortality from leukemia among workers in the styrene–butadiene rubber industry and that the increase was associated with cumulative butadiene exposure. The risk of leukemia remained but was attenuated after controlling for exposures to styrene and other potential confounding agents. However, these results were not consistent with those of two smaller studies of adequate statistical power in butadiene monomer workers. In a recent study, air exposure as well as biomarkers of exposure and genetic toxicity were evaluated in workers occupationally exposed to low levels of butadiene monomer. Air exposures and hemoglobin adducts were well correlated, but no correlation existed with any genetic effect biomarkers. Thus, while there may be an increased risk of cancer in the styrene–butadiene industry, it is unclear as to what portion of the risk is related to butadiene alone.

In Vitro Toxicity Data

Butadiene was mutagenic with metabolic activation in the Salmonella reverese mutation assay, while

mutagenic responses in the mouse lymphoma assay were equivocal. In cultured mammalian cells, butadiene dissolved in ethanol was clastogenic while gaseous butadiene was not. Both EB and DEB are mutagenic and clastogenic in the absence of exogenous metabolic activation.

Clinical Management

The primary toxicity of butadiene is CNS depression at high concentrations. Treatment involves removal from exposure and support of respiratory function.

Environmental Fate

Butadiene is a gas under normal environmental conditions with limited water solubility (735 mg l^{-1} at 25°C). Butadiene released to the atmosphere will remain there with very small amounts being distributed to water and soil. In air, butadiene will be removed by reaction with photochemically produced hydroxyl radicals (0.24–1.9 day half-life), nitrate radicals, and ozone. When released to water, butadiene will be removed by volatilization to air (Henry's law constant of 7460 Pa m^3 mol^{-1}), biodegradation (aerobic half-life of 7–28 days), and reaction with singlet oxygen. Based on its estimated organic-carbon partition coefficient (K_{oc} of 72–228), butadiene will not exhibit significant adsorption to soil. Due to volatilization to air and degradation in soil, butadiene is not expected to leach to groundwater. Similarly, it is not expected to bind significantly to or suspended particulate matter. As butadiene is readily metabolized, it is not expected to pose a significant bioaccumulation hazard.

Ecotoxicology

Butadiene exhibited a 24 h LC_{50} of 71.5 mg l^{-1} in an estuary fish species; estimated LC_{50} values for a variety of freshwater fish ranged from 21.4 to 49.8 mg l^{-1}. A 96 h EC_{50} (immobilization) of 24.8 mg l^{-1} was reported in an aquatic invertebrate (*Daphnia magna*).

Other Hazards

Butadiene is a highly flammable gas at standard temperature and pressure. In air, butadiene will form explosive peroxides that are sensitive to shock or heating above 27°C and will explode upon contact with aluminum tetrahydroborate. Butadiene has lower and upper explosive limits of 2% and 11.5% by volume in air, respectively.

Exposure Standards and Guidelines

International occupational exposure limits (OEL) for butadiene generally range from 0.5 to 15 ppm as an 8 h time-weighted average (TWA), with 2 ppm being the TWA OEL established by the American Conference of Governmental Industrial Hygienists (ACGIH). The US Occupational Safety and Health Administration lists a permissible exposure limit of 1 ppm for butadiene (TWA) with a 15 min short-term exposure limit of 5 ppm. The National Institute of Occupational Safety and Health indicates 2000 ppm butadiene is immediately dangerous to life or health. Butadiene is classified as probably carcinogenic to humans (group 2A) by the International Agency for Research on Cancer and as known to be a human carcinogen by the US National Toxicology Program.

See also: Carcinogen Classification Schemes; Styrene.

Further Reading

Hughes K, Meek ME, Walker M, and Beauchamp R (2003) 1,3-Butadiene: Exposure estimation, hazard characterization, and exposure–response analysis. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews* 6(1): 55–83.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Butadiene.

Butane

Michael A Kamrin

- Chemical Abstracts Service Registry Number: CAS 106-97-8
- Synonyms: *n*-Butane; Butyl hydride; Methylethylmethane; Liquefied petroleum gas
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon
- Chemical Formula: $CH_3(CH_2)_2CH_3$

Uses

Butane is used as a fuel in lighters, small blow torches, and camping stoves. It is also used in calibrating instruments and as a food additive. In addition, it is a raw material for organic synthesis.

Exposure Routes and Pathways

Since butane is a gas, the major routes of exposure are inhalation and contact with skin and eyes. It is a widely used substance of abuse by inhalation.

Mechanism of Toxicity

Gaseous butane acts as a simple asphyxiant, which means that it causes toxicity by displacing oxygen and preventing it from reaching important tissues and organs. In its liquid state, it causes frostbite due to rapid cooling on evaporation.

Acute and Short-Term Toxicity (or Exposure)

Animal

An LC_{50} of $658\,g\,m^{-3}$ has been established in rats for a 4 h inhalation exposure.

Human

Because of its asphyxiant properties, high doses of inhaled butane can affect the central nervous system and lead to a variety of symptoms. These include euphoria, excitation, vomiting, confusion, hallucinations, drowsiness, and coma. Skin contact with liquid butane can cause frostbite.

Clinical Management

The affected person should be removed from exposure and provided fresh air. Symptomatic and supportive treatment should be administered. This may include support of both the cardiovascular and respiratory systems.

Environmental Fate

Butane is relatively nonpersistent in the environment and has a low leaching potential. It is moderately volatile from water and it does not bioaccumulate.

Other Hazards

Butane poses severe fire and explosion hazards. It should be stored and used distant from any ignition sources.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists time-weighted average (TWA) for butane is 800 ppm ($1900\,mg\,m^{-3}$) and the TWA for liquefied petroleum gas is 1000 ppm ($1800\,mg\,m^{-3}$).

See also: Drugs of Abuse.

Further Reading

International Program on Chemical Safety (1998) *Butane*. INCHEM Poisons Information Monograph 945, Geneva, Switzerland.

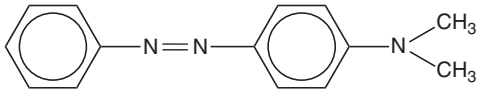
Relevant Website

http://www.inchem.org – Chemical Safety Information from Intergovernmental Organization.

Butter Yellow

Kashyap N Thakore

- Chemical Abstracts Service Registry Number: CAS 60-11-7
- Synonyms: *p*-Dimethylaminoazobenzene (DAB); *N*,*N*-Dimethyl-4-(phenylazo) benzenamine; Methyl yellow; C.I. solvent yellow 2; C.I. 11020
- Chemical/Pharmaceutical/Other Class: Food dye
- Chemical Formula: $C_{14}H_{15}N_3$
- Chemical Structure:



Uses

Early in last century, butter yellow was largely used as a food coloring. It is also used for the determination

of free HCl in gastric juice, spot test identification of peroxidized fats, as a pH indicator, and as a laboratory reagent.

Exposure Routes and Pathways

Inhalation is the most common route of exposure. When heated to decompose, it emits toxic fumes of nitrous oxides.

Toxicokinetics

Butter yellow may be rapidly absorbed by various routes including ingestion, inhalation, and dermal contact. Biotransformation involves reduction (catalyzed by at least two types of cytochrome P450) and cleavage of the azo group, demethylation, ring hydroxylation, *N*-hydroxylation, *N*-acetylation, and *O*-conjugation of metabolites in liver. The metabolites can bind to proteins and nucleic acids. When [^{14}C-dimethyl]-aminoazobenzene was fed to rats, most of the radioactivity was found in expired carbon dioxide. Urine of rats administered butter yellow contained 50–60% of it in the form of sulfates or glucuronides of *N*-acetylated metabolites.

Mechanism of Toxicity

It is metabolized *in vivo* to a reactive form that covalently binds to cellular macromolecules, such as proteins and DNA, to cause toxicity. Agents that prevent these bindings can decrease the toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Butter yellow is poisonous by the intravenous route. It is moderately toxic by the oral, intraperitoneal, intramuscular, and subcutaneous routes. It is an antihypertensive agent.

Human

The only occupational health observation in humans was of contact dermatitis in factory workers handling butter yellow. The target organs for toxicity are skin, liver, and bladder. Potential symptoms of overexposure are enlarged liver, hepatic and renal dysfunction, contact dermatitis, coughing, wheezing, difficulty in breathing, bloody sputum, bronchial secretions, frequent urination, hematuria, and dysuria.

Chronic Toxicity (or Exposure)

Animal

It shows mutagenic properties after activation. It is carcinogenic by various routes in the rat and mouse (liver carcinoma). By the oral route, it causes carcinoma of the bladder and lungs. Its carcinogenic action is influenced by diet. It is shown to be teratogenic. The LD_{50} in the rat is $200\,mg\,kg^{-1}$ orally and $230\,mg\,kg^{-1}$ intraperitoneally. The LD_{50} in the mouse is $300\,mg\,kg^{-1}$ orally and $230\,mg\,kg^{-1}$ intraperitoneally.

Human

Butter yellow can also cause adverse reproductive effects.

In the United States, Occupational Safety and Health Administration lists butter yellow as a suspected human carcinogen. Human mutation data are also reported.

Workers exposed to butter yellow should wear personal protective equipment and their work should be carried out only in restricted areas. Technical measures should prevent any contact with the skin and mucous membranes. After use, clothing and equipment should be placed in an impervious container for decontamination or disposal. Preemployment and periodic medical examination should focus on liver function.

In Vitro Toxicity Data

Butter yellow is active in inducing unscheduled DNA synthesis in Hela human cervical cancer cells. It was mutagenic in *Salmonella typhimurium* TA100 and TA98.

Clinical Management

In case of contact, the eyes and skin should be flushed with water for 15–20 min. For inhalation exposure, the victim should be moved to fresh air. Oxygen and artificial respiration should be administered, if necessary. If the patient is in cardiac arrest, cardiopulmonary resuscitation should be given. Life-support measures should be continued until medical assistance has arrived. In the case of an unconscious or convulsing person, liquids should not be administered and vomiting should not be induced.

Environmental Fate

It may bind to the soil. It may bioconcentrate in aquatic organisms, adsorb to sediment, and may be subject to direct photolysis.

See also: Food Additives.

Relevant Websites

http://ntp.niehs.nih.gov – National Toxicology Program (NTP) (2002) 10th *Report on Carcinogens*. Research Triangle Park, NC: US Department of Health and Human Services, Public Health Service.

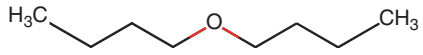
http://www.cdc.gov/niosh – National Institute for Occupational Safety and Health (NIOSH) (2003) *Pocket Guide to Chemical Hazards*, Cincinnati, OH.

http://www.iarc.fr – IARC (1987) Monographs on the Evaluations of Carcinogenic Risks to Humans: Complete List of Agents, Mixtures and Exposures Evaluated and their classification; *para*-Dimethylaminoazobenzene; Suppl. 7; p. 62.

Butyl Ether

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 142-96-1
- Synonyms: Dibutyl ether; 1-Butoxybutane, dibutyl oxide, di-*n*-butyl ether, *n*-dibutyl ether
- Chemical/Pharmaceutical/Other Class: Ether
- Chemical Formula: $C_8H_{18}O$
- Chemical Structure:



Uses

Butyl ether is used mainly as a solvent for organic materials such as resins, oils, hydrocarbons, esters, gums, and alkaloids. It is also used as an extracting agent in metal separation and as a reacting medium in organic synthesis processes. It is a solvent commonly found in teaching, research, and analytical laboratories.

Exposure Routes and Pathways

Exposure to butyl ether can occur through inhalation of vapor or mist, dermal contact, or oral ingestion of liquid dibutyl ether. Oral ingestion of dibutyl ether has been practiced to produce an 'alcoholic' euphoria. Occupational exposure to dibutyl ether may occur through inhalation and dermal contact with this compound at workplaces where dibutyl ether is produced or used. The general population may be exposed to dibutyl ether through the use of consumer products, such as latex paints, containing this compound.

Toxicokinetics

Dibutyl ether is rapidly adsorbed and eliminated from the body. Dibutyl ether can cause irritation to the skin, mucous membranes, eyes, and respiratory and gastrointestinal tracts. Systemically, dibutyl ether causes central nervous system (CNS) depression and transient liver changes.

Mechanism of Toxicity

Butyl ether has the ability to dissolve lipids. As a result, it may cause irritation and pain upon contact with eyes and nose mucosa. It also causes dermal irritation and dermatitis upon contact with the skin. Damage caused by butyl ether appears to be scattered loss of epithelial cells due to dissolution of phospholipid cell membranes. At the CNS level, butyl ether, like other volatile organic solvents, depresses the CNS by dissolving in the cell lipid membrane and disrupting the lipid matrix. These effects are known as membrane fluidization. At the molecular level, membrane fluidization disrupts solute gradient homeostasis that is essential for cell function.

Acute and Short-Term Toxicity (or Exposure)

Animal

Butyl ether is moderately toxic by the oral route. The oral LD_{50} in rats has been reported to range from 3200 to 7400 mg kg^{-1}. The inhalation LC_{50} in rats has been found to be 4000 ppm in air for 4 h. The skin LD_{50} in rabbits is 10 000 mg kg^{-1}.

Human

Signs and symptoms of excessive exposure to dibutyl ether resemble those of ethanol intoxication except that symptoms are seen shortly after exposure and the effects are short lived. Typical symptoms include dizziness, giddiness, headache, euphoria, and CNS depression.

Chronic Toxicity (or Exposure)

In humans, chronic, repeated dermal exposure may cause dermal irritation, defatting of skin, and

dermatitis. Excessive consumption of dibutyl ether as an intoxicating agent has been reported to produce ether jags, respiratory depression, and death.

Clinical Management

Given the CNS and respiratory depression properties of dibutyl ether, treatment is directed at maintaining respiration and treating irritation at the site of exposure. Patient should be monitored for respiratory distress and apnea, hyperglycemia, as well as hepatic and renal dysfunction.

Environmental Fate

Dibutyl ether's production and use as an extracting agent and as a solvent may result in its release to the environment through various waste streams. If released to air, a vapor pressure of 6.0 mmHg at 25°C indicates dibutyl ether will exist solely as a vapor in the ambient atmosphere. Vapor-phase dibutyl ether will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 13 h. Direct photolysis is not expected to be an important removal process since aliphatic ethers do not absorb light in the environmental spectrum. If released to soil, dibutyl ether is expected to have high mobility based upon an estimated K_{oc} of 51. Volatilization from moist soil surfaces may be an important fate process based upon a Henry's law constant of 6.0×10^{-3} atm m^3 mol^{-1}. Dibutyl ether is expected to volatilize from dry soil surfaces based upon its vapor pressure. If released into water, dibutyl ether is not expected to adsorb to suspended solids and sediment in water based on its K_{oc}. Aqueous screening studies indicate biodegradation may be an important fate process in both soil and water; 16% BODT was observed over a period of 5 days using acclimated microbial cultures and dibutyl ether reached 3–4% of its theoretical BOD over 4 weeks using an activated sludge seed. Volatilization from water surfaces is expected to occur based on this compound's estimated Henry's law constant. Estimated volatilization half-lives for a model river and model lake are 3.5 h and 4.6 days, respectively. Bioconcentration factors (BCFs) ranging from 30 to 114 in carp suggest that bioconcentration in aquatic organisms is moderate to high. Dibutyl ether is not expected to undergo hydrolysis in the environment due to the lack of hydrolyzable functional groups.

See also: Ethanol.

Further Reading

Sax NI and Lewis RJ (eds.) (1989) *Dangerous Properties of Industrial Materials*, 7th edn. New York: Van Nostrand Reinhold.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Butyl Ether.

Butyl Nitrite

Kashyap N Thakore

- Chemical Abstracts Service Registry Number: CAS 544-16-1
- Synonyms: NBN; NCI-C56553; Nitrous acid-*n*-butyl ester
- Chemical Formula: $C_4H_9NO_2$
- Chemical Structure: $CH_3CH_2CH_2CH_2ONO$

Uses

Butyl nitrite is used in the manufacture of rare earth azides. It is also used as a recreational drug (for vasodilatation).

Exposure Routes and Pathways

Butyl nitrite is a poison by ingestion and intraperitoneal routes. It is mildly toxic by inhalation. When heated to decompose, it emits toxic fumes of nitrogen oxides.

Toxicokinetics

Butyl nitrite is ineffective by ingestion because it is degraded in the gastrointestinal tract. A 44% uptake of butyl nitrite was observed when rats were atmospherically exposed for 5 min periods. It is very rapidly transformed in the body. The likely products of butyl nitrite *in vivo* might be butyl alcohol, methemoglobin, nitrite ion, nitrate ion, nitrosothiols, and possibly other nitroso compounds. Butyl nitrite

is also very rapidly distributed to various parts of the body such as muscles and vascular and circulating systems. The metabolites bind to hemoglobin, glutathione, and other plasma proteins. Metabolites such as nitrite ions can be eliminated in exhaled air.

Mechanism of Toxicity

Following exposure, butyl nitrite causes rapid *S*-nitrosyl glutathione formation, then a concomitant decrease in protein thiols, followed by a marked adenosine triphosphate depletion. It also causes lipid peroxidation. It produces methemoglobinemia in which oxidized hemoglobin has no oxygen carrying capacity. Also in the clinical state of methemoglobinemia, the unaltered hemoglobin shows an increased affinity for oxygen resulting in symptoms of tissue hypoxia. Cyanosis occurs when methemoglobin levels are greater than 10%. Levels above 70% are potentially lethal.

Acute and Short-Term Toxicity (or Exposure)

Animal

The formation of butyl alcohols from butyl nitrite in experimental mice produced hepatotoxicity. The oral LD_{50} is 83 mg kg^{-1} in rats and 171 mg kg^{-1} in mice; the intraperitoneal LD_{50} is 158 mg kg^{-1} in mice. The LC_{50} is 420 ppm/4 h in rats and 567 ppm/1 h in mice.

Human

Butyl nitrite is harmful if swallowed, inhaled, or absorbed through skin. It causes irritation of eyes, skin, mucous membranes, and the upper respiratory tract. Overexposure by ingestion can cause methemoglobinemia–carboxyhemoglobinemia, lowered blood pressure by vasodilatation, headache, pulse throbbing, and weakness. It can also cause behavioral changes such as altered sleep time, excitement, change in motor activity, ataxia, and rigidity. It also causes dyspnea, cyanosis, and changes in liver and kidneys. It is immunosuppressive for human lymphocytes *in vitro*.

Workers exposed to butyl nitrite should wear personal protective equipment and their work should be carried out only in restricted areas. Clothing and equipment after use should be placed in an impervious container for decontamination or disposal. Technical measures should prevent any contact with the skin and mucous membranes.

Chronic Toxicity (or Exposure)

It has not been tested for cancer, reproductive, and other long-term effects. It can initiate tumors via *in vivo* formation of *N*-nitroso compounds from butyl nitrite following exposure.

Clinical Management

In case of contact, affected eyes and skin should be flushed with water for 15–20 min. If inhaled, the affected person should be moved to fresh air. If necessary, oxygen and artificial respiration should be administered. If patient is in cardiac arrest, cardiopulmonary resuscitation should be administered. Life-support measures should be continued until medical assistance has arrived. Liquids should not be administered and vomiting should not be induced in an unconscious or convulsing person.

See also: Lipid Peroxidation.

Relevant Website

http://www.drugabuse.gov – National Institute on Drug Abuse (NIDA) (1988) Health Hazards of Nitrite Inhalants, Research Monograph Series 83, pp. 27–39.

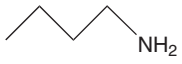
Butylamines

Janice McKee

- Representative Chemicals: *n*-Butylamine; *s*-Butylamine; *t*-Butylamine
- Chemical Abstracts Services Registry Numbers:
  - *n*-Butylamine (CAS 109-73-9)
  - *s*-Butylamine (CAS 13952-8-6)
  - *t*-Butylamine (CAS 75-64-9)
- Synonyms:
  - *n*-Butylamine: 1-Aminobutane; 1-Butanamine; Aminobutane; Butyl amine; Monobutylamine; mono-*n*-Butylamine; *n*-Butylamine; Norralamine; Tutane;
  - *s*-Butylamine: 2-Butylamine; 2-Butanamine; 2-Aminobutane; Frucote; Deccotane; 1-Methylpropane; 1-Methylpropylamine;

- *t*-Butylamine: Isobutylamine; 2-Methyl-2-propanamine; 2-Aminoisobutane; 2-Amino-2-methyl-propane; 1,1-Dimethylethylamine; 2-Methyl-2-aminopropane; Trimethylaminomethane

- Chemical/Pharmaceutical/Other Class: Amine
- Chemical Formulas:
  - *n*-Butylamine: $CH_3CH_2CH_2CH_2NH_2$
  - *s*-Butylamine: $CH_3CH_2CH(NH_2)CH_3$
  - *t*-Butylamine: $(CH_3)_2CHCH_2NH_2$
- Chemical Structure:



Uses

Butylamines are used for many purposes, including as intermediates in the manufacture of textiles, plastics, dyes and tanning agents, corrosion inhibitors, lubricating oil additives, antioxidants, fungicides, herbicides, rubber products, and emulsifying agents. They are also used in pharmaceuticals, photographic materials, and as flavors in seafood and chocolate. In addition, they are reportedly used in alcoholic beverages, ice cream, candy, baked goods, etc., and can occur naturally in some plants and foods.

Exposure Routes and Pathways

Butylamines are primary irritants and may be encountered as vapor, liquid, or as components of mixtures. They may cause damage at the point of contact (i.e., skin, eyes, lungs, and gastrointestinal tract) and also may be absorbed into the body through the intact skin, when inhaled or ingested.

Occupational exposure may occur through inhalation and dermal contact where these chemicals are produced or used. Most exposures to the general population are through various foods, from water, and from inhalation of ambient air.

Toxicokinetics

Butylamines are well absorbed from the gut and respiratory tract. Butylamines are expected to be readily metabolized, and the metabolic pathway is similar to that of other lower amines. Amines may be metabolized by monoamine oxidase and diamine oxidase (histaminase).

Mechanism of Toxicity

Butylamines are strong alkalis and potent skin, eye, and mucous membrane irritants. Contact may cause minor irritation to severe tissue damage. Butylamines may be neutralized (strong alkali to a weak acid/base) by hydrochloride, for example, in the stomach. Cellular changes may include hyperplasia, squamous metaplasia, and necrosis. Amines may cause a selective blockade of lysosomal degradation of protein. Exposure to pregnant animals has been shown to harm the fetus. The mechanism of fetotoxicity may be free radical production, metabolic acidosis, and lysosomotrophy.

Acute and Short-Term Toxicity (or Exposure)

Butylamines are primary irritants. Direct contact with liquid or sufficiently high concentrations of vapor may cause severe irritation, blistering, burns, and tissue necrosis. Contact with the eye may result in loss of vision. Ingestion of *n*-butylamine at sufficiently high concentrations may cause irritation to the mouth, throat, and gastrointestinal tract and may cause nausea, vomiting, and possibly death. Skin absorption may cause damage at the site of contact, as well as nausea, vomiting, and shock. Central nervous system effects have been observed after exposure.

Animal

Ingestion of butylamines has been shown to affect the reproductive process and cause harm to the fetus. Reported effects include increased early postimplantation losses, reduced fetal and placental weight, retarded skeletal development, and malformations.

Inhalation exposure produced maternal effects, but no developmental or fetoxicity effects were reported. Rats have been administered *n*-butylamine hydrochloride by gavage on days 6–15 postcoitum (sperm-positive = day 0), or inhaled *n*-butylamine (whole-body exposure), 6 h day^{-1} on days 6–19 postcoitum. Oral *n*-butylamine HCl 1000 mg kg^{-1} reduced maternal feed consumption, increased early postimplantation losses, reduced fetal and placental weight, retarded skeletal development, and produced malformations; 100 mg kg^{-1} was the no-observed-adverse-effect level (NOAEL) for prenatal developmental toxicity. Inhaled *n*-butylamine produced concentration-dependent nasal epithelial hyperplasia and squamous metaplasia, inflammation, and necrosis; the maternal NOAEL was less than 17 ppm. There were no treatment-related signs of embryo fetotoxicity; particularly, no effects on fetal morphology. The developmental NOAEL was 152 ppm.

Severe skin irritation with necrosis has been reported after dermal contact in the guinea pig. The LD_{50} by dermal exposure in rabbits was reported to be

850 mg kg^{-1} for *n*-butylamine and 2500 mg kg^{-1} for *s*-butylamine.

Butylamines are severely damaging to the eye when directly applied; however, the vapor is only mildly irritating to the eyes. At 3000–5000 ppm, *n*-butylamine produces an irritant response, labored breathing, and pulmonary edema, with death following in a matter of minutes or hours. An inhalation LC_{50} for *n*-butylamine was reported to be 800 mg m^{-3} for 4 h in mice.

At near lethal concentrations of *n*-butylamine administered orally, rats and rabbits exhibited increased reflex excitability, increased pulse and respiration, dyspnea, convulsions, cyanosis, and coma. The LD_{50} in rats was reported to be 147 mg kg^{-1} for *s*-butylamine and 78 mg kg^{-1} for *t*-butylamine. The oral LD_{50} in rats was reported to be 366 mg kg^{-1} for *n*-butylamine, with death due to pulmonary edema. Prior to death, the rats exhibited sedation, ataxia, nasal discharge, gasping, salivation, and convulsions.

Human

The principal hazard of concentrated *n*-butylamine to human health is its capacity to produce severe burns of the skin and eyes, as well as respiratory tract irritation (the maximal effect being pulmonary edema). Harm may occur due to direct contact with liquid or vapor at sufficiently high concentrations. Signs of toxicity include tissue damage at the site of contact, sedation, ataxia, nasal discharge, gasping, and salivation, followed by convulsions and death at very high doses. A minimum lethal human dose of *n*-butylamine has not been defined.

Vapors may be irritating to the respiratory tract at concentrations greater than 5 ppm. Workers with daily exposures from 5 to 10 ppm may complain of nose, throat, and eye irritation, and headaches. Concentrations greater than 25 ppm are difficult to tolerate for even short periods of time and concentrations greater 300 ppm may immediately threaten life. Exposure to *n*-butylamine vapors may result in erythema, particularly about the face. The face and neck may become florid within 3 h after exposure, and desquamation of the facial skin may follow in 3 days. A burning, itching sensation accompanies these symptoms. Exposure to vapors may induce allergic asthma.

Chronic Toxicity (or Exposure)

Animal

Data reviewed indicate that chronic effects may be related to the changes related to irritation. Chronic exposure to skin and mucous membranes at sufficiently high levels may cause inflammation, hyperplasia, metaplasia, and necrosis. No effects were reported as related to *s*-butylamine exposure in a 2 year feeding study in rats and dogs exposed to 2500 ppm in the diet.

Human

Generally, workers exposed to 1–5 ppm do not report symptoms. Individuals with chronic respiratory, skin, or eye disease are at increased risk from *n*-butylamine exposure. Chronic exposure to irritating levels of butylamines can cause symptoms and/or increase the severity of symptoms in people with preexisting conditions.

In Vitro Toxicity Data

Butylamine was not shown to be mutagenic in *Salmonella* in tests reviewed. Millimolar concentrations of various primary aliphatic monoamines have been shown to cause the release of lysosomal beta-glucuronidase in cultured mouse peritoneal macrophages. In selected culture systems exposed to butylamine, lysosomal enzymes were selectively released.

Clinical Management

Degree of injury should be considered when determining initial treatment. Exposed skin and eyes should be immediately irrigated with copious amounts of tepid water. The extent of damage to the eye may not be fully evident until 48–72 h after exposure.

After inhalation exposure, the victim should be moved to fresh air and monitored for respiratory distress. Also, 100% humidified supplemental oxygen with assisted ventilation should be administered as required. If coughing or breathing difficulties are noted, the patient should be evaluated for irritation, bronchitis, or pneumonitis, including chest X-rays and determination of blood gasses. If pulmonary edema is present, positive end expiratory pressure ventilation and steroids should be considered. For ingestion exposures, copious amounts of water should be given to dilute stomach contents. Because of the potential for gastrointestinal tract irritation or burns, do not induce emesis. Significant esophageal or gastrointestinal tract irritation or burns may occur following ingestion. The possible benefit of early removal of some ingested material by cautious gastric lavage must be weighed against potential complications of bleeding or perforation.

Environmental Fate

n-Butylamine's production and use as a chemical intermediate, as well as its presence in animal waste,

may result in its release to the environment through various waste streams. When released into the soil, *n*-butylamine is expected to leach into groundwater. When released into the soil, it may biodegrade and evaporate to a moderate extent. When released into water, it may biodegrade to a moderate extent and is expected to quickly evaporate. *n*-Butylamine is not expected to significantly bioaccumulate. It has an estimated bioconcentration factor (BCF) of less than 100. When released into the water, it is expected to have a half-life between 1 and 10 days. When released into the air, it is expected to be readily degraded by reaction with photochemically produced hydroxyl radicals, is expected to have a half-life between 1 and 10 days, and is expected to be readily removed from the atmosphere by wet deposition. If released to air, a vapor pressure of 92.9 mmHg at 25°C indicates *n*-butylamine will exist solely as a vapor in the ambient atmosphere.

Other Hazards

n-Butylamine has an almost unlimited shelf-life in unopened, original containers if protected from heat and properly stored in a protected storage area. It is neither explosive nor spontaneously flammable in air. However, it is flammable, malodorous and corrosive, and may corrode some metals in the presence of water.

Contact with strong acids may cause spattering and may corrode some metals in the presence of water. Liquid butylamine will attack some forms of plastics, rubber, and coatings. Some forms react violently with water. Contact with strong oxidizers may cause fires and even explosions under the right conditions. Toxic oxides of nitrogen may form in fire. Vapors may travel to source of ignition and flash back. Most vapors are heavier than air. Vapors can present an explosion hazard indoors or in enclosed spaces at high enough concentrations. Some butylamines may polymerize explosively when heated.

One should always refer to the Material Safety Data Sheet for detailed information on handling and disposal.

Exposure Standards and Guidelines

Butylamines have numerous occupational exposure standards and guidelines. *n*-Butylamine occupational exposure criteria include the following:

- USA: Occupational Safety and Health Administration permissible exposure limit ceiling value of 5 ppm ($15\ mg\ m^{-3}$).
- USA: National Institute for Occupational Safety and Health values include a recommended exposure limit of 5 ppm as a 15 min ceiling value, and an immediately dangerous to life or health value of 300 ppm.
- USA: American Conference of Governmental Industrial Hygienists ceiling limit of 5 ppm, with a skin notation.
- Australia: 5 ppm, peak limitation, with a skin notation.
- Federal Republic of Germany: 5 ppm, short-term level 25 ppm, 30 min, two times per shift.
- Sweden: 5 ppm ceiling limit, with a skin notation.
- United Kingdom: 5 ppm, 10 min, short-term exposure limit 15 ppm, with a skin notation.

Miscellaneous

n-Butylamine may exist as a liquid or vapor. The liquid is clear and colorless. Its odor is described as 'fish-like' and 'ammonia-like'. Odor may be detected at concentrations slightly less than 1 ppm, is noticeable at 2 ppm, moderately strong at 2–5 ppm, and strong at 5–10 ppm.

n-Butylamine occurs naturally in some foods. These include: kale (7 ppm); pickles; cucumbers in aromatic vinegar (0.6 ppm); cucumbers pickled with mustard (5.3 ppm); Tilsiter cheese (3.7 ppm); brown bread (1.1 ppm); mulberry leaves; fish and seafood. *n*-Butylamine has been identified as a volatile component of boiled beef. Butylamines have been reported to be a component of animal waste, perhaps from decomposition of manure.

See also: Corrosives; Respiratory Tract; Skin.

Further Reading

Clansky KB (ed.) *Suspect Chemicals Sourcebook: A Guide to Industrial Chemicals Covered Under Major Federal Regulatory and Advisory Programs*, section 3, p. 44. Burlington, CA: Roytech Publications.

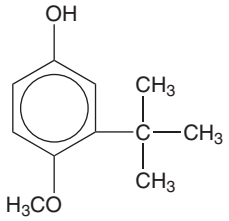
Clayton GD and Clayton FE (eds.) (1993–1994) *Patty's Industrial Hygiene and Toxicology*, vols. 2A, 2B, 2C, 2D, 2E, 2F: *Toxicology*, 4th edn. New York: Wiley.

FAO and WHO (1981) Pesticide Residues in Food. In: Lewis RJ Sr and Tatken RL (eds.) *Registry of Toxic Effects of Chemical Substances*. DHEW (NIOSH) Publication No. 79-100. EO 2975000. Cincinnati, OH: National Institute for Occupational Safety and Health.

Butylated Hydroxyanisole

Kashyap N Thakore

- Chemical Abstracts Service Registry Number: CAS 25013-16-5
- Synonyms: (1,1-Dimethylethyl)-4-methoxyphenol; 2(3)-*t*-Butyl-4-hydroxyanisole; BHA; Anthracine 12
- Chemical Formula: $C_{11}H_{16}O_2$
- Chemical Structure:



Uses

Butylated hydroxyanisole (BHA) is an antioxidant and preservative, especially in foods, cosmetics, and pharmaceuticals, and also in rubber and petroleum products.

Exposure Routes and Pathways

There is a widespread human exposure to BHA by ingestion and skin application. When heated to decompose, it emits acrid and irritating fumes and causes inhalation exposure.

Toxicokinetics

In experimental animals and in humans, BHA is absorbed rapidly after oral administration. The major metabolic pathways are conjugation (phase II) reactions, oxidative metabolism (*O*-demethylation) being relatively unimportant. BHA is metabolized to main metabolites, 4-*O*-conjugates, *O*-sulfates, and *O*-glucuronides. In dogs, oxidative metabolism is more important. It also induces both phase I and phase II drug metabolizing enzyme mRNA, protein activity, and hepatic and intestinal glutathione *S*-transferases.

BHA is distributed to various organs such as liver, lungs, and gastrointestinal tract. The metabolites are rapidly excreted through urine with little evidence of long-term tissue storage. When human volunteers were given a single oral dose of ^{14}C-labeled BHA ($\sim$0.5 mg kg^{-1} body weight), 60–70% of the radioactivity was excreted in the urine within 2 days and 80–86.5% by day 11. After administration of a single dose of 1000 mg BHA to New Zealand White rabbits, 46% of the dose was excreted in the urine as glucuronides, 9% as etherial sulfates, and 6% as free phenols. Excretion of glucuronides was inversely dose dependent.

Mechanism of Toxicity

The metabolites can bind to cellular macromolecules, such as proteins and DNA, to cause toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the oral LD_{50} is 2000 mg kg^{-1} and the intraperitoneal LD_{50} is 881 mg kg^{-1}. The oral LD_{50} is 1100 mg kg^{-1} in mice and 2100 mg kg^{-1} in rabbits.

Human

BHA is harmful if swallowed, inhaled, or absorbed through skin. It is irritating to the eyes, skin, mucous membranes, and upper respiratory tract. Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals. The target organs for toxicity are liver, lungs, and forestomach.

Chronic Toxicity (or Exposure)

Animal

BHA induces benign and malignant tumors of the forestomach in rats and hamsters by administration through diet. It is toxic to the reproductive system and embryo in rats but not toxic to rabbits, pigs, or rhesus monkeys.

Human

BHA may cause cancer.

Workers exposed to BHA should wear personal protective equipment and take measures to prevent any contact with the skin and mucous membranes.

Approximately 50 countries reportedly permit the use of BHA as a food additive. BHA is classified as Generally Recognized as Safe by the US Food and Drug Administration, when the total content of antioxidants represents not more than 0.02% w/w of the total fat or oil content of the food. It is also permitted at maximum levels of 0.001–0.02% in other specific products.

In Vitro Toxicity Data

It is not mutagenic to *Salmonella typhimurium*, *Drosophila melanogaster*, or Chinese hamster cells *in vitro* and does not cause chromosomal effects.

Clinical Management

In case of contact, eyes and skin should be flushed with water for 15–20 min. In the case of inhalation exposure, the victim should be moved to fresh air. If necessary, oxygen and artificial respiration should be administered. Cardiopulmonary resuscitation should be administered, if the patient is in cardiac arrest. Life-supporting measures should be continued until medical assistance has arrived. An unconscious or convulsing person should not be given liquids or induced to vomit.

Environmental Fate

It has low soil mobility and volatilizes slowly from water. It may bioconcentrate in aquatic organisms, adsorb to sediment, and may be subject to direct photolysis.

Exposure Standards and Guidelines

BHA or its residues are exempted from the requirement of a tolerance when used as an antioxidant in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest. BHA used as a chemical preservative in food for human consumption, in animal drugs, feeds, and related products is generally recognized as safe when used in accordance with good manufacturing or feeding practice.

See also: Food Additives.

Relevant Websites

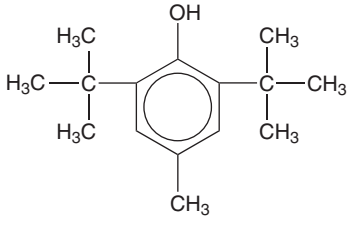
http://ntp.niehs.nih.gov – National Toxicology Program (NTP) (2002) *10th Report on Carcinogens*, Research Triangle, NC: US Department of Health and Human Services, Public Health Service.

http://www.iarc.fr – IARC (1986) Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation, vol. 40, p. 123.

Butylated Hydroxytoluene

Kashyap N Thakore

- Chemical Abstracts Service Registry Number: CAS 128-37-0
- Synonyms: 2,6-Bis(1,1-dimethylethyl)-4-methylphenol; 2,6-Di-*t*-butyl-*p*-cresol; BHT; Anthracine 8
- Chemical Structure:



Uses

Butylated hydroxytoluene (BHT) is an antioxidant for food, animal feed, petroleum products, synthetic rubbers, plastics, animal and vegetable oils, and soaps. It is also used as an antiskinning agent in paints and inks.

Exposure Routes and Pathways

Ingestion is the most common route of exposure in addition to inhalation and skin absorption. BHT is combustible when exposed to heat or flame and can emit acrid smoke and fumes.

Toxicokinetics

In BALB/c mice, 40% of an intragastric dose of BHT was taken by the tissues within 30 min by males, whereas only 10% was absorbed in females. Oxidative metabolism (phase I reactions) mediated by the microsomal monooxygenase system is the major route for degradation; oxidation of the ring methyl group predominates in rat, rabbit, and monkey and oxidation of the *t*-butyl groups in man. The predominant metabolic pathway involves oxidation of the 4-methyl group. The major metabolites are 3,5-di-*t*-butyl-4-hydroxybenzoic acid, both free and as a glucuronide, and *S*-(3,5-di-*t*-butyl-4-hydroxybenzyl)-*N*-acetylcysteine. Moreover, BHT-quinone methide (2,6-di-*t*-butyl-4-methylene-2,5-cyclohexadienone), a reactive metabolite, has been identified in the liver and bile of rats. Metabolites produced in

mice are similar to those produced in rats, except that the major biotransformation in mice was by oxidation of *t*-methyl groups.

Accumulation of BHT is greatest in tissues. In male and female BALB/c mice, a single intragastric dose was widely distributed to various tissues within 30 min, primarily to the small intestine, stomach, liver, kidneys, and lungs. Enterohepatic circulation of BHT has been reported in rats. BHT is also converted by cytochrome P450 monooxygenases to a chemically reactive metabolite – possibly BHT-quinone methide, which forms BHT–glutathione by nonenzymatic conjugation with glutathione.

BHT is cleared less rapidly from most species, enterohepatic circulation being partly responsible for the delay. The major metabolites of BHT in rat urine are 3,5-di-*t*-butyl-4-hydroxybenzoic acid (BHT acid; III), both free (90% of the dose) and as a glucuronide (15%), and *S*-(3,5-di-*t*-butyl-4-hydroxybenzyl)-*N*-acetylcysteine. The ester glucuronide and mercapturic acid were major metabolites in rat bile, while free BHT acid was the main component in the feces. In addition, 1,2-bis(3,5-di-*t*-butyl-4-hydroxyphenyl) ethane has been identified in rat bile. In BALB/c mice, ~75% of a single oral dose was excreted in the urine during the first 24 h; this was followed by a slower phase during which an additional 10% was excreted over the next 4 days. The total amount found in the feces was less than 1%. Female rats have greater urinary excretion of BHT than male rats, whereas male BALB/c mice excreted BHT more rapidly than females.

Mechanism of Toxicity

The metabolites can bind to cellular macromolecules, such as proteins and DNA, to cause toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, BHT is poisonous by intraperitoneal and intravenous routes and moderately toxic by ingestion. The oral LD_{50} is $890\,mg\,kg^{-1}$ in rats and $1040\,mg\,kg^{-1}$ in mice. In mice, the intraperitoneal LD_{50} is $138\,mg\,kg^{-1}$ and the subcutaneous LD_{50} is $650\,mg\,kg^{-1}$. In the guinea pig the oral LD_{50} is $10\,700\,mg\,kg^{-1}$.

Human

BHT is harmful if swallowed, inhaled, or absorbed through skin. It causes irritation of the eyes, skin, mucous membranes, and upper respiratory tract. Prolonged or repeated contact can damage the eyes and cause nausea, dizziness, and headache.

Chronic Toxicity (or Exposure)

Animal

BHT has produced reproductive effects in animal experiments. It is a questionable carcinogen based on experimental carcinogenic and neoplastigenic data. It induces liver tumors in long-term experiments.

Human

BHT is a possible carcinogen with the target organ being the lungs. It does not represent a relevant mutagenic/genotoxic risk to humans.

Approximately 40 countries reportedly permit the use of BHT as a direct or indirect food additive. BHT was approved and classified as 'Generally Recognized as Safe' by the US Food and Drug Administration. Regulated food products could contain a combined total of up to 0.02% BHT and butylated hydroxyanisole, based on the fat content of the food. It is also permitted at maximum levels of 0.001–0.01% in other specific products.

Workers exposed to BHT should wear personal protective equipment and take measures to prevent any contact with the skin and mucous membranes. The American Conference of Governmental Industrial Hygienists recommends that occupational exposure to airborne BHT not exceed $10\,mg\,m^{-3}$ (threshold limit value) as an 8 h time-weighted average or $20\,mg\,m^{-3}$.

In Vitro Toxicity Data

In vitro studies on bacterial, yeast, and various mammalian cell lines, and primary hepatocytes demonstrate the absence of interactions with or damage to DNA.

Clinical Management

In case of contact, eyes and skin should be flushed with water for 15–20 min. In the case of inhalation exposure, the victim should be moved to fresh air. If necessary, oxygen and artificial respiration should be administered. Cardiopulmonary resuscitation should be administered if the patient is in cardiac arrest. Life-supporting measures should be continued until medical assistance has arrived. An unconscious or convulsing person should not be given liquids or induced to vomit.

Exposure Standards and Guidelines

BHT and its residues are exempted from the requirement of a tolerance when used as an antioxidant in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulation applied to animals. BHT used as a chemical preservative in food for human consumption, in animal drugs, feeds, and related products is generally recognized as safe when used in accordance with good manufacturing or feeding practice.

See also: Food Additives; Respiratory Tract.

Relevant Websites

http://www.cdc.gov/niosh – NIOSH (2003) Pocket Guide to Chemical Hazards. Cincinnati, OH: National Institute for Occupational Safety and Health.

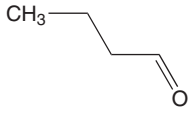
http://www.iarc.fr – IARC (1986) Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation, vol. 40, p. 161.

Butyraldehyde, *n*-

Sanjay Chanda and Harihara M Mehendale

This article is a revision of the previous print edition article by Michael J Brabec, volume 1, pp. 201–202, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 123-72-8
- Synonyms: Butyraldehyde; Butal; Butaldehyde; Butyl aldehyde; *n*-Butyl aldehyde; Butyral; *n*-Butyraldehyde; Butyric aldehyde; Butyrylaldehyde; *n*-Butanal; Butanaldehyde
- Chemical/Pharmaceutical/Other Class: Aliphatic aldehyde
- Chemical Structure:



Uses

Butanal is used in the manufacture of rubber accelerators, synthetic resins, solvents, and plasticizers. *n*-Butyraldehyde is used as an intermediate in the manufacturing of plasticizers, alcohols, solvents, and polymers (such as 2-ethylhexanol, *n*-butanol, trimethylolpropane, *n*-butyric acid, polyvinyl butyral, methyl amyl ketone). It is also used as an intermediate to make pharmaceuticals, agrochemicals, antioxidants, rubber accelerators, textile auxiliaries, perfumery, and flavors. It has no therapeutic use at the present time.

Background Information

n-Butyraldehyde is a clear, mobile, flammable, liquid with a pungent odor. It is miscible with all common solvents, for example, alcohols, ketones, aldehydes, ethers, glycols, and aromatic and aliphatic hydrocarbons, but is only sparingly soluble in water.

Exposure Routes and Pathways

Butanal is a liquid at room temperature, with a relatively low vapor pressure. Limited contact could occur by exposure to butanal vapors. Butanal has appreciable solubility in water; therefore, exposure would be expected to be primarily through ingestion of or through skin contact with the compound or a solution of the compound.

Toxicokinetics

Butanal is readily metabolized to carbon dioxide by conversion to butyryl CoA and subsequent metabolism via the pathways of short-chain fatty acid oxidation. Detoxication by reaction with glutathione also occurs. Clearance is rapid and complete.

Mechanism of Toxicity

Butanal does not possess high acute toxicity but is a potent irritant of the skin, eyes, and upper respiratory tract. The mechanism of toxicity probably involves direct reaction between the active aldehyde group and cellular components.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} value for rat is $5.9\,g\,kg^{-1}$, whereas the LC_{50} is 60 000 ppm (30 min exposure). Acute exposures to butanal vapors induce inflammation of the alveolar and bronchial regions of the lung, with death due to pulmonary edema. Severe irritation of

the eyes and nose are noted. Relatively high levels of butanal in the drinking water of mice for 50 days produced abnormal sperm morphology. Exposure of rodents to low concentrations of butanal allowed rapid recovery after exposure is ceased.

Human

Butanal has low acute toxicity. Exposure to a large dose may have a temporary narcotic effect. Exposure to low concentrations of butanal vapors produces irritation of the eyes, nose, and throat. The compound has an unpleasant odor. Impurities (butyric acid) may be present that make the smell even more objectionable. Health effects attributed to chronic exposure to low doses of butanal vapors have not been described. Dermatitis may be expected after prolonged and repeated exposures to solutions containing butanal.

Chronic Toxicity (or Exposure)

Animal

Not a recognized carcinogen.

Human

Not a recognized carcinogen.

Clinical Management

Support should be given to the patient until butanal has been cleared from the body, which occurs in a relatively short time. Recovery is uneventful.

Environmental Fate

Terrestrial Fate

The primary degradation process in soil is expected to be biodegradation. A number of biological screening studies have demonstrated that butyraldehyde is readily biodegradable. Butyraldehyde's vapor pressure of 111.4 mmHg at 25°C indicates that it will evaporate rapidly from surfaces.

Aquatic Fate

The major environmental fate processes for butyraldehyde in water are biodegradation and volatilization. A number of biological screening studies have demonstrated that butyraldehyde is readily biodegradable. Volatilization half-lives of 9 h and 4.1 days have been estimated for a model river (1 m deep) and an environmental pond, respectively. Aquatic hydrolysis, adsorption to sediment, and bioconcentration are not expected to be important fate processes.

Atmospheric Fate

In excess of 99% of the butyraldehyde present in the atmosphere will occur in the vapor phase, although a small fraction has been shown to occur in the particulate aerosol. Vapor-phase butyraldehyde will degrade relatively rapidly in an average ambient atmosphere by reaction with photochemically produced hydroxyl radicals (estimated half-life of 16.4 h). Direct photolysis may also be a major degradation process. During intense smog-pollution episodes, the natural formation rate of butyraldehyde can exceed the degradation rate. The detection of butyraldehyde in cloud and fog water indicates that physical removal from air can occur through wet deposition.

Exposure Standards and Guidelines

Workplace environmental exposure level (WEEL): 8 h time-weighted average (25 ppm).

See also: Butyric Acid; Pollution, Soil; Pollution, Water.

Relevant Website

http://www.epa.gov – Butyraldehyde Fact Sheet (from the US EPA's OPPT) (EPA 749-F-95-005a), 1994.

Butyric Acid

James Deyo

- Chemical Abstracts Service Registry Number: CAS 107-92-6
- Synonyms: Butanoic acid; Acide butyrique; *n*-Butanoic acid; *n*-Butyric acid; Butanic acid; Ethylacetic acid; Acido butirico; 1-Propanecarboxylic acid; Propylformic acid; Kyselina maselna; Buttersaeure; RTECS ES5425000; UN2820; FEMA number 2221
- Chemical/Pharmaceutical/Other Class: Industrial intermediate; Food additive
- Chemical Structure: $HOCH(=O)–CH_2–CH_2–CH_3$

Uses

Most butyric acid is consumed in the manufacture of cellulose acetate butyrate (CAB) plastics. CAB sheets are used for thermoformed sign faces, blister packaging, and goggles and face shields, while molded CAB is used to make pen barrels, eyeglass frames, and screwdriver handles. CAB is a component in acrylic enamel for automotive original equipment manufacturing coatings. Some butyric acid is used to make butyroperoxides and herbicides. It is also used as an intermediate for pharmaceuticals, emulsifiers, and disinfectants, as a leather tanning agent, and a sweetening agent in gasoline. It is used in the synthesis of butyrate ester perfumes and in the manufacture of esters, some of which serve as the bases of artificial flavoring ingredients of certain liquors, soda-water syrups, candies. Another use is as a food additive in butter, cheese, butterscotch, caramel, fruit and nut flavors (butyric acid is a (US) Food and Drug Administration generally considered as safe (GRAS) material). Butyric acid is also used in the preservation of high moisture wheat grains to prevent fungal deterioration.

Exposure Routes and Pathways

Exposure to butyric acid may occur by inhalation, dermal contact, or ingestion.

Toxicokinetics

Butyric acid is rapidly metabolized in the liver to acetic acid and ketone bodies (acetone, acetoacetate, beta-hydroxybutyrate). In humans, the butyric acid elimination curve can be divided into two parts corresponding to two half-lives: for the first (0.5 min), the slope suggests an accelerated excretion; for the second (13.7 min), a slow plateau is observed.

Mechanism of Toxicity

The most probable mechanism of toxicity is the formation of an acid proteinate following exposures to high concentrations. Such complexes result in an inhibition of protein function and disruption of cellular homeostasis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Data in laboratory animals suggest that *n*-butyric acid is only slightly acutely toxic with an oral LD_{50} value in rats ranging from 2940 to 8790 mg kg^{-1}, an inhalation LC_{50} of >40 mg l^{-1} in rabbits, and a dermal LD_{50} value in rabbits of 530 mg kg^{-1}. Butyric acid is a moderately strong irritant to skin and may induce severe eye irritation.

Human

Acute exposure to butyric acid would be anticipated to induce irritation or burns following contact with skin or eyes, or if inhaled. Such effects will occur in a concentration-dependent manner.

Chronic Toxicity (or Exposure)

Animal

Repeated inhalation or oral exposures to moderate to high doses of *n*-butyl acetate and *n*-butanol are well tolerated. These aforementioned molecules are readily and rapidly metabolized to *n*-butyric acid. The no-observed-effect level (NOEL) for repeated dose oral exposure to *n*-butanol was 125 mg kg^{-1} day^{-1}. In a 90 day inhalation study in rats with *n*-butyl acetate a NOEL of 500 ppm was reported for systemic effects, and a NOEL of 3000 ppm (highest dose tested) was reported for postexposure neurotoxicity based on functional observational battery endpoints, quantitative motor activity, neuropathy, and scheduled-controlled operant behavior endpoints. Results of inhalation studies conducted on *n*-butanol and *n*-butyl acetate were negative for inducing reproductive and developmental toxicity. The NOEL for female reproductive toxicity was 6000 ppm with *n*-butanol and 1500 ppm for *n*-butyl acetate. In a 90 day repeated-dose inhalation toxicity study with butyl acetate the NOEL for male reproductive toxicity was 3000 ppm. For developmental toxicity, a NOEL of 3500 ppm was observed with *n*-butanol and a NOEL of 1500 ppm (the highest exposure tested) was seen in both rats and rabbits following exposure to *n*-butyl acetate.

Human

Butyric acid is generally recognized as safe (GRAS) as a food additive for chronic consumption when used in accordance with good manufacturing practice. Chronic exposure also occurs through endogenous production as *n*-butyric acid is an important metabolite in the breakdown of carbohydrates, fats, and proteins and is produced in the human colon by fermentation. *n*-Butyric acid is present in butter as an ester to the extent of 4–5%.

In Vitro Toxicity Data

Data indicate butyric acid is not genotoxic. Negative results were observed in assays assessing for both mutations (Ames test) and chromosomal aberrations.

Clinical Management

Exposure should be terminated as soon as possible by the removal of the victim to fresh air. The skin, eyes, and mouth should be washed with copious amounts of water. A 15 to 20 min wash may be necessary to neutralize and remove all residual traces of the contaminant. Contaminated clothing and jewelry should be removed. Contact lenses should be removed from the eyes to avoid prolonged contact of the acid with the area. A mild soap solution may be used for washing the skin and as an aid to neutralize the acid, but should not be placed into the eyes. No cream, ointment, or dressing should be applied to the affected area. If a large quantity has been swallowed, then gastric lavage should be considered. Dilution with water may be the solution for small quantities swallowed. The victim should be kept quiet and normal body temperature should be maintained.

Environmental Fate

n-Butyric acid is not environmentally persistent or likely to bioaccumulate.

Ecotoxicology

The LC_{50} (48 h) value for fish (*Oryzias latipes*) is 90 mg l^{-1}; the EC_{50} (24 h) value for *Daphnia magna* is 1950 mg l^{-1}; and the EC_3 (3% or greater reduction in growth; 8 day) for *Scenedesmus quadricauda* is 2600 mg l^{-1} *Microcystis aeruginosa* 318 mg l^{-1}.

Other Hazards

Forms carbon dioxide and carbon monoxide during combustion.

Exposure Standards and Guidelines

None established.

See also: Food Additives; Generally Recognized as Safe (GRAS).

Relevant Website

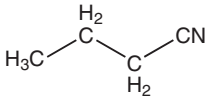
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Butyric Acid.

Butyronitrile

Carey N Pope

This article is a revision of the previous print edition article by Rhonda S Berger and Wendy Khune, volume 1, p. 203, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 109-74-0
- Synonyms: Butanenitrile; Butyric acid nitrile; Cyanopropane; Propyl cyanide; *n*-Butyronitrile; 1-Butyronitrile; 1-Cyanopropane
- Chemical/Pharmaceutical/Other Class: Alkyl nitrile
- Chemical Formula: C_4H_7N
- Chemical Structure: $CH_3CH_2CH_2C{\equiv}N$



Uses

Butyronitrile is an industrial solvent.

Exposure Routes and Pathways

Dermal, oral, and inhalation routes are all primary exposure pathways.

Toxicokinetics

Toxicokinetic data are available only for propionitrile. When administered as a ^{14}C radioisotope, 92.5% of the compound was recovered. The majority was eliminated in air or urine within 24 h. About 27% was recovered as volatile organic material within 0.5 h of gavage exposure. By 3 h, either carbon dioxide or cyanide exhalation was estimated at 38–49% of the total. At 24 h, the total ^{14}C recovery in the urine was 0.76–5.83%. A small amount (<2%) was found in liver and kidneys at 72 h after dosing. It was concluded that propionitrile is rapidly absorbed from the gastrointestinal tract and eliminated through expired air as the parent compound, CO_2, or cyanide.

Mechanism of Toxicity

The acute toxicity of butyronitriles is thought to be due to release of cyanide through metabolism of the parent compound. Signs of acute butyronitrile intoxication including dyspnea, ataxia, and convulsions are similar to those noted with acute cyanide intoxication. The onset and duration suggests that these nitriles require metabolism to elicit toxicity. Cyanide and thiocyanate have both been found in urine and

blood after butyronitrile exposure. Butyronitrile toxicity is antagonized by sodium thiosulfate and sodium nitrite and blockade of hepatic metabolism. All of these support the hypothesis that cyanide is the ultimate toxicant following butyronitrile exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values in rats for all butyronitriles are from 40 to 270 mg kg^{-1}. Inhalation LD_{50} values (1–4 h exposure) were from 1000 to 1465 ppm. Dermal LD_{50} values of *n*-butyronitrile and isobutyronitrile in rabbits were 239–389 mg kg^{-1}. Butyronitrile is a mild eye and skin irritant.

Human

Butyronitrile exposure may cause dizziness, dysnpea, nausea, vomiting, weakness, confusion, and unconsciousness.

Chronic Toxicity (or Exposure)

Animal

Butyronitriles do not elicit reproductive toxicity. In a developmental toxicity study, rats exposed to 50, 100, or 200 ppm butyronitrile for 6 h a day during gestation showed no teratogenic effects but did exhibit decreased fetal weights at the highest dosage. Little is known regarding toxicity of prolonged exposures to butyronitrile. Repeated exposures to propionitrile led to neurotoxicity (ataxia, tremors, convulsions) at high dosage levels. Dyspnea, nasal and ocular discharge, increased salivation, reduced motor activity, and alopecia were observed. Significant reduction in red blood cells and hemoglobin and increases in spleen weights were also noted.

Human

Little is known regarding chronic effects of butyronitrile exposure in humans.

In Vitro Toxicity Data

Butyronitriles were negative in *in vitro* mutagenicity and cytogenicity assays.

Clinical Management

For mild signs of intoxication (nausea, dizziness, drowsiness) with blood cyanide concentrations <2 mg l^{-1}, oxygen and bed rest should be given. With more severe intoxication exhibiting short-lived periods of unconsciousness, convulsions, vomiting, and/or cyanosis and with blood cyanide concentrations of 2–3 mg l^{-1}, l00% oxygen should be provided for not more than 24 h and observation should be made in an intensive care area. Fifty milliliters of 25% sodium thiosulfate solution (1.5 g) should be given intravenously over 10 min.

Environmental Fate

Butyronitriles undergo microbial degradation. Butyronitrile does not significantly hydrolyze at environmentally relevant pHs.

Ecotoxicology

Concentrations near 100 mg l^{-1} (96 h static) of both isobutyronitrile and *n*-butyronitrile were without effect on fathead minnows. Daphnia treated with 94.3 mg l^{-1} isobutyronitrile showed no abnormal behavior or movement changes. The same concentration of *n*-butyronitrile resulted in 1/20 daphnids becoming immobile at 48 h, but this was not considered treatment related. According to the Environmental Protection Agency assessment criteria, these values correspond to a 'low concern level'.

Exposure Standards and Guidelines

The 10 h time-weighted average for butyronitrile is 8 ppm. The proposed ERPG-3 (maximum air concentration below which all individuals could be exposed for up to 1 h without developing life-threatening health effects) for butyronitriles is 100 ppm (280 mg m^{-3}) and the ERPG-2 (maximum air concentration below which nearly all individuals could be exposed for up to 1 h without developing irreversible or serious health effects or symptoms that could impair an individual's ability to take protective action) is 30 ppm (84 mg m^{-3}).

See also: Cyanide; Neurotoxicity.

Further Reading

National Institute for Occupational Safety and Health (1978) *NIOSH Criteria for a Recommended Standard, Occupational Exposure to Nitriles* (DHEW Publication No. 78-212). Washington, DC: Government Printing Office.

Relevant Website

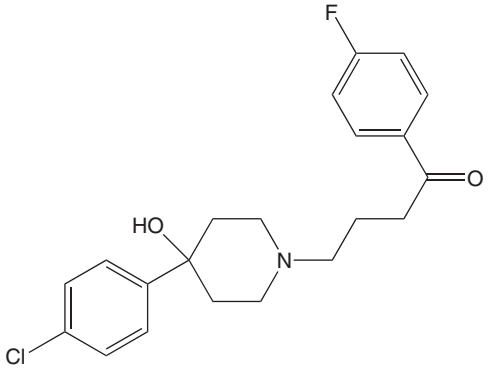
http://www.epa.gov – US Environmental Protection Agency.

Butyrophenones

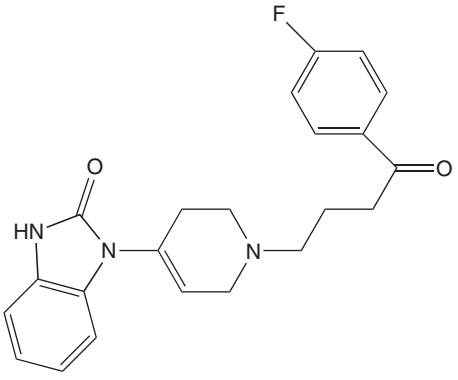
Jaya Chilakapati and Harihara M Mehendale

This article is a revision of the previous print edition article by Douglas J Borys, volume 1, pp. 203–205, © 1998, Elsevier Inc.

- Representative Compounds: Haloperidol; Droperidol
- Chemical Abstracts Service Registry Numbers: CAS 495-40-9; CAS 52-86-8 (haloperidol); CAS 548-73-2 (droperidol)
- Synonyms: Haloperidol-haldol; 4-(4-(*p*-Chlorophenyl)-4-hydroxypiperidino)-4′-fluorobutyrophenone; Droperidol-dehydrobenzperidol; Inapsine; 1-(1-(3-*p*-Fluorobenzoylprpyl)-1,2,3,6-tetrahydropyrid-4-yl)-2-benzimidazolinone
- Chemical/Pharmaceutical/Other Class: Neuroleptics; Antipsychotics; Major tranquilizers
- Chemical Formula: $C_{10}H_{12}O$
- Chemical Structures:



Haloperidol



Droperidol

Uses

Butyrophenones are used to treat psychoses including schizophrenia, organic psychosis, paranoid syndrome, acute idiopathic psychotic illnesses, and the manic phase of manic depressive illness. Other uses include treatment of aggressive behavior, delirium, acute anxiety, nausea and vomiting, pain, organic brain syndrome, and Tourette's syndrome.

Exposure Routes and Pathways

Haloperidol is available both in an injectable form and in oral dosage form. The principal exposure pathway is intentional ingestion by adults or accidental ingestion by small children. Pharmacists, physicians, and nurses dispensing or administering haloperidol could be exposed through dermal contact. Droperidol is available only as an injectable drug. The most common route of exposure is an accidental injection.

Toxicokinetics

Haloperidol is well absorbed orally with a bioavailability of 60–65% due to first-pass hepatic metabolism. It has a reversible oxidation/reduction metabolic pathway: it is metabolized via reduction to reduced haloperidol, which is biologically inactive. Both agents are rapidly absorbed after intramuscular injection, peaking within 10 min. Butyrophenones are metabolized in the liver to inactive metabolites. Concentrations of butyrophenones are found in the liver, central nervous system, and throughout the body. Haloperidol is 92% protein bound. Haloperidol is 15% eliminated through the bile. The elimination half-life is 14–41 h. The half-life of droperidol is 2 h; 10% is recovered unchanged in the urine.

Mechanism of Toxicity

Butyrophenones work primarily by blocking dopamine-mediated synaptic neurotransmission by binding to dopamine receptors. In addition to significant antidopaminergic action, butyrophenones also possess anticholinergic, α-adrenergic blockade, and quinidine-like effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Signs of toxicity reported in animals have included sedation, dullness, photosensitivity, weakness, anorexia, fever, icterus, colic, anemia, and hemoglobinuria. Treatment consists of gastric decontamination and aggressive supportive care.

Human

Clinical signs of toxicity most commonly include extrapyramidal effects, somnolence, coma, respiratory

depression, cardiac dysrhythmias, hypotension, and sedation. Neuroleptic malignant syndrome has been reported after therapeutic use and acute intoxication. The most commonly reported dystonic reactions include akathesias, stiff neck, stiff or protruding tongue, and tremor. Children appear to be more sensitive than adults to the extrapyramidal effects of butyrophenones with facial grimacing and oculogyric crisis noted. Anticholinergic effects, including dry mouth, blurred vision, and tachycardia, may occur. Other cardiac effects include prolonged QT interval and mild hypotension. Hypokalemia has also been noted. Since haloperidol may lower the seizure threshold, the drug should be used with caution in patients receiving anticonvulsant agents and in those with a history of seizures of electroencephalographic abnormalities. Possible sequelae include neuroleptic malignant syndrome and acute renal failure. Adverse reactions following therapeutic use include sedation, dysphoria, anorexia, nausea, vomiting, constipation, diarrhea, and dyspepsia.

Chronic Toxicity (or Exposure)

Animal

Rats chronically treated with haloperidol (1.5 mg kg^{-1} ip) significantly developed vacuous chewing movements and tongue protrusions.

Human

Chronic poisoning by ingestion may induce neurological syndromes, the most severe of which are parkinsonism, akathisia, and tardive dyskinesia, a syndrome which is characterized by rhythmical, involuntary movements of the tongue, face, mouth, or jaw (e.g., protrusion of tongue, puffing of cheeks, puckering of mouth, chewing movements). Sometimes these may be accompanied by involuntary movements of extremities.

Clinical Management

All basic and advanced life-support measures should be implemented. Gastric decontamination should be performed. Butyrophenones are readily absorbed by activated charcoal. Aggressive supportive care should be instituted. Dystonic reactions respond well to intravenous benztropine or diphenhydramine. Oral therapy with diphenhydramine or benztropine should be continued for 2 days to prevent recurrence of the dystonic reaction. For patients suffering from neuroleptic malignant syndrome, a potentially fatal condition associated with the administration of antipsychotic drugs, dantrolene sodium, and bromocriptine have been used in conjunction with cooling and other supportive measures. Arrhythmias should be treated with lidocaine or phenytoin. Diazepam is the drug of choice for seizures; phenytoin is used to prevent recurrence. Hemodialysis and hemoperfusion have not been shown to be effective.

Environmental Fate

If released to air, haloperidol will exist primarily in the particulate phase; physical removal from air will occur through wet and dry deposition processes.

See also: Anxiolytics; Neurotoxicity

Further Reading

Richards JR and Schneir AB (2003) Droperidol in the emergency department: Is it safe? *The Journal of Emergency Medicine* 24(4): 441–447.

BZ

Harry Salem*

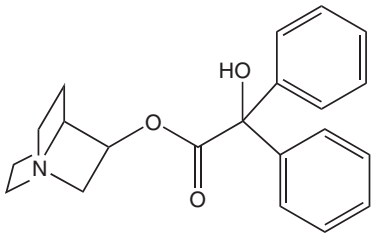
Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 6581-06-2
- Synonyms: TNB; α-Hydroxy-α-phenylbenzeneacetic acid; 1-Azabicyclo[2,2,2]octan-3-yl ester; Agent 15; 3-QNB; QNB; Agent buzz
- Description: BZ is a glycolate anticholinergic chemical related to atropine, scopolamine, and hyoscyamine. It is odorless, nonirritating, and is stable in most solvents. It has a half-life of 3–4 weeks in moist air and is extremely persistent in soil and water as well as on most surfaces. Agent 15, believed to have been stockpiled in Iraq, is speculated either to be identical to BZ or a closely

*The views of the author do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

related derivative, and has similar physicochemical properties as BZ. BZ has a slow onset and a long duration of action

- Chemical/Pharmaceutical/Other Class: Potent anticholinergic psychomimetic that produces incapacitation and is considered a hallucinogenic chemical warfare agent
- Chemical Formula: $C_{21}H_{23}NO_3$
- Chemical Structure:



Uses

BZ is considered an incapacitating chemical warfare agent. It is a nonlethal glycolate anticholinergic psychomimetic that produces incapacitation and is hallucinogenic.

Exposure Routes and Pathways

Exposure is through inhalation of aerosolized solid or BZ dissolved in a solvent such as propylene glycol, dimethyl sulfoxide, and other solvents. Exposure can also occur through the skin and via the gastrointestinal tract.

Toxicokinetics

BZ is a competitive inhibitor of muscarinic receptors associated with the parasympathetic nervous system that innervate the eyes, heart, respiratory system, skin, gastrointestinal tract, and bladder. The sweat glands, innervated by the sympathetic nervous system, are also modulated by muscarinic receptors. By any route of exposure, the onset of action is approximately 1 h, with peak effects occurring 8 h postexposure. Signs and symptoms gradually subside over 2–4 days. Most of the absorbed BZ is excreted via the kidney.

Mechanism of Toxicity

BZ acts by blocking the action of acetylcholine on the central and peripheral nervous systems. It is a tertiary amine and crosses the blood–brain barrier. BZ on acute exposure increases both heart and respiratory rates, dilates the pupils, and causes paralysis of the eye muscles necessary for near focusing. It also causes dry mouth and skin, elevates body temperature, impairs coordination, and causes flushing of the skin, hallucinations, stupor, forgetfulness, and confusion. Within 15 min to 4 h following exposure, the principal effects are dizziness, involuntary muscle movements, near vision difficulty, and total incapacitation. From 6 to 10 h after exposure, the effects are psychotropic and full recovery is expected after 4 days.

The peripheral nervous system effects are considered as under-stimulation of the end organs. This decreased stimulation of eccrine and apocrine sweat glands in the skin results in dry skin and a dry mouth, and is considered 'dry as a bone'. The reduction in the ability to dispel heat by evaporative cooling decreases sweating, and the compensatory cutaneous vasodilation causes the skin to become warm or 'hot as a hare' and 'red as a beet'. This is similar to the atropine flush. The decreased heat loss also results in an increased core temperature.

The peripheral effects described above usually precede the central nervous system effects and have been summarized by the mnemonic 'dry as a bone, hot as hares, red as a beet, and blind as a bat'.

The central nervous system effects of BZ and agent 15 result in dose-dependent 'mad as a hatter' mental changes. These effects fluctuate between a conscious state and delirium that ranges from drowsiness to coma. Disorientation, decreased social restraints, inappropriate behavior, and decreased short-term memory are common. Speech becomes slurred and indistinct.

The human estimated incapacitation ICt_{50} is reported to be 100 mg min m^{-3} and the LCt_{50} to be 200 000 mg min m^{-3}.

Acute and Short-Term Toxicity (or Exposure)

Animal

Species	*LCt_{50} (mg min m^{-3})*	*IV LD_{50} (mg kg^{-1})*
Mouse	12 000	14.1
Rats	64 000	14.0
Guinea pig	123 000	10.0
Rabbits	32 000	10.0
Dogs	25 000	9.6

See also: Atropine; Chemical Warfare Agents.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

C

Cadmium

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst, Shirley B Radding, and Kathryn A Wurzel, volume 1, pp. 207–209, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-43-9
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Cd^{2+}

Uses

Cadmium is primarily used for electroplating and galvanizing other metals because it is relatively resistant to corrosion. It is also used in electrical contacts, in soldering alloys, in nickel–cadmium storage batteries, in television phosphors, and as a stabilizer for polyvinyl chloride. Given its brilliant orange color, it has been used extensively as a pigment in paints, plasters, and plastics. Cadmium is also a by-product of zinc, lead, and copper mining and smelting.

Exposure Routes and Pathways

Due to the wide use of cadmium-based products, cadmium is widely distributed in the environment. The cadmium content in soil and water has been increasing as a result of disposal of cadmium-contaminated waste and the use of cadmium-containing fertilizers (particularly on cereal crops). Commercial sludge, contaminated with cadmium, has been used to fertilize agricultural fields. Cadmium concentrations in urban air are quite low, because of regulation of industrial air emissions. Lead and zinc smelters and waste incineration account for the majority of cadmium present in ambient air.

Ingestion and inhalation are the primary routes of exposure to cadmium. Dermal contact is not a significant route of exposure. Exposure to cadmium via foodstuffs is common since plants and animals accumulate cadmium from soil or water, especially fish and crustaceans. Cigarette smoke is a major source of cadmium exposure via inhalation.

Toxicokinetics

Absorption of cadmium in the gastrointestinal tract is ~4–7% in adults; absorption is probably higher in children. Diets low in calcium, iron, and protein enhance cadmium absorption. Zinc is an antagonist to cadmium (decreases cadmium absorption). Cadmium absorption by the lungs is dependent on particle size and the solubility of the cadmium compound, but is generally between 15% and 30%. Dermal absorption of cadmium is insignificant.

Cadmium is a classic cumulative poison that accumulates in the kidneys over a lifetime. It is transported in the blood by erythrocytes and by albumin, and it is stored mainly in the liver and kidneys as metallothionein (50–75% of the body burden). Cadmium binds to many proteins at the sulfate and carbonyl sites. The half-life of cadmium in these two organs may be as long as 30 years. The correlation between years of exposure and blood levels does not appear to be significant. Cadmium also accumulates in the bones and the placenta of pregnant women.

Urine is the most important excretion mechanism in humans. Urine concentration of cadmium increases with age and following kidney damage. Cadmium found on examination of hair is generally due to external contamination rather than internal absorption and distribution to the hair.

Mechanism of Toxicity

Cadmium inhibits plasma membrane calcium channels and Ca^{2+}-ATPases. It also inhibits repair of DNA damaged by various chemicals, an effect which is believed to be associated with the induction of tumors. Although cadmium forms a metallothionein, the preformed cadmium metallothionein is nephrotoxic (toxic to the kidneys); it is suggested that effects occur when, at some stage in the kidney, the cadmium is dissociated from the metallothionein. In *Itai-Itai* disease (see 'Chronic Toxicity, Human' section), patients were found to have chromosome abnormalities.

Cadmium has an affinity for sulfhydryl groups and hence, can inhibit enzymes; however, cells treated

with cadmium showed proliferation of peroxisomes, which contain catalase, an enzyme. It appears that cadmium at first inhibits catalase activity and then, after a time, enhances that activity. In addition, cadmium inhibits enzymes involved in gluconeogenesis (the generation of glycogen for energy production from noncarbohydrate precursors). It also inhibits oxidative phosphorylation (energy production) and depresses trypsin inhibitor capacity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cardiac effects (electrical and biochemical changes in the myocardium) were observed in rats exposed to cadmium in drinking water.

Human

Acute toxicity may result from ingestion of relatively high concentrations of cadmium from contaminated food or beverages (e.g., $16\,mg\,l^{-1}$ cadmium in a beverage). Cadmium exhibits local irritant effects on the gastrointestinal tract such as nausea, vomiting, diarrhea, abdominal pain, and a choking sensation. The effects of acute toxicity are apparent immediately.

Inhalation of cadmium fumes produces local irritant effects and may result in chemical pneumonitis and pulmonary edema, possibly resulting in death.

Chronic Toxicity (or Exposure)

Animal

Animal studies have shown cadmium to be a teratogen and a reproductive toxin; however, the results of mutagenesis experiments are equivocal. Cadmium produced local sarcomas in a number of rodent species when the metal, sulfide, oxide, or salts were administered subcutaneously. Intramuscular injection of cadmium powder and cadmium sulfate also produced local sarcomas. Injection of cadmium chloride into the ventral prostate resulted in a low incidence of prostatic carcinoma. Exposure via inhalation of cadmium chloride produced a dose-dependent increase in lung carcinomas in rats.

Human

Chronic exposure to cadmium from any route will have adverse effects on the heart, lungs, bones, gonads, and especially, the kidneys. The principal long-term effects of low-level cadmium exposure are generally chronic obstructive pulmonary disease, emphysema, and chronic renal tubular disease. Cardiovascular and skeletal effects are also possible. The initial symptoms of chronic inhalation exposure are those associated with metal fume fever (e.g., fever, headache, chest pain, sore throat, coughing, and rhinitis). Metal fume fever is most often associated with inhalation of zinc oxide but may occur following exposure to other metals such as cadmium. Although inconclusive, there is evidence that the cadmium burden in the body can lead to hypertension.

Since cadmium can displace zinc, cadmium accumulation in the testes can suppress testicular function. Evidence obtained in the past several years appears to relate cadmium to prostate cancer in young men who work with cadmium. Additional investigation (such as epidemiological studies with a larger cohort) needs to be performed to investigate this apparent association of cadmium with prostate cancer.

Skeletal changes due to cadmium accumulation are probably related to calcium loss, which can be influenced by diet and hormonal status. These skeletal changes include osteomalacia (softening of bone resulting from loss of minerals) and pseudofractures. In Japan, people who ate fish contaminated with cadmium experienced skeletal changes, especially in their backs. This very painful effect was called the '*Itai-Itai*' ('ouch-ouch') disease. Postmenopausal women with low calcium and vitamin D intake were apparently most susceptible.

Since the kidneys are the main depot for cadmium, they are of greatest concern for cadmium toxicity. Cadmium interferes with the proximal tubule's reabsorption function. This leads to abnormal actions of uric acid, calcium, and phosphorus. Amino aciduria (amino acids in the urine) and glucosuria (glucose in the urine) result; in later stages, proteinuria (protein in the urine) results. When this happens, it is assumed that there is a marked decrease in glomerular filtration. Long-term exposure to cadmium leads to anemia, which may result from cadmium interfering with iron absorption.

Cadmium metallothionein has also been studied extensively. This metalloprotein is high in the amino acid cysteine ($\sim 30\%$) and is devoid of aromatic amino acids. Metallothionein itself may function to help detoxify cadmium. For some experimental tumors, cadmium appears to be anticarcinogenic (e.g., it reduces the induction of tumors).

Clinical Management

For treatment of oral poisoning, administration of syrup of ipecac is indicated, followed by gastric

lavage. The chelating agent calcium EDTA (calcium disodium salt of ethylenediaminetetraacetic acid) is indicated for acute exposure if administered shortly after cadmium exposure before new metallothionein is synthesized. BAL (British antilewisite; 2,3-dimercaptopropanol) is contraindicated as it may enhance kidney toxicity. Newer dimercapto compounds dimercaptosuccinic acid (DMSA) and dimercaptopropane sulfonate (DMPS) are being evaluated as are derivatives of dithiocarbamates. Delayed pulmonary edema may result from inhaled cadmium dusts; therefore, supportive measures are indicated.

The apparent affinity for zinc metallothionein may someday be found to be useful as an antidote for cadmium toxicity. Antagonists to cadmium toxicity include a pretreatment with selenium and zinc. It is believed that this pretreatment allows cadmium to displace zinc in the zinc metallothionein.

Environmental Fate

As indicated in the Exposure section, cadmium is widely distributed in the environment from a variety of natural and anthropogenic sources. Cadmium emitted into the air is often found bound to small particulates and can travel with these particulates over long distances. As a result, cadmium can remain in the atmosphere for long periods of time until it is deposited by gravitational settling or in rain and snow. Cadmium tends to be more mobile in water than other heavy metals although it will complex with humic substances and can precipitate out under certain conditions. Cadmium can bioaccumulate in aquatic organisms; the degree of accumulation is associated with the pH and humic content of the water. It can also bioaccumulate in plants and in the animals that feed on these plants; for example, cattle and wildlife. However, terrestrial bioaccumulation is much lower than that in water and cadmium concentrations at the top of the terrestrial food chain are not much higher than those at the lower end of the chain.

Exposure Standards and Guidelines

American Conference of Governmental Industrial Hygienists (ACGIH) lists cadmium as a suspected human carcinogen. The ACGIH threshold limit value – time-weighted average (TLV – TWA) is $0.01\,mg\,m^{-3}$ for elemental cadmium and inorganic compounds as total dust/particulate. The ACGIH TLV – TWA for the respirable fraction of cadmium particulate is $0.002\,mg\,m^{-3}$.

See also: Cardiovascular System; Kidney; Metallothionein; Metals; Pollution, Air; Pollution, Soil; Pollution, Water; Respiratory Tract; Sensory Organs.

Further Reading

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Relevant Website

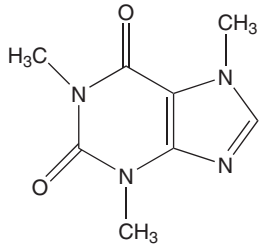
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Cadmium.

Caffeine

Christopher P Holstege

This article is a revision of the previous print edition article by Michael J Hodgman, volume 1, pp. 209–211, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 58-08-2
- Synonyms: 1,3,7-Trimethylxanthine; Guaranine; Methyltheobromine; Thein
- Chemical/Pharmaceutical/Other Class: Methylxanthine
- Chemical Structure:



Uses

Caffeine is used as a central nervous system (CNS) stimulant, anorexiant, diuretic, and in a number of analgesic and cold medication compounds. It is also used in the treatment of spinal headaches and has been used as a respiratory stimulant in preterm infants.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Caffeine is consumed in wide variety of beverages, such as coffee, tea, and soda. It is found alone or in combination with other pharmaceutical products. It is also available for injection.

Toxicokinetics

Caffeine is rapidly absorbed after an oral dose, with peak levels reached within 1–2 h at therapeutic doses. Onset of clinical effects occurs within 60 min. In adults, caffeine is extensively metabolized by the liver primarily by *N*-demethylation. It is excreted in the urine primarily as 1-methyluric acid and 1-methylxanthine. Theophylline (1,3-dimethylxanthine) is a minor product of caffeine metabolism in adults ($<1\%$). After massive caffeine overdoses, serum levels of theophylline are measurable. The elimination half-life of caffeine is 3–6 h at therapeutic doses. The half-life is shorter in smokers and is prolonged by oral contraceptives, cimetidine, late pregnancy, and in overdose. The half-life of caffeine is much longer in infants and does not approximate that seen in adults until 6 months of age. The half-life of caffeine may exceed 100 h in preterm infants. Only 1–10% of caffeine appears unchanged in the urine in adults. Neonates may excrete up to 85% of caffeine unchanged.

Mechanism of Toxicity

Caffeine can have profound effects on the cardiovascular system. At least four mechanisms have been proposed for the pro-arrhythmic potential of caffeine in overdose. First, caffeine increases circulating catecholamines. Second, caffeine inhibits phosphodiesterase. Increased circulating catecholamines after caffeine overdose increase β1-receptor stimulation. Stimulation of β1-receptors increases intracellular cAMP by G protein stimulation of adenylate cyclase. The activity of cAMP is prolonged due to its decreased metabolism as phosphodiesterase is inhibited by caffeine. Subsequently, β1-receptor effects are exaggerated and tachydysrhythmias are induced. Third, caffeine increases myocardial intracellular calcium. Caffeine both induces release of calcium from the sarcoplasmic reticulum and blocks calcium's reuptake into the sarcoplasmic reticulum. This resulting increase in cytosolic calcium may provoke dysrhythmias. Fourth, caffeine blocks cardiac adensosine receptors, which have been shown to be antiarrhythmic.

The hypotension that has been noted with overdoses of caffeine is primarily due to two mechanisms. First, caffeine-induced tachydysrhythmias lead to inadequate filling of the heart and subsequent decrease in cardiac output. Second, caffeine augments β2-effects and causes subsequent vasodilation with resulting hypotension.

Caffeine in overdose also acts as a nonselective antagonist of neuronal adenosine receptors that may lead to seizures. Caffeine is also a mild diuretic and it stimulates gastric acid secretion, respiration, and lipolysis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity in animals is similar to that found in humans. Dehydration and hyperthermia may occur.

Human

Acute toxicity manifests primarily in the CNS, cardiovascular system, and gastrointestinal system. CNS signs include restlessness, tremor, nervousness, headache, insomnia, tinnitus, confusion, delirium, psychosis, and seizures. Cardiac manifestations of overdose include sinus tachycardia, various dysrhythmias, asystole, and cardiovascular collapse. Other findings include tachypnea, nausea, vomiting, hematemesis, diarrhea, and fever. Case reports also include rhabdomyolysis and pulmonary edema. Laboratory findings include metabolic acidosis, respiratory alkalosis, ketosis, hypokalemia, and hyperglycemia. The estimated lethal dose in adults is 150–200 mg kg^{-1}, whereas doses of 10–15 mg kg^{-1} may produce early signs of toxicity. Serum levels greater than 30 mg ml^{-1} have been associated with adverse symptoms. Levels exceeding 80 mg ml^{-1} have been associated with death, although levels as high as 405 mg ml^{-1} have been reported in survivors.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies in rats do not seem to produce increased levels of anxiety compared with

controls. When added to the diets of overweight, diabetic rats, caffeine produced slight increases in heart rate and blood pressure, but more profound changes in the kidney. Proteinuria increased dramatically and creatinine clearance was reduced compared to matched controls.

Human

No definite association has been demonstrated between habitual caffeine use and hypertension, myocardial infarction, carcinogenicity, or teratogenicity. Abrupt cessation of chronic caffeine ingestion may cause withdrawal headaches.

In Vitro Toxicity Data

Caffeine has been found to be weakly mutagenic in some nonmammalian animal models. It is not been found to be mutagenic in Ames *Salmonella* assays.

Clinical Management

After assessment of airway, breathing, and circulation with necessary supportive care, decontamination of the gastrointestinal tract should be undertaken for substantial recent ingestions. The patient should be placed on continuous cardiac monitoring with pulse oximetry. The initial treatment of hypotension consists of intravenous fluids. If hypotension persists, then pressors may be considered. Frequent neurological checks should be made. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered, but vomiting may make retention difficult. Beta-blocking agents have been used to treat caffeine tachydysrhythmias; however, one report described cardiovascular collapse following β-blocker administration. Standard therapy for seizures should be employed. Monitoring should be performed for fluid and electrolyte imbalances.

Various techniques to enhance elimination of caffeine have been reported in the literature. Multidose activated charcoal has been advocated to both prevent further absorption of drug and enhance elimination by gut dialysis. Hemodialysis has been reported in the literature for the treatment of caffeine toxicity. The mean plasma protein binding of caffeine (36%), the molecular size (194), and the volume of distribution ($0.6–0.8\,l\,kg^{-1}$) make hemodialysis a possible modality to enhance elimination. There have also been cases of severe caffeine toxicity treated with peritoneal dialysis, but this modality is less efficient at drug clearance than hemodialysis.

See also: Catecholamines; Theophylline.

Further Reading

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Calcium Channel Blockers

Shayne C Gad

- Representative Compounds: Amlodipine; Bepridil; Diltiazem; Felodipine; Isradipine; Nicardipine; Nifedipine; Nimodipine; Verapamil
- Chemical Abstracts Service Registry Numbers: Norvasc (CAS 88150-42-9); Vascor (CAS 74764-40-2); Cardizem (CAS 42399-417); Plendil (CAS 72509-76-3); DynaCirc (CAS 75695-93-1); Cardene (CAS 55985-32-5); Procardia (CAS 21829-25-4); Nimotop (CAS 66085-59-4); Isoptin (CAS 15211-4)
- Chemical/Pharmaceutical/Other Class: Antiarythmics

Uses

Calcium channel blockers are used in the management of angina pectoris, hypertension, supraventricular arrhythmias, and subarachnoid hemorrhage.

Exposure Routes and Pathways

Ingestion is the most common route for both accidental and intentional exposures. Verapamil and diltiazem

are both available for parenteral administration, and toxicity can occur via the parenteral route.

Toxicokinetics

Following oral administration, absorption is rapid and almost complete (80–100%), but the ultimate bioavailability is limited and variable (15–94%) following oral administration due to significant first-pass metabolism in the liver. Protein binding is high and ranges from 70% to 99%. Volumes of distribution for some calcium channel blockers are as follows: verapamil, 5 l kg^{-1}; diltiazem, 3.1 l kg^{-1}; nifedipine, 0.78 l kg^{-1}; and nicardipine, 1.1 l kg^{-1}. Extensive hepatic metabolism occurs. Only small amounts (0–10%) are excreted unchanged in the urine. Elimination half-life ranges from 1 h (nimodipine) to 50 h (amlodipine).

Mechanism of Toxicity

The pharmacologic and toxicologic mechanisms of the calcium channel blockers are complex. They include interference with electrical conduction through the atrioventricular node, decreased myocardial contractility, and direct vasodilation. Calcium channel blockers also interfere with pancreatic release of insulin.

The interference with electrical conduction through the atrioventricular node is caused by interference with the influx of calcium in phase II of the action potential and manifest by bradycardia, lengthening of the PR interval, QRS widening, and QTc prolongation.

Decreased myocardial contractility is due to calcium influx into the cell, which results in increased release of calcium from the sarcoplasmic reticulum. The overall effect of this calcium influx and release is the bridging of actin and myosin and subsequent myocardial contraction. The negative inotropic effect of the calcium blockers is due to interference with this process.

Vasoconstriction occurs when calcium activates vascular myosin kinase, which in turn allows for phosphorylation of myosin and subsequent bridging with actin. Administration of calcium channel blockers will interfere with this process and produce vasodilation.

Acute and Short-Term Toxicity (or Exposure)

Human

The clinical effects of the calcium channel blockers are primarily cardiovascular in nature. Due to their interference with conduction, they can cause a variety of dysrrhythmias including sinus bradycardia, all degrees of atrioventricular block, junctional rhythms, pulseless electrical activity, and asystole. The negative inotropic effects of the calcium channel blockers cause significant decreases in cardiac output. Profound hypotension is observed following calcium channel blocker poisoning due to their vasodilatory properties. Renal failure secondary to decreased perfusion may be seen. The neurologic toxicities of the calcium channel blockers are most likely secondary to their cardiovascular effects. The most common neurologic effects are lethargy and coma. Neurologic deterioration can be rapid. Some patients with significant hypotension may have intact neurologic examinations initially. Seizure activity has also been observed in calcium channel blocker toxicity. The most common metabolic effects that occur in calcium channel blocker toxicity are metabolic acidosis, hyperglycemia, and hypokalemia. Hyperkalemia has also been reported.

Clinical Management

Advanced supportive care is a primary component of patient management. Emergent intubation and assisted ventilation are often necessary in these patients. Pulse oximetry should be utilized to assess respiratory status. Extensive cardiovascular monitoring is also necessary. Arterial blood gases, serum electrolytes, and glucose measurements should be obtained. Serum concentrations of specific calcium channel blockers are difficult to obtain and have limited clinical utility. Syrup of ipecac-induced emesis is contraindicated due to the rapid decreases in level of consciousness that may occur as well as emesis-induced vasovagal effects. Gastric lavage and activated charcoal can be used if warranted by the history of the ingestion and the patient's neurologic status. Whole bowel irrigation along with activated charcoal should be utilized in ingestions involving sustained-release products. Calcium salts are often administered as antidotes for calcium channel blocker toxicity although they have been used with limited success. Calcium chloride is preferred over calcium gluconate since it contains more elemental calcium on a milligram-per-milligram basis. Doses of up to 4 g of calcium have been recommended in this setting. Glucagon, which has been used in β-adrenergic blocker toxicity, has been recommended in calcium blocker toxicity. This agent has positive inotropic properties due to activation of cyclic adenosine monophosphate. It has limited beneficial effects in calcium channel blocker toxicity. Control of heart rate and rhythm present a significant challenge in this patient population. Transcutaneous pacemakers should be utilized to stabilize rate and enhance

atrioventricular conduction. A vagolytic, atropine, has also been used to increase heart rate. It has limited effect since it primarily affects the sinoatrial node. The negative inotropic effects of these agents must also be treated aggressively. Positive inotropic agents, such as dopamine, dobutamine, amrinone, and isoproterenol, can be utilized to increase contractility. Isoproterenol should be used with caution due to its vasodilatory properties. Vasopressors, such as dopamine, epinephrine, and norepinephrine, may be effective. Cardiopulmonary bypass has been used experimentally to treat patients with calcium channel blocker toxicity who do not respond to traditional therapy. Sodium bicarbonate should be administered to treat acidosis.

Seizure activity should be initially treated with benzodiazepines. If benzodiazepines are not effective, phenytoin and barbiturates can be administered. Insulin replacement may be necessary to correct hyperglycemia.

See also: Cardiovascular System.

Further Reading

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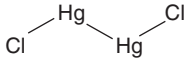
Eisenberg MJ, *et al.* (2004) Calcium channel blockers: An update. *American Journal of Medicine* 116(1): 35–43.

Calomel

Kashyap N Thakore

- Chemical Abstracts Service Registry Number: CAS 7546-30-7
- Synonyms: Mercurous chloride; Mercury(I) chloride; Mercury monochloride
- Chemical/Pharmaceutical/Other Class: Heavy metals
- Chemical Formula: Hg_2Cl_2
- Chemical Structure:



Uses

Calomel is used as a laboratory reagent, as a fungicide, and as a depolarizer in dry batteries.

Exposure Routes and Pathways

The primary routes of entry are ocular and dermal contact, inhalation, and ingestion. Calomel is found in environmental and occupational settings, such as in mercury mining operations, battery plants, paints and dyes, photography, perfumes and cosmetics, and chemical laboratories. It is poisonous by ingestion through food and intraperitoneal routes. Calomel is moderately toxic by skin contact. When heated to decompose, it emits very toxic fumes of Cl^- and Hg.

Toxicokinetics

After inhalation, ~70–80% of metallic vapor is retained and absorbed. Little is taken up in the gastrointestinal tract, and less than 10% is absorbed. In the body, it is oxidized to mercuric mercury, which binds to reduced sulfhydryl groups. The kidney is the main depository following exposure to both metallic and mercuric mercury. In addition to other organs, it passes into the brain and fetus.

The metabolite is eliminated mainly in urine and feces; it is also excreted in milk. In humans, inorganic mercury compounds have two elimination half-lives: one lasts for days or weeks and the other much longer.

Mechanism of Toxicity

Calomel can generate reactive oxygen species and deplete glutathione levels. Both genotoxic and nongenotoxic mechanisms may contribute to renal carcinogenic effect of mercury.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, intense exposure causes lung damage, intestinal and renal tubular necrosis, immunosuppression, and possible cytogenetic effects. The oral LD_{50} is $210\,mg\,kg^{-1}$ in rats and $180\,mg\,kg^{-1}$ in mice. The intraperitoneal LD_{50} is $10\,mg\,kg^{-1}$ in mice.

Human

Calomel is harmful and may be fatal, if swallowed or inhaled. When swallowed, it causes central nervous

system depression; when inhaled, it causes tightness and pain in the chest, coughing, and breathing difficulties. Ocular and dermal exposures cause irritation of the eyes and skin. In cases of chronic exposure, mercury builds up in the brain, liver, and kidneys and causes headache, shakes, loose teeth, loss of appetite, skin ulceration, and impaired memory. Mercury concentration in urine, blood, and plasma is useful for biological monitoring.

The recommended health-based limits are $0.05\,mg\,m^{-3}$ for occupational exposure, $50\,\mu g\,g^{-1}$ creatinine in urine for long-term occupational exposure to mercury vapors, and $1\,\mu g\,l^{-1}$ for exposure by drinking water (WHO report, 1980).

Chronic Toxicity (or Exposure)

Animal

There is limited evidence for carcinogenicity. Calomel causes renal adenoma and adenocarcinoma in male mice and female rats.

Human

There is inadequate evidence for carcinogenicity.

Clinical Management

In case of contact, eyes and skin should be flushed with water for 15–20 min. If inhaled, the victim should be removed to fresh air. If necessary, oxygen and artificial respiration should be administered. If the patient is in cardiac arrest, cardiopulmonary resuscitation should be provided. These life-supporting measures should be continued until medical assistance has arrived. An unconscious or convulsing person should not be given liquids or induced to vomit.

Other Hazards

It is not flammable.

See also: Mercury; Metals.

Further Reading

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US Environmental Protection Agency (US EPA) (1997) Mercury Study Report to Congress: Health Effects of Mercury and Mercury Compounds, vol. V.

Relevant Websites

http://www.iarc.fr – International Agency for Research on Cancer.

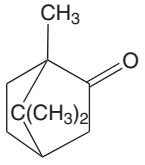
http://www.epa.gov – US Environmental Protection Agency.

Camphor

Fermin Barrueto Jr.

This article is a revision of the previous print edition article by Bonnie S Dean, volume 1, pp. 213–214, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: CAS 464-48-2 and CAS 464-49-3 (optical isomers); CAS 21368-68-3 (racemic mixture)
- Synonyms: Campho-phenique; Musterole; Ben-Gay children's vaporizing rub; Vicks Vaporub; Vicks Vaposteam; Heet; Sloan's Liniment; Camphorated oil; Camphor spirits
- Chemical/Pharmaceutical/Other Class: Cyclic ketone of the hydroaromatic terpene group
- Chemical Formula: $C_{10}H_{16}O$
- Chemical Structure:



Uses

Camphor is employed externally as a rubefacient, mild analgesic, antipruritic, and counterirritant in commercially available products that contain 1.0–10% camphor. It is currently produced synthetically. It has a characteristic odor and a pungent aromatic taste.

Exposure Routes and Pathways

Ingestion is the most common route of both intentional and unintentional exposure to camphor.

Ocular exposures may also occur as can transdermal exposure.

Toxicokinetics

Camphor in liquid form is rapidly absorbed through the skin, mucous membranes, and gastrointestinal tract. Symptoms may appear within 5–90 min following ingestion. The absorption is highly dependent on the presence of food and other chemicals that may influence the rate of camphor absorption. Camphor is metabolized to a campherol, which is conjugated with glucuronic acid in the liver. It is unclear whether camphor toxicity is attributed to the parent compound, a metabolite, or both. Camphor-related metabolites are fat soluble. Thus, significant concentrations may accumulate in fat tissue. Camphor is distributed widely in all tissues. Measurable serum levels are apparent within minutes after ingestion of $\sim$0.5–1.0 g. The volume of distribution is $\sim$2–4 l kg^{-1}. The glucuronide form is excreted in the urine. The half-life of a 200 mg dose is known to be 167 mm.

Mechanism of Toxicity

Its action has been postulated to be intraneuronal on the oxidation cycle at a phase above the cytochrome *b* level of the cytochrome oxidase system though its precise mechanism has not been elucidated. It is primarily a neurotoxin with a chemical structure that allows for easy penetration of the blood–brain barrier.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal toxicity corresponds to human toxicity.

Human

Upon ingestion, an initial burning sensation may be noted in the mouth and throat. Spontaneous nausea and vomiting may occur within minutes of ingestion. Confusion, vertigo, restlessness, delirium, hallucinations, tremors, and convulsions are all directly related to the central nervous system involvement and may be predictors of serious toxicity. More severe intoxications may result in hepatic failure. Death may be caused by respiratory depression or may follow status epilepticus. Camphor should be considered an eye irritant.

Chronic Toxicity (or Exposure)

Animal

Chronic camphor dosing in a mouse model has lead to development of neuronal necrosis.

Human

The chronic ingestion of camphor may produce similar toxicity but in a more insidious fashion. Liver failure is a more pronounced clinical manifestation.

In Vitro Toxicity Data

Camphor inhibited catecholamine secretion from bovine adrenal chromaffin cells.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be used for substantial recent ingestions. Activated charcoal is marginally effective in adsorbing camphor. Oils, alcohols, and other lipophilic substances enhance intestinal absorption and are contraindicated. Ocular exposures necessitate flushing with a gentle system of tepid water for a minimum of 15 min. If signs of irritation persist, an ophthalmology consultation is required. The seizure activity is often singular and self-limiting and responsive to benzodiazepines.

See also: Benzodiazepines; Catecholamines; Charcoal.

Further Reading

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Smith AG and Margolis G (1954) Camphor poisoning: Anatomical and pharmacologic study; report of a fatal case; experimental investigation of protective action of barbiturate. *American Journal of Pathology* 30: 857–868.

Cancer *See* Carcinogenesis.

Cancer Chemotherapeutic Agents

David S Fischer

Cancer is a general term used to describe 100 or more malignant neoplasms that invade other tissues and may metastasize to distant sites and then grow there. The defining characteristic of the cancer cell is uncontrolled proliferation and multiple genetic alterations. A tumor is a circumscribed noninflammatory growth arising from existing tissue but growing independently of the normal rate or structural development of such tissue and serving no physiological function. It may be malignant or benign. The benign tumor does not invade or metastasize.

A chemotherapy drug is a chemical agent used to treat diseases. The term may be applied to a drug used to treat infection, but more frequently is used to refer to drugs used to treat cancer. The term, cancer chemotherapy, is used by some to include biological agents that are used to treat cancer while others prefer to use the more specific terms biotherapy, cancer biotherapy, or biologic therapy of cancer.

Historical Development

For several centuries, the only useful treatment for tumors was surgical removal. With the development of cellular and tissue pathology in the mid-nineteenth century, malignant tumors could be identified without demonstrating distant metastases, and malignancies of the blood were identified and called leukemia. In 1865, Lissauer, a German physician, used potassium arsenite (Fowler's solution) by chance and found that it restored to health two near moribund patients with chronic myeloid leukemia. This was the first chemical agent effective in the treatment of a malignant disease and it continued to be used for 70 years. Recently, arsenic trioxide has been used as an effective drug for treating acute promyelocytic leukemia (APL).

After Roentgen discovered X-rays in 1895, they were used for many medical purposes and were particularly effective in shrinking Hodgkin's disease tumors and the enlarged spleens of chronic leukemias with a resultant drop in their high white cell counts, results similar to those produced by potassium arsenite. Paul Ehrlich used organic arsenicals in his search for a 'magic bullet' to cure syphilis. Other investigators were frustrated by their inability to find effective agents to treat cancers because they did not understand the biology of cancer and the search was largely abandoned for many decades.

Modern Chemotherapy

The development of effective antibacterial agents, for example, sulfanilamide and penicillin in the 1930s, aroused interest in chemical and biological agents in the treatment of cancer. During World War II, a number of investigators studied the effects of chemical warfare agents that might be used by adversaries. Nitrogen mustard, then known by the wartime code name HN2, was extensively studied in the laboratory and in mice and rabbits before the first near moribund patient with lymphoma was treated in early December 1942 at the New Haven Hospital affiliated with the Yale School of Medicine.

The treatment resulted in a dramatic regression of disease and the era of cancer chemotherapy began. Several books relate the story that the use of nitrogen mustard as a chemotherapeutic agent was suggested by the serendipitous finding of marrow and lymphoid hypoplasia in seamen exposed to mustard gas following the sinking of a ship in Bari Harbor, Italy, containing chemical warfare agents. That event is well documented but it occurred on December 2, 1943, one year after the Yale human trials. This is an interesting story but there is no direct connection.

Nitrogen mustard will hereafter be referred to by its generic name, mechlorethamine, and generic names will be used for all drugs. Trade names for some are listed in Appendix 1.

As a therapeutic agent, mechlorethamine has many toxic effects. Acutely, it causes nausea and vomiting, skin blistering, and ulceration. After a week or two, it causes leukopenia, lymphopenia, anemia, thrombocytopenia, diarrhea, oral ulcers, and hyperuricemia. It can cause sterility and after a few years, leukemia. The most susceptible tissues are those with renewable cell populations, bone marrow, lymphoid tissues, and gastrointestinal (GI) epithelium. The therapeutic dose of mechlorethamine and most of the cytotoxic chemotherapy drugs is very close to the toxic dose. The therapeutic index (ratio of beneficial effect to toxic effect) is small.

Both the benefits and toxicities of mechlorethamine stimulated a worldwide search for new antineoplastic agents. In the United States, the National Cancer Institute (NCI) had been established in 1937 and was already empirically studying plant extracts for anticancer activity. In 1955, the NCI established the Cancer Chemotherapy National Service Center to systematically screen drugs *in vitro* and *in vivo*. NSC numbers were assigned to each new drug screened and the number now exceeds 720 000. Over the past half century, a growing understanding

of the biology and metabolism of proliferating cells has led to the development of about 100 active anticancer drugs that have been FDA approved and marketed, and many more are in the pipeline. The groups have similar or related toxicities. The mechanism of action that is successful in injuring or eliminating the cancer cell is usually the same mechanism of action that injures or destroys the normal cell leading to the adverse effects that we call toxicity. Of course, drugs in the same group also have some dissimilar and unique toxicities. The goal is to develop drugs that are able to differentially damage or kill neoplastic cells and spare benign cells. Penicillin is effective because it destroys the cell wall of plants. Bacteria are plants and are susceptible to destruction when the plant wall is injured sufficiently. Animals have a cell membrane but no cell wall and therefore penicillin has minimal toxicity in those humans who are not allergic to it. The goal in cancer chemotherapy is to develop similarly targeted precision drugs and a few new drugs appear to fill that role.

Cell Kinetics and the Cell Cycle

The rate of growth of a tumor is a reflection of the proportion of actively dividing cells (the growth fraction), the length of the cell cycle (doubling time), and the rate of cell loss. Acute leukemias, some lymphomas, germ cell tumors, Wilms' tumor, neuroblastoma, and choriocarcinoma are characterized by a rapid growth fraction as demonstrated by tritiated thymidine uptake and turnover studies. Most solid cancers are not characterized by rapid growth. For example, breast, lung, and colon cancer cells may take up to 100 days to double their population. The growth and division of normal and neoplastic cells occur in a sequence of events called the cell cycle. The cell cycle is divided into several different phases (**Figure 1**). Many of the antineoplastic drugs have been and many continue to be classified based on whether their activity is cell cycle specific or nonspecific. Alkylating agents are nonspecific. Antimetabolites, vinca alkaloids, taxanes, podophyllotoxins, and a few others are specific (**Table 1**).

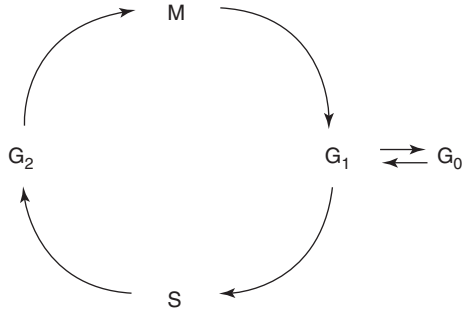

Figure 1 Phases of the cell cycle. G_0, resting phase (nonproliferation of cells): G_1, pre-DNA synthetic phase (12 h to a few days). S, DNA synthesis (usually 2–4 h); G_2, post-DNA synthesis (2–4 h; cells are tetraphoid in this stage); M, mitosis (1–2 h).

Synthesis of ribonucleic acid (RNA) and protein occurs during the G_1 phase. When cells are in G_1 for prolonged periods of time, they are often said to be in a resting phase, referred to as G_0. Synthesis of deoxyribonucleic acid (DNA) occurs during the S phase. During G_2, DNA synthesis halts, and RNA and protein synthesis continue. The final steps of chromosome replication and segregation occur during the mitotic or M phase. The cell undergoes cell division and produces two daughter cells. The rate of RNA and protein synthesis slows during this phase as the genetic material is transferred into the daughter cells. Also located within the cell cycle of normal cells are check points. These are biochemically designated areas that can be activated during the cell cycle process. They prevent the cell from moving forward from one phase to the next if adverse genetic conditions have occurred in the previous phase. Many cancer cells have lost these check points. Drugs that exert their cytotoxic effects during a specific phase of the cell cycle (i.e., phase-specific agents) are usually not effective against cells that are predominantly in a dormant phase (G_0). In contrast, nonphase-specific agents are theoretically more likely to be effective against a tumor population that is not in a state of rapid division. The antineoplastic drugs can best be studied in groups related either to their mechanism of action or by their source of origin (**Table 2**).

Table 1 Cell-cycle-phase-specific drugs

S phase-dependent	*M phase-dependent*
Antimetabolites	Vinca alkaloids[a]
Capecitabine	Vinblastine
Cytarabine	Vincristine
Doxorubicin	Vinorelbine
Fludarabine	Podophyllotoxins
Floxuridine	Etoposide
Gemcitabine	Teniposide
Hydroxyurea	Taxanes
Mercaptopurine	Docetaxel
Methotrexate	Paclitaxel
Pemetrexed	G_2 phase-dependent
	Bleomycin
Procarbazine	Irinotecan
Thioguanine	Mitoxantrone
	Topotecan
	G_1 phase-dependent
	Asparaginase

[a] Have greatest effect in S phase and possibly late G_2 phase; cell blockade or death, however, occurs in early mitosis.

Table 2 Classification of anticancer drugs by mechanism of action or derivation

Alkylating agents
Antimetabolites
Natural products
Hormonal agents
Biotherapeutic agents
Miscellaneous agents

Table 3 Alkylating agents

Nitrogen mustards
Chlorambucil
Cyclophosphamide
Estramustine
Ifosfamide
Mechlorethamine
Melphalan
Aziridine
Thiotepa
Alkyl sulfonate
Busulfan
Nitrosoureas
Carmustine
Lomustine
Streptozocin
Platinum complexes
Carboplatin
Cisplatin
Oxaliplatin
Nonclassical alkylators
Altretamine
Dacarbazine
Procarbazine
Temozolomide

The Alkylating Agents

Alkylating agents are highly reactive compounds that easily attach to DNA and cellular proteins. The primary mode of action for most alkylating drugs is via cross-linking of DNA strands. They can be classified as either monofunctional alkylating agents, implying reactions with only one strand of DNA, or bifunctional alkylating agents, which cross-link two strands of DNA. Replication of DNA and transcription of RNA are prevented by these cross-links.

Many alkylating agents have been developed (**Table 3**). Although these drugs have similar mechanisms of action, there are major differences in spectrum of activity, pharmacokinetic parameters, and toxicity. Alkylating agents play a significant role in the treatment of lymphoma, Hodgkin's disease, breast cancer, multiple myeloma, and other malignancies. In addition to conventional chemotherapy, the linear dose–response curve of alkylating agents expands their role for incorporation into transplant regimens.

The major clinical toxicities of most of the alkylating agents are similar to those of mechloramine, primarily bone marrow depression (including anemia, leukopenia, and thrombocytopenia) and nausea and vomiting. Individual drugs have additional toxicities. Chlorambucil, mechlorethamine, melphalan, and procarbazine can cause gonadal dysfunction and occasionally, late leukemias. Busulfan, carmustine, chlorambucil, and lomustine can cause pulmonary fibrosis. Cyclophosphamide and ifosfamide can cause hemorrhagic cystitis and in a small percent of patients, bladder cancer. Cisplatin, carmustine, lomustine, and streptozocin can cause renal damage. Carboplatin and cisplatin can cause ototoxicity and peripheral neuropathy. Procarbazine is a weak monoamine oxidase inhibitor. It can cause hypertensive reactions if used concurrently with sympathomimetic agents, tricyclic antidepressants, foods with high tyramine content, and with the narcotic meperidine.

Antimetabolites

The interest in antibacterial chemotherapy and its mechanisms of action had direct consequences for antineoplastic drug development. After sulfanilamide was found to be an antimetabolite of paraminobenzoic acid, an essential growth factor for streptococci, the group at Lederle Laboratories synthesized antimetabolites of folic acid – first aminopterin and later amethopterin now known generically as methotrexate. In 1948, Farber and the Harvard Childrens Hospital group used these antimetabolites to palliate acute lymphoblastic leukemia in children. This led to further studies of anticancer drugs based on the biochemistry and metabolism of cancer cells. In 1954, Hitchings and Elion at the Burroughs Wellcome laboratories developed the antipurine drugs, 6-mercaptopurine and 6-thioguanine for leukemias. In 1957, Heidelberger and his group at the McArdle Institute at the University of Wisconsin introduced the first antipurine, 5-fluorouracil for GI tumors. Additional antimetabolites have been developed (**Table 4**).

Antimetabolites interfere with the synthesis of DNA, RNA, and ultimately proteins. They exert their effects largely in the synthetic (S) phase of the cell cycle. Some antimetabolites are structural analogs of normal metabolites essential for cell growth and replication. This property allows some of them to be incorporated into DNA and/or RNA so that a false message is transmitted. Other antimetabolites inhibit enzymes that are necessary for the synthesis of essential compounds. The action and toxicity of the antimetabolites are significantly

Table 4 Antimetabolites

Folate analogs
Methotrexate
Pemetrexed
Trimetrexate
Purine analogs
Cladribine
Fludarabine
Mercaptopurine
Pentostatin
Thioguanine
Pyrimidine analogs
Capecitabine
Cytarabine
Floxuridine
Fluorouracil
Gemcitabine
Ribonucleotide reductase inhibitor
Hydroxyurea

Table 5 Natural products

Camptothecin analogs
Irinotecan
Topotecan
Epipodophyllotoxins
Etoposide
Teniposide
Antitumor antibiotics
Bleomycin
Dactinomycin
Daunorubicin
Doxorubicin
Epirubicin
Idarubicin
Mitomycin
Mitoxantrone
Valrubicin
Microtubule agents
Docetaxel
Paclitaxel
Vinblastine
Vincristine
Vinorelbine
Enzymes
Asparaginase
Pegasparaginase
Metals
Arsenic trioxide
Gallium nitrate

modified by the duration of exposure as well as the dose. Prolonged infusions or prolongation of absorption by pegylation or incorporation into liposomes can change both the response and the toxicity. Since this is a large subject, it will suffice to note here that some of the anticancer antibiotics and biotherapy drugs are also available in pegylated or liposomal forms.

The toxicity of antimetabolites is, as expected, due to their incorporation into the metabolism of normal cells, which is nearly identical to that of the malignant cells that they were designed to injure. The normal cells injured most severely are the rapidly proliferating cells of the bone marrow, the lymphoid system, and the GI epithelium. Thus, the common toxicities are bone marrow depression, nausea and vomiting, diarrhea, and mucositis. Cytarabine and pentostatin can cause conjunctivitis. Capecitabine and prolonged use of fluorouracil or cytarabine can cause cerebellar ataxia and the hand-foot syndrome, that is, palmar-plantar erythrodysesthesia or acral erythema. Pentostatin and high-dose methotrexate can cause renal toxicity.

Natural Products

The natural products may be divided into six primary groups (**Table 5**): campothecin analogs, epipodophyllotoxins, antitumor antibiotics, microtubule agents, enzymes, and metals. The first three act primarily on the topoisomerases. Topoisomerases are enzymes that break and reseal DNA strands. The plant alkaloid campothecin and its analogs (topotecan and irinotecan) are nonclassic enzyme inhibitors of topoisomerase I. These agents are no longer referred to as inhibitors but are instead classified as topoisomerase I targeting agents or topoisomerase I poisons. The epipodophyllotoxins (etoposide and teniposide) and the antitumor antibiotics (dactinomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, and valrubicin) are inhibitors of topoisomerase II. The drugs form a stable complex by binding to DNA and topoisomerase enzymes, resulting in DNA damage that interferes with replication and transcription.

Mitotic Inhibitors

A group of mitotic inhibitors (vinblastine, vincristine, and vinorelbine) exert their cytotoxic effects by binding to tubulin. This inhibits formation of microtubules, causing metaphase arrest. Their mechanism of action and metabolism are similar, but the antitumor spectrum, dose and clinical toxicities of vinblastine, vincristine, and vinorelbine are very different. Paclitaxel and docetaxel are also mitotic inhibitors. However, they differ from the vinca alkaloids by enhancing microtubule formation. As a result, a stable and nonfunctional microtubule is produced.

The major toxicities of these four groups are bone marrow depression, nausea and vomiting, mucositis, and diarrhea. Daunorubicin, doxorubicin, epirubicin, idarubicin, and to a lesser extent, mitoxantrone, cause cardiac toxicity. Mitomycin and bleomycin cause

pulmonary fibrosis. Paclitaxel and vincristine cause peripheral neuropathy, and paclitaxel (or its vehicle) can cause anaphylaxis. Dactinomycin, daunorubicin, docetaxel, doxorubicin, epirubicin, etoposide, idarubicin, mitoxantrone, mitomycin, paclitaxel, teniposide, and vinblastine all cause alopecia to varying degrees. Etoposide and topotecan can cause leukemia. Other individual unique toxicities will be noted in Appendix 2.

Enzymes

L-Asparaginase is an enzyme product that acts primarily by inhibiting protein synthesis by depriving tumor cells of the amino acid asparagine. Cells that have the ability to form their own asparagine, such as many normal cells, are not affected by L-asparaginase.

L-Asparaginase is a foreign protein, is antigenic, and can cause serious hypersensitivity reactions. Included in this category are the long-acting pegylated asparaginase and Erwinia-derived asparaginase, both similar in mechanism to L-asparaginase.

Metals

Of the two metals used in cancer chemotherapy, only one is significant. At one time, gallium nitrate was used for the treatment of hypercalcemia and bladder cancer, but it causes nausea, vomiting, and renal toxicity and has been largely replaced by superior drugs. Arsenic trioxide has been available for a century and was sometimes used instead of potassium arsenite for treatment of chronic myeloid leukemia. Both arsenicals were abandoned for this purpose after superior agents became available. Arsenic trioxide was recently reintroduced into cancer chemotherapy by the Chinese. Its efficacy for inducing remissions in APL has been confirmed in Europe and the United States. Its major toxicities are nausea, vomiting, abdominal pain, diarrhea, pruritis, headache, dermatitis, hyperpigmentation, some skin exfoliation, and some bone marrow depression. 'Retinoic acid syndrome' (RAS) occurs in ~30% of patients treated and is characterized by high fever, dyspnea, respiratory distress, pulmonary infiltrates, and pericardial and/or pleural effusions. Some patients have required intubation and mechanical ventilation. Initiation of corticosteroid treatment at the first sign of dyspnea is advised and then maintained until symptoms resolve.

Hormonal Agents

The palliation of breast and prostate cancer by means of endocrine manipulation is an effective and relatively nontoxic therapy. Toward the end of the nineteenth century, it was noted that the ovaries influenced mammary physiology. In 1896, Beatson, an English surgeon, removed the ovaries in some premenopausal women with breast cancer and reported striking palliation in a few. This was the first use of cancer therapy that involved hormonal manipulation although the term hormone and the concept of a humoral regulator were not developed until 1902. Subsequent studies of oophorectomy showed temporary improvement in one-third of premenopausal patients and it is still used in some selected patients although it causes a prompt menopause with all its side effects.

In 1941, Huggins, Stevens, and Hodges showed that bilateral orchiectomy could lead to shrinkage of prostatic cancer and its metastases and relieved the pain of bone metastases in many patients. This approach is still used, although less frequently since the availability of medical alternatives. As expected, orchiectomy leads to impotence, loss of libido, gynecomastia, softening of the skin and beard, fatigue, loss of muscle tone, changes in personality, decreased bone mineral density, and hot flashes. In recent years, long-acting gonadotropin-releasing hormone (GnRH) analogs also known as leuteinizing-hormone releasing hormone (LHRH) analogs alone or in combination with androgen antagonists, have offered an alternative therapy with equal efficacy and more control and reversibility of the side effects with intermittent therapy. These drugs are listed in **Table 6** and their individual toxicities in Appendix 2.

Hormonal management of breast cancer used to depend on androgens, estrogens, and progestins. In recent years, they have been largely replaced by estrogen antagonists, aromatase inhibitors, and LHRH analogs. These new groups of agents have the side effects one would expect from estrogen deprivation, such as hot flashes, decreased energy, a variable decrease in bone mineral density, variable nausea, and in some cases an increased incidence of thromboembolic phenomena.

Corticosteroids are widely used throughout medical practice. In cancer therapy, prednisone and dexamethasone are the most frequently used. They have a lytic effect on lymphoma and myeloma cells, reduce the edema associated with brain metastases, reduce immunological and allergic reactions and exert an antiemetic effect alone and with 5-HT_3 blockers. The many side effects of corticosteroids are often the consequence of the desired effect on the disease process being treated also impacting the normal tissues adversely. These toxicities are well known as they are seen throughout clinical medicine.

Table 6 Hormonal agents

Androgens
Fluoxymesterone
Testosterone
Androgen antagonists
Bicalutamide
Flutamide
Ketoconazole
Nilutamide
Aromatase inhibitors
Aminoglutethimide
Anastrazole
Exemestane
Letrozole
Corticosteroids
Dexamethasone
Prednisone
Estrogens
Diethylstilbesterol
Estradiol
Estrogen antagonists
Fulvestrant
Raloxifene
Tamoxifen
Toremifene
Luteinizing hormone-releasing hormone (LHRH) analogs
Abarelix
Goserelin
Leuprolide
Triptorelin pamoate
Progestins
Medroxyprogesterone acetate
Megestrol acetate

Biotherapeutic Agents

The immune system is responsible for protecting the body from bacteria, viruses, and cancer. Early work with nonspecific stimulators of the immune system failed to demonstrate any reliable benefit. More recent investigations of immunological responses have increased our knowledge of tumor biology and coupled with recombinant DNA technology have led to the development of the biologic response modifiers and monoclonal antibody targeting agents that are effective as targeted cancer treatment options (**Table 7**). More treatment options are in the pipeline.

Interferons were originally isolated from human leukocytes as antiviral agents, but the interferon alfa-2 that we use today in cancer therapy is a recombinant product. It is used primarily in the treatment of Hairy Cell leukemia, the Kaposi sarcoma of AIDS, melanoma, and renal cell carcinoma. The major toxicity is a flu-like syndrome with fever, chills, rigors, and myalgias. Longer term toxicities include profound fatigue, confusion, neurologic side effects, and depression, sometimes severe enough to lead to suicide.

Table 7 Biotherapeutic agents

Interferon alfa-2
Interleukin-2 (aldesleukin)
Monoclonal antibodies
Alemtuzumab
Bevacizumab
Cetuximab
Gemtuzumab
Ibritumomab tiuxetan
Rituximab
Tositumomab
Trastuzumab

Interleukins are a family of cytokines, substances secreted by T-cells (lymphocytes), monocytes, macrophages, and other cells. Recombinant IL-2, known generically as aldesleukin, is effective in the therapy of a small percent of patients with renal cell carcinoma and melanoma, sometimes with very gratifying results. Its toxicity is dose-, route-, and time-dependent. At its worst, high-dose intravenous prolonged infusions cause fever, fluid retention, hypotension, respiratory distress, capillary-leak syndrome, suppression of hematopoiesis, nephrotoxicity, and hepatotoxicity.

Monoclonal antibodies were made possible by the development of the hybridoma methodology in 1986. Monoclonal antibodies are classified and named based on their derivation. Murine monoclonal antibodies having the suffix ending 'momab,' are cleared quickly from the body, and have a greater chance of inducing a HAMA reaction (human antimouse antibody). Chimeric antibodies are a human-mouse antibody mixture; they possess the suffix ending 'imab' and are more efficient and effective at destroying cells via CDC (complement-dependent cytotoxicity) and ADCC (antibody-dependent cell-mediated antibody). Chimeric antibodies circulate longer in the human body and are less likely to invoke a HAMA reaction. Humanized monoclonal antibodies possess the suffix ending 'umab' and are not likely to invoke a HAMA reaction.

Monoclonal antibody therapy is based on the ability to target markers and bind to cell membrane antigens with great specificity. Many times the enhanced specificity demonstrated toward the tumor antigens allows normal cells to be protected against harmful effects, unlike conventional chemotherapy. There are several mechanisms by which monoclonal antibodies destroy or prevent further replication of malignant cells. Some monoclonal antibodies utilize tumor immunology and components of the host natural defense mechanism to exert their desired effect. For example, monoclonal antibodies can utilize tumor effector cells to promote tumor cell lysis or have the ability to directly modulate tumor function.

Conjugated monoclonal antibodies can be used as carriers of toxic therapy, such as radionuclides, (e.g., yttrium-90 ibritumomab tiuxetan and iodine-131 tositumomab), cytotoxic drugs, or cell toxins to specific cell targets. They are also being employed to create tumor vaccines by stimulating a host antibody reaction causing the production of anti-idiotype antibodies.

In the last 2–5 years, selected monoclonal antibodies have become a routine part of care for certain malignancies. Rituximab, a chimeric monoclonal antibody used against CD 20 positive B-cell non-Hodgkin's lymphoma, is now utilized in combination with the CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone). Trastuzumab, a humanized monoclonal antibody, is a weekly maintenance therapy for HER2neu-positive metastatic breast cancer patients.

A common toxicity of monoclonal antibodies that react with antigen is the potential to produce a side effect referred to as an infusion-related symptom complex. The probability of this reaction occurring increases in patients with a large tumor burden. This reaction is generally observed with the first or second dose of the monoclonal antibody, however, it is important to note that mild to severe latent reactions have occurred. The symptom complex is characterized by one or more of the following: fever, chills, rigors, dyspnea, bronchospasm, headache, hypotension, rash, nausea, throat tightness, flushing, and urticaria. This reaction can range from very mild symptoms to a severe and/or fatal reaction. It is vital to assess each patient on an individual basis due to the variability of reactions. The management of infusion-related reactions begins with stopping the infusion, assessing the patient, and administering hypersensitivity medications as needed (e.g., diphenhydramine, meperidine, H2 blockers, corticosteroids, and epinephrine). Once patient symptoms have resolved, many patients can have the infusion restarted at a slower rate, under clinical observation.

Patients need to be aware of the side effects that are likely to occur, and they must be informed about what they can do to prevent or minimize the severity of these side effects. They also need to know that virtually all of the side effects are reversible, most subsiding within a few days after stopping treatment. The severity of symptoms varies from patient to patient. The lack of acute side effects is not usually predictive of adverse effects that may occur after weeks to months of treatment. Many of the side effects are subjective (i.e., fatigue, bone pain) and accurate documentation requires frequent communication and the cooperation of the patient and healthcare provider.

Miscellaneous Agents

Several agents are difficult to classify (**Table 8**).

Denileukin diftitox is a fusion protein that combines portions of the IL-2 molecule with the diphtheria toxin to destroy cells with the IL-2 receptor by inhibition of protein synthesis. It is used primarily in cutaneous T-cell lymphoma (CTCL) in patients whose disease expresses the CD 25 component of the IL-2 receptor. Its major toxicity is hypersensitivity reactions and the vascular leak syndrome.

Mitotane is an adrenal cytotoxic agent for the treatment of adrenocortical cancer. It has been suggested that it damages the mitochondria of adrenocortical cells. The major toxicity is nausea and vomiting and central nervous system effects like lethargy, somnolence, dizziness, and vertigo.

Octreotide is a long-acting somatostatin analog that inhibits the secretion of serotonin, vasoactive intestinal peptide, gastrin, motilin, insulin, glucagons, secretin, and pancreatic polypeptide. It is used for the control of symptoms in patients with carcinoid and vasoactive intestinal peptide-secreting tumors (VIPomas). Its major toxicity is nausea and vomiting.

Retinoids are differentiation agents related to or derivative of vitamin A. They bind to a cellular protein that facilitates their transfer from the cytoplasm to the nucleus where they are believed to increase DNA, RNA, and protein synthesis and to affect cellular mitosis.

Alitretinoin is dispensed as a gel, which is applied topically to treat the skin lesions of Kaposi's sarcoma secondary to AIDS. Except for mild skin irritation and a rash, it has no significant toxicity.

Betarotene is used in the treatment of refractory CTCL and the treatment of AIDS-related Kaposi's sarcoma. It may cause headache, rash, bone marrow depression, and photosensitivity.

Isotretinoin is widely used for the treatment of severe disfiguring acne. It is being evaluated for the

Table 8 Miscellaneous agents

Denileukin diftitox
Mitotane
Octreotide
Retinoids
Alitretinoin
Bexarotene
Isotretinoin
Tretinoin
Thalidomide
Tyrosine kinase inhibitors
Gefitinib
Imatinib

treatment of head and neck cancer, CTCL, and, neuroblastoma and as a prevention agent for myelodysplastic syndromes. Isotretinoin is teratogenic, and fetal abnormalities can result if used during pregnancy, particularly in the first trimester. Its toxicities include bone pain, myalgia, arthralgia, nausea, vomiting, headache, cheilitis, and elevated serum lipids. Although depression is uncommon, it has been associated with suicides, especially in teenage patients receiving it for the treatment of acne.

Tretinoin is better known as all-transretinoic acid or ATRA. It is a derivative of vitamin A and binds to a chromosomal receptor that is near the chromosomal lesion that is associated with APL. Differentiation of APL cells occurs after administration of tretinoin and remissions occur but the treatment is not curative and must be followed with cytotoxic chemotherapy for consolidation. Tretinoin is teratogenic and should not be used during pregnancy. General toxicity can also be severe and includes headache, xerosis, pruritis, arthralgia, myalgia, cheilitis, hypertriglyceridemia, and RAS which was described in relation to arsenic trioxide. It may be that the APL contributes to the drug effect in causing RAS. In either case, corticosteroid therapy with dexamethasone can control it.

Tyrosine kinase inhibitors are targeted to interfere with a crucial metabolic pathway that is more vulnerable in certain tumor cells than in normal cells.

Imatinib mesylate inhibits the Bcr-Abl tyrosine kinase and can induce apoptosis (programmed cell death) and inhibit further proliferation of the cell lines that are positive for Bcr-Abl. These cell lines are prominent in Philadelphia chromosome-positive chronic myeloid leukemia and in GIST (gastrointestinal stromal tumors). In many cases the initial responses have been very good. Toxicity consists mainly of nausea, vomiting, diarrhea, abdominal pain, skin rash, neutropenia, fluid retention, arthralgia, fatigue, fever, muscle cramps, myalgia, and muscle pain. In most cases therapy has not been interrupted due to the symptoms.

Gefitnib is a signal transduction inhibitor that is thought to exert its antitumor effects primarily by preventing activation of tyrosine kinase, which is necessary for the function of epidermal growth factor. Gefitnib exerts its primary antineoplastic effect by inhibiting EGFR. The EGFR is located in varying amounts in tumor cells of the colon, lung, head, and neck. Gefitinib is FDA approved for the treatment of nonsmall cell lung cancer, and is being investigated for activity in other malignancies. The major side effects are diarrhea and skin rash.

Thalidomide is best known as a drug that caused an international medical disaster. In 1957 it was marketed in Europe as a hypnotic, particularly for use by pregnant women. After a short period, it became apparent there was an increased incidence of a relatively rare birth defect, phocomelia, in which the hands and feet are attached close to the body resembling flipper of a seal or develop only as limb buds with no digits. It soon reached epidemic proportions, and retrospective epidemiologic research firmly established the causative agent to be thalidomide taken early in the course of pregnancy. Thalidomide was not licensed in the United States and was withdrawn from the European market in 1961. There were some cases in the United States in children born to women on investigational studies. In 1962, the Food Drug and Cosmetic Act was amended to give the FDA more authority in requiring evidence of both efficacy and relative safety before marketing new drugs.

In 1998, the FDA approved the marketing of thalidomide for erythema nodosum leprosy. Subsequently it has demonstrated activity against multiple myeloma, myelodysplastic syndrome, AIDS wasting syndrome, melanoma, and renal cell carcinoma.

To prevent severe birth defects and possible death of the newborn child, when thalidomide is used in women of child-bearing age or in sexually active men (due to levels of thalidomide in semen) adherence to strict guidelines adopted by the FDA is required. The specific guidelines fall under the term 'S.T.E.P.S. program' or 'System for Thalidomide Education and Prescribing Safety'. All prescribers (physicians) and distributors (pharmacists, etc.) must register and adhere to these guidelines. Other toxicities include headache, dizziness, rash, pruritis, drowsiness, somnolence, peripheral neuropathy, leucopenia, and venous thrombosis.

Combination Cancer Chemotherapy

Just as combination antibiotic chemotherapy has been found to be more efficacious in the treatment of tuberculosis and serious Gram-negative sepsis, as compared to single antibiotics, in a similar fashion, combination anticancer chemotherapy has been achieving better results than single agents in many of the tumors tested. Possible exceptions include some of the more sensitive neoplasms such as gestational trophoblastic tumors and African Burkitt's lymphoma where a single agent is often curative. Still, combination regimens seem to have higher response rates and longer durations of disease-free survival in many instances when compared to single agents.

It is best to select drugs with different mechanisms of cell destruction. One can combine an alkylating agent to kill cells in G_0 or any other phase of the

cycle, an antimetabolite to kill rapidly developing tumors in M phase, and a corticosteroid or other hormone to control cell growth without definitive cell kill. These agents with differing mechanisms reduce the chances of cell resistance.

While combination chemotherapy and high-dose therapy (with or without stem cell transplantation) can increase cancer response rates, they generally increase toxicity significantly and sometimes in unanticipated ways when drugs interact with each other. Hence, the toxicity of each combination chemotherapy protocol and each high-dose therapy protocol must be considered individually.

Management of Organ System Toxicity

It has been previously emphasized that cancer chemotherapy involves a process of differential and selective toxicity. Agents are used that injure neoplastic cells and normal cells and the goal is to damage the neoplastic cells irreversibly and allow the normal cells and tissues to recover sooner. In addition, it is important to ameliorate the unpleasant side effects and to support the patient. Discussion of a few major toxicities is in order.

Bone Marrow Suppression

All elements of the bone marrow are injured by cytotoxic drugs.

Neutrophils are depressed first because they renew their population every day. Neutropenia is defined as an absolute neutrophil count (ANC) 500 cells μl^{-1}. Patients with an ANC of less than 100 cells μl^{-1} or those with prolonged neutropenia (more than 7 days) are at significantly high risk for serious infection. That risk can be reduced with prophylactic antibiotics and the administration of colony-stimulating factors (CSFs). Current evidence does not support the routine use of CSFs (filgrastim, pegfilgrastim, and sargramostim) in afebrile neutropenic patients unless the patient is at high risk because of bone marrow compromise or comorbidity, for example, previous radiation to large areas of bone marrow, recurrent febrile neutropenia with similar dose chemotherapy, extensive prior chemotherapy, or active tissue infection. The exceptions to this guideline include administration of trimethoprim-sulfamethoxazole for immunosuppressed patients at risk for Pneumocystis carinii pneumonitis, and antifungal therapy (with fluconazole) and antiviral therapy (with acyclovir or gancyclovir) for prophylaxis of patients undergoing allogeneic stem cell transplantation. The development of fever (a single temperature of 101°F or 38.3°C or persistent temperature greater than or equal to 100.4°F or 38°C) in a neutropenic patient represents an urgent clinical problem requiring a prompt infectious agent assessment and intervention with appropriate antibiotics. Leukocyte transfusions are seldom, if ever, indicted.

Thrombocytopenia (platelet count of less than 10 000 μl^{-1} is a frequent consequence of cytotoxic chemotherapy. A moderate risk of bleeding exists when the platelet count falls to less than 50 000 μl^{-1} and a major risk is associated with platelet counts less than 10 000 μl^{-1}. Adequate coagulation can be further compromised by drugs that interfere with platelet function, like aspirin, nonsteroidal anti-inflammatory drugs, ginkgo biloba, and anticoagulants like warfarin and heparin. Platelet transfusions can reduce or eliminate fatal consequences in patients at high risk because of thrombocytopenia. Generally accepted guidelines for platelet transfusions are summarized in **Table 9**. An infrequently used approach is to stimulate the production of

Table 9 Summary highlights of ASCO's clinical practice guidelines for platelet transfusions[a]

Indication	*Guideline*
Platelet product	Use random donor pooled platelets unless histocompatible platelets are needed, then use single donor platelets
Prophylactic platelet transfusion: acute leukemia and hematopoietic cell transplant	A threshold of 10 000 μl^{-1} is recommended for asymptomatic patients. Transfusions at levels above this threshold are indicated for patients with complicating clinical conditions
Prophylactic transfusions: solid tumors	A threshold of 20 000 μl^{-1} is recommended for patients with bladder cancer receiving aggressive therapy and those with necrotic tumors. For all others, a threshold of 10 000 μl^{-1} is recommended
Surgical or invasion procedures	A platelet count of 40 000–50 000 μl^{-1} is deemed sufficiently safe to perform invasive procedures in the absence of coagulation problems
Prevention of alloimmunization with leukoreduced blood products	Recommended for patients with AML from time of diagnosis; consider for all other patients

[a] Data from: Shiffer CA, Anderson KC, Bennet CL, *et al.* (2001) Platelet transfusion for patients with cancer: Clinical practice guidelines of the American Society of Clinical Oncology. *Journal of Clinical Oncology* 19: 1519–1538.

platelets before administering chemotherapy by the administration of oprelvekin, a recombinant IL-11. This drug stimulates megakaryocytopoeisis and thrombopoiesis and platelet increases are observed 5–9 days after initiation of treatment.

Anemia is associated with cancer and may be multifactorial. It may be due to bleeding, hemolysis, or bone marrow suppression secondary to the malignancy or it may be due to chemotherapy. Treatment for an acute need is generally by red cell transfusion. For chronic anemia in patients due to cancer chemotherapy who are not hemolyzing and not iron deficient, epoietin alfa or the longer-acting darbepoietin alfa, can raise hemoglobin levels and relieve some of the fatigue of malignancy.

Nausea, Vomiting, and Antiemetic Therapy

There are three patterns of nausea and vomiting associated with chemotherapy: acute, delayed, and anticipatory. Acute occurs within the first 24 h of treatment, delayed occurs or is a continuation beyond 24 h, and anticipatory is the experience of nausea or vomiting before receiving another chemotherapy treatment. It is a conditioned or learned response to previous effects from therapy. It may be prevented by minimizing the adverse effects of the first and subsequent treatments. The incidence and severity of nausea and vomiting are related to the emetogenic potential of the drug (**Table 10**), dose, route of administration, schedule, infusion rate, time of day drug is given, patient characteristics, and combination of drugs. It is easier to prevent nausea and vomiting than to treat. Hence, antiemetics are given shortly before chemotherapy administration. In general, one should use aggressive antiemetic therapy for chemotherapy naïve patients, give an adequate duration of coverage for the predicted risk period and select the appropriate agents and dosing according to the emetic potential of the chemotherapy. While **Table 10** is a good guide, combination chemotherapy will frequently move the potential antiemetic effect higher, that is, one group to the left.

Therapy for nausea and vomiting is directed at blocking the effect on the chemoreceptor trigger zone of the brain and the receptors in the GI tract. For low-risk emetogenic chemotherapy, dexamethasone, metoclopromide, or prochlorperazine are most useful. A psychotropic agent like lorazepam may be helpful if one suspects a degree of apprehension. There are other antiemetics available (e.g., butyrophenones and the cannabinoids), but they are of low therapeutic efficacy and are not recommended as first line therapy. For moderate or high-risk emetogenic therapy, a 5-HT_3 antagonist (dolasetron, ganisetron, ondansetron, and palonosetron) with dexamethasone is recommended. For delayed emesis due to moderately emetogenic chemotherapy, a single dose of the longer-acting palonosetron (with dexamethasone) may be more effective than the other 5-HT_3 inhibitors. For both acute and delayed emesis due to highly emetogenic drugs, aprepitant plus a 5-HT_3 inhibitor plus dexamethasone is the current treatment of choice. For breakthrough emesis despite optimal prophylactic pretreatment, an agent from another pharmaceutical class may be added and antiemetic doses increased.

Renal and Bladder Toxicity

Major risk factors for renal toxicity in cancer patients include nephrotoxic chemotherapy drugs, age, nutritional status, concurrent use of other nephrotoxic drugs (e.g., aminoglycoside antibiotics), and preexisting renal dysfunction. Drugs with a high risk for renal toxicity include cisplatin, ifosfamide,

Table 10 Emetic potential of chemotherapy drugs as single agents

Very high (> 90%)	*High (60–90%)*	*Moderate (30–60%)*	*Low (10–30%)*
Carmustine[a]	Azacitidine	Altretamine	Cytarabine
Cisplatin	Carboplatin	Daunorubicin	Docetaxel
Cyclophosphamide[a]	Carmustine	Doxorubicin	Etoposide
Cytarabine[a]	Cyclophosphamide	Epirubicin	5-Fluorouracil
Mechlorethamine	Dacarbazine	Idarubicin	Gemcitabline
Melphalan[a]	Dactinomycin	Ifosfamide	Irinotecan
Streptozocin	Lomustine	Mitomycin	Paclitaxel
		Mitoxantrone	
		Oxaliplatin	Thiotepa
		Plicamycin	Topotecan
		Procarbazine	

[a] High dose.

methotrexate (high dose), mitomycin, and streptozocin. Carboplatin is significantly less nephrotoxic than cisplatin, but if administered in high doses (e.g., in stem cell transplantation), or given with other nephrotoxic drugs, it has the potential to contribute to renal damage. Before using a renal toxic chemotherapy agent, renal function should be evaluated with a serum creatinine or creatinine clearance as a guide to the need for dose reduction or omission.

Hemorrhagic cystitis and an increased incidence of bladder cancer are associated with use of ifosfamide and cyclophosphamide. Contact of the bladder wall with their toxic metabolites, primarily acrolein, produces mucosal erythema, inflammation, ulceration, necrosis, diffuse small-vessel hemorrhage, oozing, and a reduced bladder capacity. Symptoms include hematuria (microscopic or gross) and dysuria. The uroprotective agent 2-mercaptoethane sulfonate sodium (mesna) acts by binding to acrolein to result in a nontoxic thioether. The use of adequate mesna and hydration with ifosfamide or high-dose cyclophosphamide significantly reduces the incidence of bladder toxicity.

Cardiopulmonary Toxicity

Chemotherapy drugs can directly or indirectly cause: acute pneumonitis (bleomycin, carmustine, gemcitabine, methotrexate, mitomycin, procarbazine, and vinca alkaloids); pulmonary fibrosis (bleomycin, carmustine, cyclophosphamide, methotrexate, and mitomycin); hypersensitivity pneumonitis (bleomycin, methotrexate, and procarbazine); noncardiogeneic pulmonary edema (cytarabine, cyclophosphamide, methotrexate, mitomycin, and teniposide). Docetaxel is associated with fluid retention, which may result in pulmonary edema or pleural effusion. Some of these conditions respond to corticosteroid therapy but some cases of pulmonary fibrosis are fatal.

Cardiomyopathy is the most common chemotherapy-associated cardiac toxicity. Myocardial ischemia, pericarditis, arrhythmias, miscellaneous electrocardiogram (ECG) changes, and angina occur much less frequently. The anthracyclines (daunorubicin, doxorubicin, epirubicin, and idarubicin) have the highest consistent risk for cardiomyopathy, which is cumulative dose related. There is evidence that high-dose cyclophosphamide, mitoxantrone, and fluorouracil also pose an increased risk of cardiac damage. The concurrent use of traztuzumab with an anthracycline and cyclophosphamide is associated with a risk of cardiac dysfunction, but the consequences of sequential use are not yet known.

Management of chemotherapy-induced cardiac dysfunction is conventional therapy for heart failure. Because of the limited value of this intervention in the face of existing cardiac disease, prevention of cardiac toxicity is important. This can be done by limiting the cumulative total dose, giving it more slowly, and using dexrazoxane, an intracellular iron-chelating agent that prevents iron from combining with anthracyclines to form free oxygen radicals. Dexrazoxane is initiated after two-thirds of the cumulative toxic dose is administered (i.e., at $300\,\mathrm{mg\,m^{-2}}$ for doxorubicin). Long-term follow-up is indicated because congestive heart failure may develop several years after therapy is completed.

Dermatological and Neurological Toxicity

Chemotherapy drugs can cause a variety of dermatological conditions including rashes, pruritis, swelling, hyperkeratosis, urticaria, exfoliation, photosensitivity, flushing, nail changes, and pigmentation. Extravasation of some agents, especially carmustine, dactinomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, mechlorethamine, mitomycin, and the vinca alkaloids, can lead to tissue necrosis, ulceration, and sloughing. To reduce the incidence of extravasation, central venous catheters are frequently used to administer these drugs.

Encephalopathy, peripheral neuropathy, cerebellar syndromes, autonomic neuropathy, and cranial nerve toxicity represent the range of neurological complications associated with cancer chemotherapy. Dose, route of administration, age of the patient, hepatic and renal function, prior and/or concomitant use of other neurotoxic drugs, and the concurrent use of cranial or CNS radiotherapy can each influence the incidence rate and severity of neurologic symptoms associated with selected chemotherapy drugs.

The management of the dermatological and neurological toxicities secondary to chemotherapy drugs is essentially the same as those due to other causes. Tables of drugs and their specific subtypes of these toxicities and fuller discussions of them are available in the references listed as further reading. They also include discussions of other toxicities including mucositis, diarrhea, constipation, hypercalcemia, headache, depression, anxiety, fatigue, anorexia, weight loss, impotence, sterility, premature menopause, pregnancy risks, and teratogenicity.

Appendix 1 Drugs of choice for cancer – partial list of brand names

Accutane – isotretinoin
Adriamycin[a] – doxorubicin
Adrucil[a] – fluorouracil
Alimta – pemetrexed
Alkeran – melphalan
Arimidex – anastrozole
Aromasin – exemestane
Avastin – bevacizumab
Bexxar – tositumomab
BiCNU – carmustine
Blenoxane[a] – bleomycin
Caelyx – liposomal doxirubicin
Campath – alemtuzumab
Camptosar – irinotecan
Casodex – bicalutamide
CeeNU – lomustine
Cerubidine[a] – daunorubicin
Cosmegen – dactinomycin
Cytadren – aminoglutethimide
Cytosar[a]-U–cytarabine
Cytoxan[a] – cyclophosphamide
Daunoxome – liposomal daunorubicin
Depo-Provera – medroxyprogesterone acetate
Doxil – liposomal doxorubicin
DTIC[a] – *Dome*-dacarbazine
Ellence – epirubicin
Eloxatin – oxaliplatin
Elspar – asparaginase
Emcyt – *estramustine phosphate sodium*
Erbitux – cetuximab
Eulexin – flutamide
Fareston – toremifene
Faslodex – fulvestrant
Femara – letrozole
Fludara – fludarabine
FUDR – floxuridine
Ganite – gallium nitrate
Gemzar – gemcitabine
Gleevec – imatinib
Gliadel – carmustine wafer
Halotestin – fluoxymesterone
Herceptin – trastuzumab
Hexalen – altretamine
Hycamtin – topotecan
Hydrea[a] – hydroxyurea
Idamycin – idarubicin
Ifex – ifosfamide
Intron-A[b] – interferon alfa-2b
Iressa – gefitinib
Kidrolase – asparaginase
Leukeran – chlorambucil
Leustatin – cladribine
Lupron[a] – leuprolide acetate
Lysodren – mitotane
Matulane – procarbazine
Megace[a] – megestrol acetate
Mesnex – mesna
Mustargen – mechlorethamine
Mutamycin[a] – mitomycin
Myleran – busulfan
Mylotarg – gemtuzumab
Natulan – procarbazine
Navelbine – vinorelbine tartrate
Neosar[a] – cyclophosphamide
Neutrexin – trimetrexate
Nilandron – nilutamide
Nipent – pentostatin
Nizoral[a] – ketoconazole
Nolvadex[a] – tamoxifen citrate
Novantrone – mitoxantrone
Oncaspar – pegaspargase
Oncovin[a] – vincristine sulfate
Ontak – denileukin diftitox
Panretin – alitretinoin
Paraplatin – carboplatin
Pharmorubicin – epirubicin
Platinol[a] – cisplatin
Proleukin – interleukin-2 (aldesleukin)
Provera[a] – medroxyprogesterone *acetate*
Purinethol – mercaptopurine
Rituxan – rituximab
Roferon-A – interferon alfa-2a
Rubex[a] – doxorubicin
Sandostatin – octreotide
Stilphostrol – diethylstilbestrol
Tamofen – tamoxifen
Tarabine[a] – cytarabine
Targretin – bexarotene
Taxol – paclitaxel
Taxotere – docetaxel
Tegison – etretinate
Temodar – temozolomide
Thalomid – Thalidomide
TheraCys – live BCG
Thioplex – thiotepa
TiceBCG – live BCG
Tomudex[b] – raltitrexed
Trelstar – triptorelin
Trisenox – arsenic trioxide
Uromitexan – mesna
Velban[a] – vinblastine
Velbe[a] – vinblastine
VePesid[a] – etoposide
Vesanoid – tretinoin
Vincasar[a] – vincristine
Vumon – teniposide
Wellcovorin[a] – leucovorin
Xeloda – capecitabine
Zanosar – streptozocin
Zevalin – ibritumomab tiuxetan
Zoladex – goserelin

[a]Also available generically.
[b]Available in the USA at this time for investigational use only.
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Appendix 2 Drugs of choice for cancer – toxicity of some anticancer drugs

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Alemtuzumab (*Campath* – Berlex)	Infusion reactions (fever, rigors, fatigue, musculoskeletal pain, dyspnea, hypotension, urticaria); autoimmune hemolytic anemia	**Bone marrow depression**
Alitretinoin (*Panretin* – Ligand Pharmaceuticals)	Erythema; rash; pruritus	Continued application may result in worsening of acute symptoms and also edema and vesiculation
Altretamine (hexamethymelamine; *Hexalen* – MGI Pharma)	Nausea with vomiting	**Bone marrow depression**; CNS depression; peripheral neuropathy; visual hallucinations; ataxia; tremors; alopecia; rash
Anastrozole (*Arimidex* – AstraZeneca)	Nausea; diarrhea; hot flashes; headache	Asthenia; pain (bone pain, back pain); dyspnea; peripheral edema; rash
Arsenic trioxide (*Trisenox* – Cell Therapeutics)	"Retinoic acid-like syndrome" (fever, dyspnea, pulmonary infiltrates, pleural effusions, peripheral edema, hypotension); fatigue; musculoskeletal pain; prolongation of QT interval and cardiac arrhythmias; hyperglycemia	Peripheral neuropathy; dysesthesias; rash; alopecia; renal toxocity; myelosuppression

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Asparaginase (Elspar S – Merck; *Kidrolase* in Canada)	Nausea and vomiting; fever; chills; headache; hypersensitivity, anaphylaxis; abdominal pain; hyperglycemia leading to coma	CNS depression or hyperexcitability; acute hemorrhagic pancreatitis; coagulation defects, thrombosis; renal damage; hepatic damage
Bevacizumab (Avastin-Genentech)	**Allergic reactions**	**Gastrointestinal perforations; hemoptysis**
Bexarotene (*Targretin* – Ligand Pharmaceuticals)	Headache; rash	Leukopenia; anemia; asthenia; hypothyroidism; hypertriglyceridemia; hypercholesterolemia; photosensitivity
Bicalutamide (*Casodex* – AstraZeneca)	Nausea; diarrhea; hot flashes; hematuria; increased aminotransferase activity	Gynecomastia; breast pain; hypersensitivity pneumonitis
Bleomycin (*Blenoxane* – Bristol-Myers Squibb Oncology, and others)	Nausea and vomiting; fever; anaphylaxis and other allergic reactions; phlebitis at injection site	**Pneumonitis and pulmonary fibrosis**; rash and hyperpigmentation; stomatitis; alopecia; Raynaud's phenomenon; hemorrhagic cystitis
Busulfan (*Myleran* – GlaxoSmithKline)	Nausea and vomiting (with high-dose therapy only); diarrhea (rare)	**Bone marrow depression**; pulmonary infiltrates and fibrosis; alopecia; gynecomastia; ovarian failure; hyperpigmentation; azoospermia; leukemia; chromosome aberrations; cataracts; hepatitis; seizures and veno-occlusive disease with high doses; secondary malignancy with prolonged use
Capecitabine (*Xeloda* – Roche)	Nausea and vomiting	**Palmar-plantar erythrodysesthesia (hand-foot syndrome)**; diarrhea; stomatitis; dermatitis; bone marrow depression; hyperbilirubinemia; ocular irritation and corneal deposits
Carboplatin (*Paraplatin* – Bristol-Myers Squibb Oncology)	Nausea and vomiting; hypersensitivity, anaphylaxis	**Bone marrow depression**; peripheral neuropathy; hearing loss; transient cortical blindness; hemolytic anemia
Carmustine (BCNU; BiCNU – Bristol-Myers Squibb Oncology; *Gliadel* – Guilford)	Nausea and vomiting; local phlebitis	**Bone marrow depression (cumulative) with delayed leukopenia and thrombocytopenia** (may be prolonged); pulmonary fibrosis (may be irreversible); delayed renal damage; reversible liver damage; leukemia; myocardial ischemia; veno-occlusive disease of liver after transplantation doses
Cetuximab (C225; *Erbitux* – ImClone Systems)	Rash; fever and chills	Asthenia
Chlorambucil (*Leukeran* – GlaxoSmithKline)	Nausea and vomiting	**Bone marrow depression**; pulmonary infiltrates and fibrosis; leukemia; hepatic toxicity; sterility
Cisplatin (Cis-DDP; *Platinol* – Bristol-Myers Squibb Oncology, and others)	Nausea with vomiting; diarrhea; hypersensitivity reactions	**Renal damage**; ototoxicity; bone marrow depression; hemolysis; hypomagnesemia; peripheral neuropathy; hypocalcemia; hypokalemia; Raynaud's phenomenon; sterility; hypophosphatemia; hyperuricemia; anorexia
Cladribine (2-chlorodeoxy-adenosine; 2-CdA; *Leustatin* – Ortho-Biotech)	Fever	**Bone marrow depression**; immunosuppression; peripheral neuropathy with high doses
Cyclophosphamide (*Cytoxan* – Bristol-Myers Squibb Oncology; *Neosar* – Pharmacia)	Nausea and vomiting; Type 1 (anaphylactoid) hypersensitivity; facial burning and metallic taste with IV administration; visual blurring	**Bone marrow depression**; alopecia; hemorrhagic cystitis; sterility (may be temporary); pulmonary infiltrates and fibrosis; hyponatremia; leukemia; bladder cancer; inappropriate ADH secretion; cardiac toxicity; amenorrhea
Cytarabine HCl (*Cytosar*-U – Pharmacia, and others)	Nausea with vomiting; diarrhea; anaphylaxis, sudden respiratory distress and high doses; fever	**Bone marrow depression**; conjunctivitis; megaloblastosis; oral ulceration; hepatic damage; pulmonary edema and central and peripheral neurotoxicity with high doses; rhabdomyolysis; pancreatitis when used with asparaginase; rash

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Dacarbazine (*DTIC-Dome* – Bayer)	Nausea and vomiting; diarrhea; anaphylaxis; pain on administration; phlebitis at infusion site	**Bone marrow depression**; alopecia; flu-like syndrome; renal impairment; hepatic necrosis; facial flushing; paresthesia; photosensitivity; urticarial rash
Dactinomycin (*Cosmegen* – Merck)	Nausea and vomiting; hepatic toxicity with ascites; diarrhea; severe local tissue damage and necrosis on extravasation; anaphylactoid reaction	**Stomatitis; oral ulceration; bone marrow depression**; alopecia; folliculitis; dermatitis in previously irradiated areas
Daunorubicin HCl (*Cerubidine* – Bedford, and others)	Nausea and vomiting; diarrhea; red urine (not hematuria); severe local tissue damage and necrosis on extravasation; transient ECG changes; facial flushing; anaphylactoid reaction	**Bone marrow depression**; **cardiotoxicity** (may be delayed for years); alopecia; stomatitis; anorexia; diarrhea; fever and chills; dermatitis in previously irradiated areas; skin and pigmentation; photosensitivity
Liposomal daunorubicin (*DaunoXome* – Gilead)	Less nausea and vomiting; no red urine; less local tissue damage; infusion reactions	Less cardiotoxicity; minimal alopecia
Denileukin diftitox (*Ontak* – Ligand Pharmaceuticals)	Hypersensitivity reactions (hypotension, back pain, dyspnea, rash, chest tightness, tachycardia, dysphagia); chills; fever; headache; nausea and vomiting; diarrhea; pruritus	Vascular leak syndrome (hypotension, edema, hypoalbuminemia); anemia; infection; anorexia; asthenia; increased aminotransferase activity; hypocalcemia
Diethylstilbestrol (*Stilphostrol* – Bayer)	Nausea and vomiting; abdominal cramps; headache	Gynecomastia in males; breast tenderness; loss of libido; thrombophlebitis and thromboembolism; hepatic injury; sodium retention with edema; hypertension; change in menstrual flow
Docetaxel (*Taxotere* – Aventis)	Hypersensitivity reactions; nausea and vomiting; bowel perforation (rare)	**Bone marrow depression**; peripheral neuropathy; fluid retention (including generalized edema and pleural effusions); myalgia; alopecia; mucositis; cutaneous fibrosis; onycholysis, subungual hemorrhage; increased hepatic aminotransferase activity; epiphora; phlebitis
Doxorubicin HCl (*Adriamycin* – Pharmacia, and others)	Nausea and vomiting; red urine (not hematuria); severe local tissue damage and necrosis on extravasation; diarrhea; fever; transient ECG changes; ventricular arrhythmia; anaphylactoid reaction	**Bone marrow depression; cardiotoxicity** (cumulative and may be delayed for years); alopecia; stomatitis; anorexia; conjunctivitis; acral pigmentation; dermatitis in previously irradiated areas; acral erythrodysesthesia; hyperuricemia; leukemia
Liposomal doxorubicin (*Doxil* – Alza; Caelyx – Schering in Canada)	Less nausea and vomiting; no red urine; less local tissue damage; infusion reactions	Less cardiotoxicity; minimal alopecia; palmar-plantar and acral dysesthesia; mucositis
Epirubicin (*Ellence* – Pharmacia; Pharmorubicin in Canada)	Local tissue damage; red urine (not hematuria); nausea and vomiting; ECG changes; arrhythmias; anaphyactoid reaction	**Bone marrow depression**; alopecia; paresthesias; fatigue; cardiotoxicity; leukemia
Estramustine phosphate sodium (*Emcyt* – Pharmacia)	Nausea and vomiting; diarrhea	Mild gynecomastia; increased frequency of vascular accidents; myelosuppression (uncommon); edema; dyspnea; pulmonary infiltrates and fibrosis; decreased glucose tolerance; thrombosis; hypertension
Etoposide (VP-16; *VePesid* – Bristol-Myers Squibb Oncology, and others)	Nausea and vomiting; diarrhea; fever; hypotension; hypersensitivity reactions; phlebitis at infusion site	**Bone marrow depression**; rashes; alopecia; peripheral neuropathy; mucositis and hepatic damage with high doses; leukemia
Exemestane (*Aromasin* – Pharmacia)	Hot flashes; nausea	Peripheral edema and weight gain; fatigue
Floxuridine (*FUDR* – Roche)	Nausea and vomiting; diarrhea	**Oral and gastrointestinal ulceration; bone marrow depression**; alopecia; dermatitis with hepatic infusion; jaundice; sclerosing cholangitis
Fludarabine (*Fludara* – Berlex)	Nausea and vomiting	**Bone marrow depression**; immunosuppression; CNS effects; visual disturbances; renal damage with higher doses; pulmonary infiltrates

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Fluorouracil (5-FU; *Adrucil* – Pharmacia, and others)	Nausea and vomiting; diarrhea; mucositis; hypersensitivity reaction (rare)	**Oral and GI ulcers; bone marrow depression**; diarrhea (especially with fluorouracil and leucovorin); neurological defects, usually cerebeller; cardiac arrhythmias; angina pectoris; alopecia; hyperpigmentation; **palmar-plantar erythrodysesthesia**; conjunctivitis; heart failure; seizures
Fluoxymesterone (*Halotestin* – Pharmacia)	Nausea and vomiting	Menstrual changes; gynecomastia; androgenic effects; hepatic toxicity
Flutamide (*Eulexin* – Schering; *Euflex* in Canada)	Nausea; diarrhea; hot flashes	Gynecomastia; hepatic toxicity; hypersensitivity pneumonitis
Fulvestrant (*Faslodex* – AstraZeneca)	Nausea; pain at injection site; headache; rash; hot flashes	Anorexia; anemia; dyspnea; asthenia; musculoskeletal symptoms; occasional thromboembolism
Gallium nitrate (*Ganite* – SoloPak)	Hypocalcemia; metallic taste	**Hypophosphatemia**; nephrotoxicity; anemia; optic neuritis
Gefitinib (*Iressa* – AstraZeneca)	Diarrhea; rash; dry skin; acne; nausea; vomiting; dyspnea; pruritus	Mucostis; anorexia; asthenia; interstitial pneumonitis (rarely)
Gemcitabine (*Gemzar* – Lilly)	Fatigue; nausea and vomiting; fever	**Bone marrow depression**; edema; pulmonary toxicity, anal pruritus
Gemtuzumab (*Mylotarg* – Wyeth)	Fever; chills; nausea; hypotension; hypersensitivity reactions	Bone marrow depression; increased aminotransferase activity; veno-occlusive disease
Goserelin (*Zoladex* – AstraZeneca)	Transient increase in bone pain; transient increase in tumour mass, resulting in ureteral obstruction and/or spinal cord compression in patients with metastatic prostate cancer; hot flashes	Impotence; testicular atrophy; gynecomastia; allergic reactions; peripheral edema; decreased bone mineral density
Hydroxyurea (*Hydrea* – Bristol-Myers Squibb Oncology, and others)	Nausea and vomiting; allergic reactions to tartrazine dye	**Bone marrow depression**; stomatitis; dysuria; alopecia; rare neurological disturbances; pulmonary infiltrates; secondary leukemias
Ibritumomab tiuxetan (*Zevalin* – IDEC Pharmaceuticals)	Rash; pruritus; urticaria; nausea; vomiting; fever; chills (See also Rituximab)	**Neutropenia; thrombocytopenia**; hemorrhage; anorexia
Idarubicin (*Idamycin* – Pharmacia)	Nausea and vomiting; tissue damage on extravasation	**Bone marrow depression**; alopecia; stomatitis; myocardial toxicity; diarrhea
Ifosfamide (*Ifex* – Bristol-Myers Squibb Oncology)	Nausea and vomiting; confusion; nephrotoxicity; metabolic acidosis and renal Fanconi's syndrome; cardiac toxicity with high doses	**Bone marrow depression; hemorrhagic cystitis** (prevented by concurrent mesna); alopecia; inappropriate ADH secretion; renal failure; neurotoxicity (somnolence, hallucinations, blurring of vision, coma)
Imatinab (STI-571; *Gleevec* – Novartis)	Nausea and vomiting; rash; diarrhea; muscle cramps; increased aminotransferase activity	**Bone marrow depression**, pulmonary, periorbital and pedal edema
Interferon alfa-2a (*Roferon-A* – *Roche*), alfa-2b (*Intron A* – *Schering*)	Flu-like syndrome (fever, chills, myalgias, fatigue, arthragias, headache); nausea; diarrhea; hypotension	Bone marrow depression; anorexia; neutropenia; anemia; confusion; depression; renal toxicity; possible hepatic injury; facial and peripheral edema; cardiac arrhythmias; rhabdomyolysis
Interleukin-2 (aldesleukin; *Proleukin* – Chiron)	**Fever; fluid retention; hypotension; respiratory distress**; rash; anemia; thrombocytopenia; nausea and vomiting; diarrhea; capillary leak syndrome; nephrotoxicity; myocardial toxicity; hepatotoxicity; erythema nodosum; neutrophil chemotactic defects	Neuropsychiatric disorders; hypothyroidism; nephrotic syndrome; possibly acute leukoencephalopathy; brachial plexopathy; bowel perforation
Irinotecan (*Camptosar* – Pharmacia)	Nausea and vomiting; **early diarrhea** (<24 h); fever	**Late diarrhea** (>24 h); **leukopenia**; anorexia; asthenia; alopecia; abdominal cramping and pain; thromboembolism
Isotretinoin (*Accutane* – Roche)	Fatigue; headache; nausea and vomiting; pruritus; pancreatitis; depression	**Teratogenicity**; cheilitis xerostomia; rash; conjuctivitis and eye irritation; bone and joint pain; anorexia; hypertriglyceridemia; pseudotumor cerebri; psychosis; increased aminotransferase activity

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Ketoconazole (*Nizoral* – Janssen, and others)	Nausea and vomiting	Hepatocellular toxicity; hypertension; gynecomastia, breast tenderness; impotence; nail changes; pruritus; adrenal insufficiency
Letrozole (*Femara* – Novartis)	Hot flashes; nausea and vomiting; headache	Peripheral edema and weight gain; dyspnea; fatigue; musculoskeletal pain; arthralgia; constipation; diarrhea; rare thromboembolic events
Leucovorin (*Wellcovorin* – GlaxoSmithkline, and others)	Hypersensitivity reactions; nausea; diarrhea	
Leuprolide acetate (*Lupron*, *Lupron* Depot – TAP, and others)	Transient increase in bone pain; transient increase in tumor mass, resulting in ureteral obstruction and/or spinal cord compression in patients with metastatic prostate cancer; hot flashes; hematuria	Impotence; testicular atrophy; gynecomastia; peripheral edema; allergic reactions; decreased bone mineral density
Lomustine (CCNU; *CeeNU* – Bristol-Myers Squibb Oncology)	Nausea and vomiting	**Bone marrow depression** (cumulative) with delayed leukopenia and thrombocytopenia (may be prolonged); transient elevation of transaminase activity; neurological reactions; pulmonary fibrosis; renal damage; leukemia
Mechlorethamine HCl (nitrogen mustard; *Mustargen* – Merck)	Nausea and vomiting; local reaction and phlebitis	**Bone marrow depression**; alopecia; diarrhea; oral ulcers; leukemia; amenorrhea; sterility; hyperuricemia
Megestrol acetate (*Megace* – Bristol-Myers Squibb Oncology, and others)	Nausea and vomiting; headache; weight gain	Menstrual changes; hot flashes; thrombophlebitis and thromboremolism; fluid retention; edema; impotence
Melphalan (*Alkeran* – GlaxoSmithKline)	Mild nausea; hypersensitivity reactions	**Bone marrow depression** (especially platelets); pulmonary infiltrates and fibrosis; amenorrhea; sterility; leukemia
Mercaptopurine (*Purinethol* – GlaxoSmithKline)	Nausea and vomiting; diarrhea	**Bone marrow depression**; cholestasis and rarely hepatic necrosis; oral and intestinal ulcers; pancreatitis
Mesna (Mesnex – Bristol-Myers Squibb Oncology; *Uromitexan* in Canada)	Nausea and vomiting; diarrhea; allergic reactions	
Methotrexate (MTX; *Folex* – Pharmacia, and others)	Nausea and vomiting; diarrhea; fever; hepatic necrosis; hypersensitivity reactions	**Oral and gastrointestinal ulceration**, perforation may occur; **bone marrow depression**; hepatic toxicity including cirrhosis; renal toxicity; **pulmonary infiltrates and fibrosis**; osteoporosis; conjunctivitis; alopecia; depigmentation; menstrual dysfunction; encephalopathy; infertility; lymphoma; photosensitivity
Mitomycin (*Mutamycin* – Bristol-Myers Squibb Oncology, and others)	Nausea and vomiting; tissue necrosis; fever	**Bone marrow depression** (cumulative) with delayed leukopenia and thrombocytopenia; stomatitis; alopecia; acute pulmonary toxicity; pulmonary fibrosis; cardiotoxicity; hepatotoxicity; renal toxicity; amenorrhea; hemolytic-uremic syndrome; bladder contracture (with intravesical administration)
Mitotane (*o,p′*-DDD; *Lysodren* – Bristol-Myers Squibb Oncology)	Nausea and vomiting; diarrhea	**CNS depression**; rash; visual disturbances; adrenal insufficiency; hematuria; hemorrhagic cystitis; albuminuria; hypertension; orthostatic hypotension; cataracts; prolonged bleeding time
Mitoxantrone HCl (*Novantrone* – Immunex)	Blue-green pigment in urine; blue-green sclera; nausea and vomiting; fever; phlebitis	**Bone marrow depression**; cardiotoxicity; alopecia; white hair; skin lesions; hepatic damage; renal failure; extravasation necrosis; stomatitis
Nilutamide (*Nilandron* – Aventis)	Nausea and vomiting; hot flashes; alcohol intolerance	Delayed adaptation to darkness; hepatic toxicity; gynecomastia; hypersensitivity pneumonitis
Octreotide (*Sandostatin* – Novartis)	Nausea and vomiting; diarrhea	Steatorrhea; gallstones

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Oxaliplatin (*Eloxatin* – Sanofi-Synthelabo)	Peripheral sensory neuropathy; pharyngolaryngeal dysesthesias; paresthesias; nausea and vomiting; rare cases of anaphylaxis (reduced systolic blood pressure, flushing, tachycardia, respiratory distress)	Bone marrow depression; diarrhea; persistent neuropathy
Paclitaxel (*Taxol* – Bristol-Myers Squibb Oncology)	Hypersensitivity reactions	**Bone marrow depression**; peripheral neuropathy; alopecia; arthralgias; myalgias; cardiac toxicity; mucositis; onycholysis
Pegaspargase (PEG-L-asparaginase; *Oncaspar* – Aventis)	Similar to asparaginase	Similar to asparaginase
Pemetrexed (*Alimta* – Lilly)	Diarrhea; rash	**Neutropenia**; stomatitis; thrombocytopenia
Pentostatin (2′-deoxycoformycin; *Nipent* – SuperGen)	Nausea and vomiting; rash	Nephrotoxicity; CNS depression; **bone marrow depression**; respiratory failure; hepatic toxicity; arthralgia; myalgia; photophobia; conjunctivitis
Procarbazine HCl (*Matulane* – Sigma-Tau, Natulan in Canada)	Nausea and vomiting; CNS depression; disulfiram-like effect with alcohol; adverse interactions typical of a monoamine oxidase (MAO) inhibitor	**Bone marrow depression**; stomatitis; peripheral neuropathy; pneumonitis; leukemia; rash
Raltitrexed[b] (*Tomudex* – AstraZeneca)	Vomiting; nausea; rash; diarrhea	**Leukopenia**; thrombocytopenia; mucositis; malaise; elevated liver function tests; anorexia
Rituximab (*Rituxan* – IDEC Pharmaceuticals/Genentech)	Fever; chills; nausea; vomiting; headache; myalgia; pruritus; rash; pain at sites of disease; severe hypersensitivity reactions (hypontension, bronchospasm, angioedema); cardiac arrhythmias; renal failure	Bone marrow depression; mucocutaneous reactions including Stevens-Johnson syndrome and toxic epidermal necrolysis
Streptozocin (*Zanosar* – Pharmacia)	Nausea and vomiting; local pain	**Renal damage**; hypoglycemia; hyperglycemia; liver damage; diarrhea; bone marrow depression (uncommon); fever; eosinophilia; nephrogenic diabetes insipidus
Tamoxifen citrate (*Nolvadex* – AstraZeneca, and others; Tamofen in Canada)	Hot flashes; transient increased bone or tumor pain	Thromboembolism; vaginal bleeding and discharge; hypercalcemia; thrombocytopenia; peripheral edema; headache; decreased visual acuity; cataracts; purpuric vasculitis; uterine adenocarcinoma and sarcoma
Temozolomide (*Temodar* – Schering)	Nausea and vomiting; headache	Bone marrow depression, especially thrombocytopenia and neutropenia; asthenia; fatigue
Teniposide (*Vumon* – Bristol-Myers Squibb Oncology)	Severe hypersensitivity reactions; nausea and vomiting; diarrhea; phlebitis at infusion site	**Bone marrow depression**; alpoecia; mucositis; rash; hepatic toxicity; leukemia
Thalidomide (*Thalomid* – Celgene)	Rash; pruritus; headache; dizziness; drowsiness; somnolence; constipation; rarely toxic epidermal necrolysis	Severe birth defects; peripheral neuropathy; neutropenia; venous thrombosis
Thioguanine (GlaxoSmithKline; *Lanvis* in Canada)	Occasional nausea and vomiting; diarrhea	**Bone marrow depression**; hepatic damage; stomatitis
Thiotepa (*Thioplex* – Immunex)	Nausea and vomiting; rare hypersensitivity reaction	**Bone marrow depression**; menstrual dysfunction; interference with spermatogenesis; leukemia; mucositis with high doses
Topotecan (*Hycamtin* – GlaxoSmithKline)	Nausea and vomiting; diarrhea; headache; flu-like symptoms	**Bone marrow depression**; asthenia; stomatitis; alopecia; abdominal pain
Toremifene (*Fareston* – Shire)	Hot flashes; nausea and vomiting	Vaginal bleeding and discharge; peripheral edema; dizziness; increased aminotransferase activity; hypercalcemia; thromboembolic events (rarely)
Tositumomab (*Bexxar* – Coulter)	Nausea; vomiting; pruritus; hypotension; fever; chills	**Neutropenia; thrombocytopenia**; anorexia; fatigue; myalgia; arthralgia; increased incidence of leukemia and myelodysplasia

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Trastuzumab (*Herceptin* – Genentech)	Fever; chills; rigors; nausea and vomiting; headache; rash; hypotension; hypersensitivity reactions (hypotension, angioedema, tachycardia, respiratory distress)	Cardiac dysfunction, including congestive heart failure, particularly when combined with an anthracycline; diarrhea
Tretinoin (*Vesanoid* – Roche)	**Headache; xerosis**; pruritus; "retinoic acid syndrome" (fever, dyspnea, pulmonary infiltrates, pleural effusions, peripheral edema, hypotension); arthralgias; myalgias	**Cheilitis; teratogenicity; rashes**; leucocytosis; hypertriglyceridemia; pseudotumor cerebri; thrombophlebitis
Trimetrexate (*Neutrexin* – MedImmune Oncology)	Fever; chills; malaise; rash; pruritus; hyperpigmentation	**Bone marrow depression**; mucositis
Triptorelin (*Trelstar* Depot – Pharmacia)	Pain at injection site; tumor flare; bone pain; pain at injection site; nausea; pruritus; headache; hot flashes	Decreased libido and impotence; emotional lability; depression; gynecomastia; decreased bone mineral density
Vinblastine sulfate (*Velban* – Lilly, and others; Velbe in Canada)	Nausea and vomiting; local reaction and tissue damage with extravasation	**Bone marrow depression**; alopecia; peripheral neuropathy; stomatitis; jaw pain; muscle pain; paralytic ileus
Vincristine sulfate (*Oncovin* – Lilly, and others)	Tissue damage with extravasation	**Peripheral neuropathy**; alopecia; mild bone marrow depression; constipation; paralytic ileus; jaw pain; inappropriate ADH secretion; optic atrophy
Vinorelbine tartrate (*Navelbine* – GlaxoSmithKline)	Nausea and vomiting; injection site reactions (erythema, discoloration, phlebitis, pain)	**Bone marrow depression**; alopecia; anorexia; stomatitis; asthenia; peripheral neuropathy; constipation; myalgias

[a]Dose-limiting effects are in bold type. Cutaneous reactions (sometimes severe), hyperpigmentation, and ocular toxicity have been reported with virtually all nonhormonal anticancer drugs. For adverse interactions with other drugs, see The Medical Letter *Handbook of Adverse Drug Interactions*, 2003.
[b]Available in the United States at this time for investigational use only. (Reproduced with permission from (2003) *Treatment Guidelines*, vol. 1(7), pp. 41–52; © Medical Letter.)

See also: Androgens; Arsenic; BCNU (Bischloroethyl Nitrosourea); Carcinogenesis; Cisplatin; Corticosteroids; Cyclophosphamide; Gallium; Mitomycin C; Nitrogen Mustard; Platinum.

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Cancer Potency Factor

Anna M Fan

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In the evaluation of carcinogenicity of chemicals, data obtained from human and animal studies are analyzed for hazard identification and dose–response relationships. The results are used in combination with exposure assessment and risk characterization for the assessment of cancer risks of the chemicals to humans.

Cancer risk assessment involves a quantitative estimate of the carcinogenic activity of a carcinogen. For genotoxic carcinogens, this estimate is derived from the cancer potency of the carcinogen. Cancer potency is defined as the slope of the dose–response curve for induction of tumors, and is a function of the dose and the magnitude of response, measured as a slope. The endpoint is the cancer incidence or frequency of occurrence of cancer (tumor induction) in

the test population. Potency may also be expressed in terms of an effective dose (ED) or lowest effective dose (LED). A less commonly used approach defines potency as a function of the breadth of biological effects (i.e., number of sites or species) that can be affected by the chemical. In general, data on the incremental increase in an effect proportional to an incremental increase in dose above the background is used to establish the dose–response curve for the toxic effects of chemicals. The slope of the curve expresses extra risk per dose unit, or risk per milligram per kilogram body weight per day.

Most of the data available for determining carcinogenicity of chemicals are based on the results of animal testing. Mathematical models have been developed for the calculation of the cancer potency or prediction of cancer potency at low doses from these animal bioassay data. These are statistical models designed to determine the shape and slope of the dose–response curve taking into consideration biological plausibility and mode of action. The most common one used by regulatory agencies in the United States is the multistage model constructed based on multistage carcinogenesis and the absence of a carcinogenic threshold. This is a default model used in the absence of additional information (e.g., mode of action) that warrants the inclusion of additional parameters such as for analysis.

The equation for the multistage model is as follows, estimating the probability of developing cancer from exposures equivalent to a daily dose d:

$$P(d) = 1 - \exp - (q_0 + q_1 d + q_2 d^2 + \cdots + q_k d^k)$$

where $P(d)$ is the probability or risk of cancer, 'exp' is the natural base 'e' raised to the exponent in parentheses, d is the dose, and k, q_0, q_1,…, q_k are parameters. For the low-dose range of interest the equation reduces to approximately the linear term of the exponent, represented by q_1 (the slope of the low dose, linear portion of the curve) times the dose.

Cancer potency is taken as the upper 95% confidence limit (UCL) on the linear term q_1, which is called the cancer slope factor (CSF) or q_1^*, in units of $(\text{mg/kg-day})^{-1}$, derived using maximum likelihood estimate techniques. Cancer risks at low doses equals 'dose $\times$ q_1^*' and the dose associated with a specific risk equals 'risk/q_1^*'. The calculated risks are upper bound estimates and calculated doses are lower bound estimates. These are further summarized below.

$$\begin{aligned}\text{Cancer potency} &= 95\%\ \text{UCL of the slope of the low dose, linear portion of the dose-response curve (using multistage model)}\\ &= \text{Cancer slope factor (CSF), or } q_1^*\ (\text{mg/kg-day})^{-1}\text{, which relates dosage to the probability of an individual developing cancer}\\ \text{Cancer risk} &= \text{dose} \times q_1^*\\ \text{Dose} &= \text{risk}/q_1^*(\text{mg/kg-day})^{-1}\end{aligned}$$

The choice of models for a chemical depends on two factors: (1) the hypothesis for the mechanism of carcinogenesis for that chemical; and (2) science policy. In the absence of definitive data supporting one model or another, the more conservative model among biologically plausible models may be used, or results from a range of plausible models are presented. The choice of the low-dose extrapolation model (Weibull, probit, multistage) can have a major impact on the estimate of risk at low exposure levels, sometimes varying by orders of magnitude at the same exposure level.

The linearized multistage model is used on the basis of its biological plausibility as it assumes no threshold, and its conservatism, as it is unlikely to underestimate risk at low exposure levels to develop CSFs. The probit, logistic, and Weibull models are also used to describe quantal–response toxicity data. Although these models generally provide similar fits to data from dose–response experiments within the observable response range, they can differ appreciably when extrapolated to very low doses. The shape of the dose–response curve may be linear, sublinear, or supralinear for the logistic and Weibull models, whereas the probit model exhibits a sublinear behavior in the low-dose region regardless of the values of the model parameters.

The cancer potency factor is used to predict cancer risk. Risk can be predicted by multiplying the cancer potency factor by the daily dose, averaged over lifetime (mg/kg-day).

In using animal data to extrapolate to humans, the basic assumption is that carcinogenic effects in animal studies indicate that the agent under study can have carcinogenic potential in humans. For exposure by the oral route, the default assumption is that delivered doses are related to applied dose by a power of body weight based on similarities of mammalian physiology, anatomy, and biochemistry generally observed across species. Oral slope factors incorporate a cross-species scaling factor based on equivalence of $\text{mg/kg}^{3/4}$-day. When oral potency is derived from animal data, the equivalent human oral dose uses the default procedure to scale a daily-applied dose for a lifetime in proportion to body weight

raised to the 0.75 power ($W^{0.75}$):

$$\text{Dose, human} = \text{dose} \times (\text{BW}_{\text{human}}/\text{BW}_{\text{animal}})^{0.75}$$

or

$$\text{Cancer potency, human} = q_{\text{human}} = q_{\text{animal}} \times (\text{BW}_{\text{human}} \times \text{BW}_{\text{animal}})^{0.25}$$

For dose estimation, physiologically based pharmacokinetic (PBPK) modeling has been used as a tool for tissue dosimetry, or to obtain a better estimate of the ED than the administered dose, using model parameters measured in or estimated from experiments. The models describe the pharmacokinetic behavior of toxic chemicals and predict the dose of reactive metabolites reaching the target tissues. PBPK modeling provides mathematical descriptions of the update and disposition of chemicals based on quantitative interrelations among the biological parameters of these processes. The models represent the body as compartments and require information on such determinants as partition coefficients, blood flow, tissue volume, and metabolic parameters. In cancer risk assessment, the modeling may help to establish a relationship in terms of a cancer dose–response from animal or human data, relating exposure to ED, and estimating risk as a function of exposure. Use of PBPK modeling in cancer risk prediction requires the identification of activation pathways associated with carcinogenesis and a kinetic model (e.g., Michaelis–Menten). Such models have been used in cross-species, route and dose extrapolations. Accurate parameterization is fundamental in using PBPK models.

Similarly, unit risk estimates for lifetime exposures can be made for inhalation (risk per $\mu g\,m^{-3}$ air breathed) and drinking water (risk per $\mu g\ l^{-1}$ water). Unit risks are potencies in terms of the equation above, risk = dose $\times\ q_1^*$, and in this case

$$\text{Risk} = \text{intake} \times \text{unit risk}$$

For the oral route, US Environmental Protection Agency (EPA) calculates both a slope factor (formerly referred to as potency factor) and a unit risk. The oral slope factor expresses the risk per mg/kg-day and the unit risk is a numerically equivalent term expressed as the risk associated with a drinking water concentration of the chemical at $1\,\mu g\,l^{-1}$, based on the assumption of a 70 kg body weight and $2\,l\,day^{-1}$ of water consumption. For the inhalation route, the US EPA only expresses the risk in terms of a unit risk expressed as risk per $1\,\mu g\,m^{-3}$, or the risk associated with an air concentration of $1\,\mu g\,m^{-3}$, assuming that a 70 kg adult breathes $20\,m^3\,day^{-1}$. For estimating cancer risk from inhalation or oral exposure, toxicological data specific to the exposure route of interest are preferred. Extrapolation from one route to another may be appropriate in some but not all cases. Related parameters used in estimating risk (e.g., inhalation rate versus water consumption rate, percent absorption via inhalation versus oral intake) need to be modified as appropriate.

Oral slope factors and unit risk estimates for lifetime exposures incorporate exposure factors that are based on adult parameters and adjustments have to be made when assessing risks from less than lifetime exposures that occur, for example, during childhood.

Animal studies are conducted at dose levels much higher than the environmental levels to which humans are exposed, and the accuracy of prediction in environmental level is unknown.

Potency estimates derived from such animal studies help to characterize the dose–response relationship at the low-exposure levels to which humans are likely to be exposed and to predict the quantitative estimate of the risks that humans are likely to encounter at ambient exposures. Experimental evidence for various shapes of the dose–response curve for carcinogens showed that reliable high-dose data from human studies contain examples of superlinearity, linearity, and sublinearity. These are also seen in animal studies. But there are no data to indicate the shape of the dose–response relationship corresponding to lifetime risk of one in a million, the insignificant risk level generally used by the regulatory agencies.

The US EPA in its revised draft cancer risk assessment guidelines has revised the approach to dose–response assessment in consideration of the uncertainties in dose–response modeling for low-dose cancer risk and the increasing acceptance of a threshold dose–response relationship for some carcinogens, generally nongenotoxic carcinogens. New risk assessments are being conducted consistent with the proposed guidelines.

If sufficient data are available to support the use of a biologically based dose–response model, it may represent the most appropriate method for using the observed data to extrapolate to exposure below the observed dose range. If data are not available for a biologically based model, which is the case for the majority of chemicals studied, a 'point of departure' (POD) approach is recommended. The POD represents a dose within the range of observed data associated with a 10% extra tumor

risk. It is developed using the linearized multistage model (or the most appropriate model based on the shape of the dose–response curve) and expressed at the lower 95% confidence limit on the dose with the 10% extra risk (LED_{10}). Risks below the LED_{10} are characterized either through linear extrapolation (for chemicals believed to act via a linear dose–response relationship, or genotoxic carcinogens) or through a margin of exposure analysis (for chemicals whose dose–response relationship is likely to be threshold or nonlinear). For chemicals where data might support either a linear extrapolation or a margin of exposure approach, both analyses are to be presented. Potencies calculated are based on $0.1/LED_{10}$.

Overall, cancer risk assessment involves the four steps of hazard identification, dose–response, exposure assessment, and risk characterization. The dose–response curve established for cancer potency derivation for a chemical is based on evaluation of data on the carcinogenicity and dose–response characteristics of the chemical. The pharmacokinetics and mechanistic data evaluation (e.g., genotoxic or nongenotoxic) and a dose–response review of all adequate bioassays are conducted to determine, if target dose estimates or a dose–response model different from the default may be suggested.

Inherent in the default potency derivation are the following assumptions: that the dose–response relationship in the most sensitive species is representative of that in humans; low-dose potency estimate can be derived from using the multistage polynomial to extrapolate potency outside the range of the experimental observations; surface area scaling is used as an interspecies factor; tissue dose resulting in a tumor is proportional to the administered dose; and cancer hazard increases with the third power of age. Based on the above and tumor findings, potency is derived from data on malignant tumors, combined malignant, and benign tumors or tumors that are believed to progress to malignancy.

Most of the risk assessments are performed for decision-making such as setting exposure limits for a chemical. The human cancer potency (q_{human}) derived from oral route exposure data can be used to estimate chemical exposure or intake associated with a given level of cancer risk based on the following:

$$I\ (\text{intake } \mu\text{g day}^{-1}) = \frac{10^{-6} \times 70\ \text{kg} \times 1000\ \mu\text{g mg}^{-1}}{q_{\text{human}}\ (\text{mg/kg-day})^{-1}}$$

The intake value can then be converted to an air or drinking water equivalent for the corresponding environmental standards. It is important that cancer risk assessments be performed by trained risk assessors who have the training, experience, and understanding of the toxicological principles and the risk assessment methodologies involved.

See also: Carcinogenesis; Dose–Response Relationship; Risk Assessment, Human Health; Uncertainty Factors.

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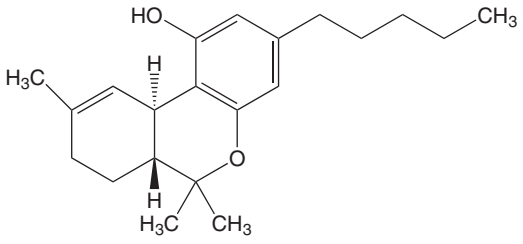
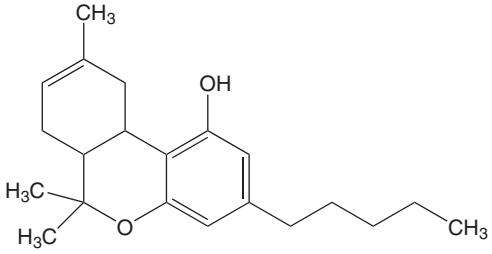
Cannabinoids

Jaya Chilakapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 8063-14-7
- Chemical/Pharmaceutical/Other Class: Hallucinogenic substances
- Chemical Structures:



[Delta]^9-tetrahydrocannabinol



[Delta]^8-tetrahydrocannabinol

Representative Compounds

Cannabinoids are aryl-substituted meroterpenes unique to the plant genus *Cannabis*. The pharmacology of most of the cannabinoids is largely unknown but the most potent psychoactive agent, [Delta]^9-tetrahydrocannabinol ([Delta]^9-THC, or THC), has been isolated, synthesized, and much studied. Other plant cannabinoids include [Delta]^8-THC, cannabinol, and cannabidiol.

Sources

Cannabinoids are present in the stalks, leaves, flowers, and seeds of the plant, and also in the resin secreted by the female plant. A 'joint' made out of skunkweed, netherweed, and other potent subspecies of *Cannabis sativa* may contain ~150 mg of THC, or 300 mg if laced with hashish oil.

Uses

Delta-9-THC and some synthetic analogs are used therapeutically, for example, for nausea and vomiting produced by antineoplastic chemotherapy, analgesic, anticonvulsant for epilepsy, anti-inflammatory agent, appetite stimulant for patients with AIDS, as well as treatment for conditions such as asthma and glaucoma. Synthetic cannabinoids used therapeutically include dronabinol, nabilone, and levonamtradol.

Exposure Routes and Pathways

The usual route of administration for medical purposes is oral. The commonest way of abusing it is by inhalation.

Toxicokinetics

About 50% of the THC in a 'joint' of herbal cannabis is inhaled in the mainstream smoke; nearly all of this is absorbed through the lungs, rapidly enters the bloodstream, and reaches the brain within minutes. Effects are perceptible within seconds and fully apparent in a few minutes. Bioavailability after oral ingestion is much less; blood concentrations reached are 25–30% of those obtained by smoking the same dose, partly because of first-pass metabolism in the liver. The onset of effect is delayed (0.5–2 h) but the duration is prolonged because of continued slow absorption from the gut. Once absorbed, THC and other cannabinoids are rapidly distributed to all other tissues at rates dependent on the blood flow. Because they are extremely lipid soluble, cannabinoids accumulate in fatty tissues, reaching peak concentrations in 4–5 days. They are then slowly released back into other body compartments, including the brain. Because of the sequestration in fat, the tissue elimination half-life of THC is ~7 days, and complete elimination of a single dose may take up to 30 days. Clearly, with repeated dosage, high levels of cannabinoids can accumulate in the body and continue to reach the brain. Within the brain, THC and other cannabinoids are differentially distributed. High concentrations are reached in neocortical, limbic, sensory, and motor areas.

Cannabinoids are metabolized in the liver. A major metabolite is 11-hydroxy-THC, which is possibly more potent than THC itself and may be responsible for some of the effects of cannabis. More than 20 other metabolites are known, some of which are psychoactive and all of which have long half-lives of

several days. The metabolites are partly excreted in the urine (25%) but mainly into the gut (65%) from which they are reabsorbed, further prolonging their actions. Because of the pharmacokinetic characteristics of cannabinoids – both the sequestration in fat and the presence of active metabolites – there is a very poor relationship between plasma or urine concentrations and degree of cannabinoid-induced intoxication.

Mechanism of Toxicity

Cannabinoids exert their effect by interaction with specific endogenous cannabinoid receptors. Neuronal cannabinoid receptors are termed CB_1 receptors and have been found in rat, guinea pig, dog, monkey, pig, and human brains and peripheral nerves. A second cannabinoid receptor, the CB_2 receptor, was identified in macrophages in the spleen and is also present in other immune cells. The distribution of CB_1 receptors is very similar to that of the distribution of injected THC and includes cerebral cortex, limbic areas (including hippocampus and amygdala), basal ganglia, cerebellum, thalamus, and brainstem.

The discovery of cannabinoid receptors naturally stimulated a search for an endogenous ligand with which the receptors naturally interact. Such a substance was isolated from the pig brain. It was found to be chemically different from plant cannabinoids: it is a derivative of the fatty acid arachidonic acid (arachidonyl ethanolamide) related to the prostaglandins. This endogenous substance was named anandamide after the Sanskrit word for bliss, *ananda*. It has a high affinity for CB_1 receptors and has most of the actions of THC.

Acute and Short-Term Toxicity (or Exposure)

Animal

With THC, the oral LD_{50} in mice is $482\,mg\,kg^{-1}$, the rat oral LD_{50} is $666\,mg\,kg^{-1}$, and the intravenous LD_{50} is $29\,mg\,kg^{-1}$. Delta-9-THC and other cannabinoids with psychoactive effects in man have particularly unusual effects on the overt behavior of dogs. At dose levels that elicit blood concentrations of THC similar to those found in regular human marijuana users, THC markedly disrupts the menstrual cycle in the rhesus monkey. Naturally occurring cannabinoids, unique to the plant *Cannabis sativa* and constituting 15% of the cannabis by weight, have been implicated as immunomodulatory. Delta-9-THC has been studied to characterize its immunosuppressive properties and studies have shown that it suppresses both humoral and cell-mediated immunity in experimental animals.

Human

High levels of intoxication are associated with decreased motor coordination, muscle strength, and hand steadiness. Lethargy, sedation, poor concentration ability, slurred speech, ataxia, and an increase in reaction time may also occur. High doses of delta 9-THC can induce frank hallucinations, delusions, and paranoid feelings. Thinking becomes confused and disorganized; depersonalization and altered time sense are accentuated. Anxiety reaching panic proportions may replace euphoria, often as a result of the feeling that the drug-induced state will never end. The most consistent effects on the cardiovascular system are an increase in heart rate, an increase in systolic blood pressure while supine, decreased blood pressure while standing, and a marked reddening of the conjunctivae.

Chronic Toxicity (or Exposure)

Animal

Under the conditions of 2 year gavage studies, there was no evidence of carcinogenic activity of 1-*trans*-delta9-tetrahydrocannabinol in male or female F344/N rats administered 12.5, 25, or $50\,mg\,kg^{-1}$. There was equivocal evidence of carcinogenic activity of THC in male and female B6C3F1 mice based on the increased incidences of thyroid gland follicular cell adenomas in rats treated with 125 mg THC per kilogram.

Human

Chronic use may be associated with the induction of 'amotivational syndrome' and loss of memory. Endocrine effects have been reported following chronic use, including impairment of gonadotrophin secretion (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)), reduction in testosterone levels, and direct effect on cytochrome P-450 of the Leydig cells with inhibition of testosterone synthesis. Abrupt discontinuation of chronic THC use has resulted in a mild abstinence syndrome, consisting of agitation, apprehension, and aggressiveness, as well as tremulousness, insomnia, and diaphoresis, and the development of common migraine headaches.

Clinical Management

Activated charcoal is administered as a slurry. Depressive, hallucinatory, or psychotic reactions

should be treated by placing the patient in a quiet area and providing them with reassurance that no permanent effects will occur. Benzodiazepines are preferred drugs for treatment of extreme agitation. When psychotic phenomena predominate, haloperidol 5 mg i.m. is recommended. The patient should be kept well hydrated.

Exposure Standards and Guidelines

FDA requirements: tetrahydrocannabinol is a chemical derivative of cannabis (marihuana), named in section 502(d) of the Federal Food, Drug, and Cosmetic Act, and is thereby designated as habit forming.

See also: Benzodiazepines; Marijuana.

Further Reading

Adams IB and Martin BR (1996) Cannabis: Pharmacology and toxicology in animals and humans. *Addiction* 91: 1585–1614.

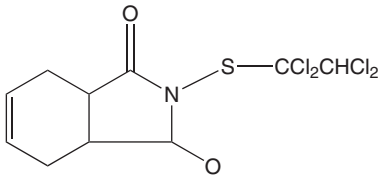
Howlett AC, Breivogel CS, Childers SR, *et al.* (2004) Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology* 47: 345–358.

Cannabis *See* Cannabinoids.

Captafol

Priya Raman

- Chemical Abstracts Service Registry Number: CAS 2425-06-1
- Synonym: 3*a*,4,7,7*a*-Tetrahydro-2-[(1,1,2,2-tetrachloroethyl)thio]-1*H*-isoindole-1,3(2*H*)-dione
- Chemical/Pharmaceutical/Other Class: Phthalimide fungicide
- Chemical Structure:



Uses

Captafol is a widely used broad-spectrum contact fungicide belonging to the class of sulfanilamides. It is effective for the control of a wide variety of fungal diseases in plants and is widely used outside the United States to control foliage and fruit diseases on apples, citrus, tomato, cranberry, sweet corn, barley, wheat, and several other plants. Captafol is also extensively used as a seed protectant in cotton, peanuts, and rice. It is also used to reduce losses from wood rot fungi in logs and wood products.

Exposure Routes and Pathways

Dermal and ocular exposures are the most common routes of exposure to captafol. Contact dermatitis has been reported after exposure to captafol. During occupational exposure, captafol has been reported to cause severe irritation of the respiratory tract, eye damage and other systemic effects. Oral ingestion of captafol is unlikely to cause acute poisoning.

Toxicokinetics

Captafol is poorly absorbed from the gastrointestinal tract. The liver and the gastrointestinal tract are the primary sites of metabolism of captafol. Captafol is eliminated via urine, feces, and expired air. The major single metabolite, tetrahydrophthalimide (THPI), was detected in blood, urine, and feces, but most of the activity in the blood and urine was in the form of more water-soluble metabolites. Following oral administration in animals, captafol is hydrolyzed to THPI and dichloroacetic acid. THPI is degraded to tetrahydrophthalimidic acid and further down to phthalic acid and ammonia.

Mechanism of Toxicity

The primary toxicity following captafol exposure probably occurs through a hypersensitivity mechanism.

Acute and Short-Term Toxicity (or Exposure)

Animal

The reported acute oral LD_{50} of captafol $6780\,mg\,kg^{-1}$ in male rats and $6330\,mg\,kg^{-1}$ in female rats. The rabbit dermal LD_{50} is reported to be $15\,400\,mg\,kg^{-1}$, showing moderate dermal irritation at 72 h with severe dermal sensitization. Another test for captafol-induced eye irritation in rabbit showed corneal opacity and iris and conjuctival irritation, all symptoms being present for 21 days. Captafol was reported to be teratogenic and to cause fetal developmental abnormalities at high (maternally toxic) doses in hamsters. Teratogenicity studies in rabbits indicated a teratogenic no-observed-effect level (NOEL) $>50\,mg\,kg^{-1}\,day^{-1}$ and a fetotoxic NOEL of $16.5\,mg\,kg^{-1}\,day^{-1}$. Captafol was, however, found to have no effect on embryonic development in rabbits and monkeys.

Human

The primary symptoms of captafol exposure reported in humans include contact dermatitis and conjunctivitis. The reaction may be severe and may include stomatitis and painful bronchitis. Persons with a skin rash following exposure to captafol were found to have systemic as well as dermal disorders. Hypertension was reported in patients with marked edema. Other findings following captafol exposure include protein and urobilinogen in the urine, depression of liver function, anemia, and depression of blood cholinesterase activity. Acute oral or dermal exposure to captafol rarely results in severe toxicity. However, due to a higher level of toxicity in animal models following intraperitoneal exposure, parenteral exposure may present a greater hazard potential. Captafol has been classified as a group 2A probable human carcinogen.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to captafol at dietary levels of 1500 and 5000 ppm demonstrated growth depression, some liver and kidney changes, as well as an increased mortality. Following exposure to 300 or $100\,mg\,kg^{-1}$ of captafol, dogs suffered frequent vomiting and diarrhea during the first 4 weeks and were observed to be slightly anemic and deficient in growth during a 2 year study. Dogs at dosages of $30\,mg\,kg^{-1}$ or greater developed both absolute and relative increases in the weights of the liver and kidney. Oral administration in mice produced a high incidence of adenocarcinomas of the small intestine, vascular tumors of the heart, and spleen and hepatocellular carcinomas. In a 2 year rat feeding study, a dose-related increased incidence of neoplastic nodules in the liver of females was reported. The US Environmental Protection Agency reported an NOEL for nononcogenic effects at 56 ppm based on a chronic toxicity study in rats. There is sufficient evidence in experimental animals for carcinogenicity of captafol.

Human

Captafol is also known to be a skin sensitizer and has been reported to cause both allergic and contact dermatitis in humans. Breakdown products may contribute to the skin irritation and sensitization associated with captafol.

Clinical Management

Exposed eyes and skin should be flushed with copious amounts of water. In case of an inhalation exposure, the patient should be monitored for respiratory distress. Artificial ventilation may be provided and symptomatic treatment may be administered as necessary.

Environmental Fate

Captafol is not persistent in the environment. Captafol is stable under ordinary environmental conditions and rapidly degrades in soil, the rate of degradation being a function of soil type and pesticide concentration. It does not leach from basic soils and is unlikely to contaminate groundwater. Captafol sprayed on most crops has a half-life of less than 5 days. Captafol and/or its metabolites and degradates are readily absorbed by roots and shoots of plants.

Ecotoxicology

Avian toxicity for captafol is low, the LD_{50} being greater than 2510 ppm. However, high levels of exposure can cause reproductive impairment. Captafol is characterized as being very highly toxic to both cold-water and warm-water fish, 96 h LC_{50} being 0.027–0.50 and $0.045–0.230\,mg\,l^{-1}$ in rainbow trout and bluegill sunfish, respectively. It is considered only moderately to very highly toxic to freshwater invertebrates. Captafol is considered nontoxic to bees.

Exposure Standards and Guidelines

Captafol is a general use pesticide with a toxicity classification of IV (relatively nontoxic). It is classified a as 'restricted use' pesticide in the United States. It is no longer sold in the United States. The Occupational Safety and Health Administration threshold limit value for captafol is reported to be $0.1\,mg\,m^{-3}$.

Miscellaneous

Captafol is a colorless to pale yellow in color with a distinct odor. It has a molecular weight of 349.1, a water solubility of 1.4 mg l^{-1} at 20°C and a melting point of 160–162°C. Some common trade names of products containing captafol include Crisfolatan, Difolatan, Difosan, Folcid, Haipen, Kenofol, Pillartan, and Sanspor.

See also: Pesticides.

Further Reading

Tamano S, Kawabe M, Sano M, Masui T, and Ito N (1993) Oral Toxicity Study of Captafol in B6C3F1Mice. *Journal of Toxicology and Environmental Health* 38(1): 69–75.

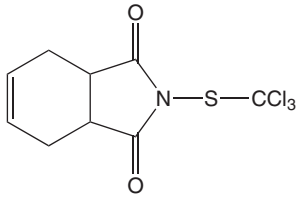
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Captafol.

Captan

Xun Song

- Chemical Abstracts Service Registry Number: CAS 133-06-2
- Synonyms: 3a,4,7,7a-Tetrahydro-2-[(trichloromethyl)thio]-1*H*-isoindole-1,3(2*H*)-dione; 1,2,3,6-Tetrahydro-*N*-(trichloromethylthio)phthalimide; Captano (Italy); Captane (France); Captex; Hexacap; Kaptan; Orthocide®; Vancide 89®
- Chemical/Pharmaceutical/Other Class: Phthalimide
- Chemical Structure:



Uses

Captan is widely used as a fungicide. The main application is for foliage protection in agriculture.

Exposure Routes and Pathways

Exposure to captan may occur through dermal, oral, or inhalation route during manufacture or application of captan, or consumption of agricultural products with captan residues.

Toxicokinetics

Captan is readily excreted after either oral or systemic dosing. Twenty-four hours after treatment in rats, ~75% of captan is eliminated in urine and 6.5% in the feces. Nearly complete elimination occurs within 36 h. There are little gender differences in biotransformation. A small portion of captan given orally was metabolized into thiozolidine-2-thione-4-carboxylic acid, a salt of dithiobis(methanesulfonic acid) and the disulfide monoxide derivative of dithiobis(methanesulfonic acid).

Mechanism of Toxicity

Liver enzymes were modulated after repeated captan exposures at relatively high dosages. Captan led to the breakdown of the inner membrane of mitochondria. *In vitro* studies showed that captan caused swelling of mitochondria in rat liver and loss of intracellular potassium in human erythrocytes. Captan inhibits mitochondrial function nonspecifically, leading to uncoupling of oxidative phosphorylation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Generally, captan has been found to have low toxicity to laboratory animals after oral dosing. Oral LD_{50} values greater than 5000 mg kg^{-1} have been reported in rats. Oral LD_{50} values of 7840 and 7000 mg kg^{-1} were reported for male and female mice. Captan was more potent by intraperitoneal administration (LD_{50} values of 462–518 and 35–40 mg kg^{-1} in rats and mice, respectively). Dermal LD_{50} values greater than 2000 mg kg^{-2} were reported in rabbits.

Human

Captan has a low acute toxicity potential. Sensitivity, e.g., dermatitis, to captan exposure has been reported. Captan is a weak eye irritant.

Chronic Toxicity (or Exposure)

Animal

Dietary captan (10 000 ppm for 54 weeks) caused marked growth depression in both male and female rats. Captan feeding at 5000 ppm for 2 years ($\sim 50\,mg\,kg^{-1}\,day^{-1}$) led to growth depression in female but not male rats. Testicular atrophy was observed at autopsy in some animals consuming 10 000 ppm captan in the diet.

At high dosages, captan increased tumorous cancer in female mice and in male rats. Captan is similar in structure to other pesticides (folpet and captafol) that also cause cancer in test species. Tumors were localized to the gastrointestinal tract and kidneys. Teratogenicity studies with rats, rabbits, hamsters, and dogs have given both negative and positive results.

Human

Evidence indicates that captan is not a teratogen in humans.

In Vitro Toxicity Data

Captan is not mutagenic in most assays.

Clinical Management

Intoxication after acute captan exposure is unlikely; treatment is symptomatic.

Environmental Fate

Captan has a low persistence in soil (half-life of 1–10 days). Captan has little mobility in soils. Captan is rapidly degraded in surface waters at neutral pH. Captan is readily taken up into plant tissues.

Ecotoxicology

Captan is practically nontoxic to birds (LD_{50} 2–$5\,g\,kg^{-1}$). High captan exposures can reduce egg production in chickens but have no effect on fertility or hatching. Captan is very highly toxic to fish. The LC_{50} (96 h) for captan was $0.056\,mg\,l^{-1}$ in cutthroat trout and Chinook salmon and $0.072\,mg\,l^{-1}$ in bluegill. The LC_{50} in *Daphnia magna* was 7–$10\,mg\,l^{-1}$. Captan has a low to moderate tendency to bioaccumulate (concentration factor = 10–1000). Captan has relatively little toxicity in bees.

Exposure Standards and Guidelines

The reference dose for captan is $0.13\,mg\,kg^{-1}\,day^{-1}$, the acceptable daily intake is $0.1\,mg\,kg^{-1}\,day^{-1}$, and the threshold limit value (8 h) is $5\,mg\,m^{-3}$.

See also: Pesticides.

Further Reading

Gordon EB (2001) Captan and folpet. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1711–1742. San Diego, CA: Academic Press.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Carbamate Pesticides*

Stephanie Padilla

Published by Elsevier Inc.

Chemical Structure and Uses

Carbamate compounds are usually subdivided into at least three main groups with respect to their structure and general use (see **Figure 1**): insecticides, herbicides, and thio- or dithiocarbamates. A variety of 'R' groups may be substituted in the molecule producing, as is the case for insecticides, a variety of alkyl or aryl esters of carbamic acid. Although technically characterized as carbamate pesticides, thio- and dithiocarbamate fungicides are not included in this

*The research described in this article has been reviewed by the National Health and Environmental Effects Research Laboratory, US EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency nor does mention of trade names and commercial products constitute endorsement or recommendation for use.

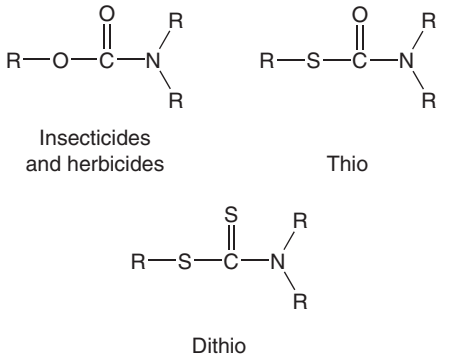


Figure 1 General formulas for carbamates.

brief overview because they have drastically different modes of action from the first group.

Background Information

Carbamate pesticides have a colorful and interesting history of discovery and development. Oral administration of calabar bean paste, which is rich in carbamate alkaloids, was used in West Africa to reveal the guilt or innocence of people accused of witchcraft. If the alleged 'witches' died after being forced to eat calabar bean paste, then they were indeed witches; if not, then they were declared innocent. Scientific investigation revealed that the active carbamate in the calabar bean was physostigmine. In fact, the synonym for physostigmine, eserine, comes from the West Africans' word for calabar bean, esere. In the mid to late 1940s, the first carbamate pesticides were synthesized in an effort to develop new insect repellents, but the insecticidal properties of this class of compound were quickly recognized and appreciated.

Exposure Routes and Pathways

Carbamates do not require hepatic activation for their toxicity. In other words, the parent compound is the active moiety. The majority of carbamate compounds are easily absorbed through mucous membranes and the respiratory and gastrointestinal tracts. Therefore, not only can carbamates be absorbed through the skin (dermal exposure) and lungs (inhalation exposure), but also through foods treated with carbamates (oral exposure). Most of the acute poisoning episodes in humans occurred via the dermal or inhalation route. Although the data are limited, the half-life of selected carbamate pesticides is short in mammals, for example, on the order of 8 h in the adult rat. In the latest NHANES survey of pesticides in the urine of Americans of various ages, there were very low levels of carbamate pesticides, with the most prevalent being carbaryl, an indication of a chronic low-level exposure to this carbamate.

Mechanism of Toxicity

Generally, carbamate compounds, like organophosphorus pesticides, exert their primary toxic action through the inhibition of acetylcholinesterase (EC 3.1.1.7), although it is well known that carbamates are inhibitors of many other esterases. The inhibition of acetylcholinesterase activity is thought to precipitate a toxic response through the short-term increase in the concentration of acetylcholine at cholinergic junctions (e.g., central nervous system, neuromuscular junction, all autonomic preganglionic and parasympathetic postganglionic synapses, and the sympathetic innervation of the adrenal and sweat glands). Carbamates interact with acetylcholinesterase in the same manner as the natural substrate, acetylcholine, except that the carbamate remains in the active site for a markedly longer period of time, thereby preventing the hydrolysis of acetylcholine and resulting in a net inhibition of the enzyme's activity. The carbamylation of the active site of acetylcholinesterase is a much more labile union than is phosphorylation by an organophosphate and does not lead to 'aging' of the enzyme as can inhibition by some organophosphorus compounds. Therefore, restoration of acetylcholinesterase activity (i.e., decarbamylation or reactivation) is highly likely with the carbamate-inhibited enzyme. That is why carbamates are often labeled 'reversible' inhibitors of acetylcholinesterase, that is, because enzyme activity is restored within hours without significant *de novo* synthesis of acetylcholinesterase. Actually, in a biochemical sense, carbamates are not 'reversible' inhibitors because the carbamate does not exit the active site intact; rather, the carbamate is hydrolyzed just as acetylcholine is hydrolyzed. Because of this unstable inhibition, great care must be taken when analyzing cholinesterase inhibition in tissues from carbamate-treated animals to prevent reactivation of the enzyme activity. Generally, carbamates do not cause peripheral neuropathy as do some organophosphorus compounds. Interestingly, carbamates may inhibit neurotoxic esterase activity (the 'first step' in the precipitation of the neuropathy), but do not 'age' (the definitive step in precipitation of the neuropathic response).

Acute and Short-Term Toxicity (or Exposure)

The overall toxicity profile of the carbamate pesticides covers a wide spectrum from virtually nontoxic to some of the most highly toxic pesticides in commercial use; carbamate LD_{50} values can range from 5000 to $1\,mg\,kg^{-1}$. More than 50 commercially available carbamate pesticides are in use today with

the highest volume usage attributed to butylate, carbofuran, methomyl, carbaryl, and benomyl. Generally, metabolites are less toxic than the parent compound, and the metabolites are commonly excreted in the urine. The general metabolic profile is basically the same in insects, plants, or animals. The first step in the catabolic scheme is usually hydrolysis to carbamic acid, but the mechanism of hydrolysis is different for *N*-methyl and *N*-dimethyl derivatives. In general, the predominant, acute effect is acetylcholinesterase inhibition, although there are reports of some carbamates causing disturbances of gonadotrophic function at relatively low doses.

Chronic Toxicity (or Exposure)

In addition to inhibition of acetylcholinesterase activity, carbamates have been reported to cause skin and eye irritation, hemopoietic alterations, degeneration of the liver, kidneys, and testes, as well as functional and histological changes in the nervous system after long-term, high-dose exposures. Moreover, some carbamates are known to produce reproductive and teratogenic effects. Fetuses of mothers dosed with a carbamate have been reported to exhibit increased mortality and decreased weight gain. Carbamates are also considered embryotoxic, and some have also been reported to be mutagenic, but they have little carcinogenic potential.

Clinical Management

Reported effects in humans have usually been confined to the expected cholinergic overstimulation. These signs and symptoms include salivation, lacrimation, diarrhea, nausea, tremors, pin-point pupils, bradycardia, tachycardia, headache, confusion, and, rarely, death. Signs and symptoms are reported within minutes of exposure and can last for hours, but because of the 'reversibility' of the inhibition, recovery is usually apparent within 24 h, depending on the severity of the dose. Metabolites in the urine or red blood cell cholinesterase activity may be used for biological monitoring (although potential for reactivation of inhibited enzyme must be carefully considered in the analysis). During the acute cholinergic crisis produced by these compounds, atropine (a muscarinic antagonist) may be used to counteract the effects, but oximes are typically contraindicated.

Environmental Fate

As a group, carbamates have a relatively low environmental persistence, in contrast to the organochlorine pesticides. Carbamates are broken down by microorganisms, plants, animals, soil, or water, and hydrolysis is accelerated by increased light intensity, temperature, and/or alkalinity. Therefore, carbamates rarely bioaccumulate in animals. The possibility exists, however, that carbamates used in agriculture may be present in groundwater, and by extension, drinking water.

Ecotoxicology

In general, carbamates are not markedly toxic to wildlife. In higher doses, however, carbamate usage has the potential to produce ecotoxicity. When applied directly to the soil, many carbamates will cause significant reduction in microflora and worms. Bees are also especially sensitive to some carbamate pesticides. Because there is very little bioaccumulation of carbamate pesticides, the threats to wildlife are usually through direct exposure after application rather than through the food chain. Note that there have been reports of wildlife morbidity and mortality even if applications of certain carbamate pesticides were made at the recommended rate.

Exposure Standards and Guidelines

For human health, carbamates are usually regulated based on their potency for inhibiting acetylcholinesterase activity in the nervous system. Within the next two years (2004–2006), carbamate pesticides in the United States will be regulated as a mixture of compounds (rather as single compounds) with a common mechanism of action under the direction of the Food Quality Protection Act.

See also: Acetylcholine; Benomyl; Carbaryl; Carbofuran; Carboxylesterases; Cholinesterase Inhibition; Dithiocarbamates; Methomyl; Organochlorine Insecticides; Organophosphates; Pesticides; Pollution, Soil.

Further Reading

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Smith GJ (1993) *Toxicology and Pesticide Use in Relation to Wildlife: Organophosphate and Carbamate Compounds*. Boca Raton, FL: CRC Press.

Relevant Websites

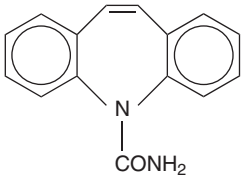
http://www.epa.gov – US Environmental Protection Agency.

http://www.inchem.org – World Health Organization (1986) *Carbamate Pesticides: A General Introduction*. Geneva: WHO.

Carbamazepine

Henry A Spiller

- Chemical Abstracts Service Registry Number: CAS 298-46-4
- Synonyms: CBZ; *5H*-Dibenz(*b*,*f*)-azepine-5-carboxamide; Tegretol
- Chemical/Pharmaceutical/Other Class: Synthetic iminostilbene derivative structurally similar to imipramine, a tricyclic antidepressant. While unrelated structurally, carbamazepine shares a similar therapeutic action with phenytoin
- Chemical Formula: $C_{15}H_{12}N_2O$
- Chemical Structure:



Uses

Carbamazepine is used in the treatment of epilepsy and trigeminal neuralgia. Unlabeled uses include treatment of postherpetic pain syndrome, neurogenic diabetes insipidus, bipolar disorder, alcohol withdrawal, and cocaine dependence.

Exposure Routes and Pathways

The exposure pathway for carbamazepine is exclusively oral (ingestion of tablets or suspension).

Toxicokinetics

Carbamazepine is slowly and incompletely absorbed during therapeutic use. With large ingestions, absorption may be delayed and unpredictable, producing peak levels from 4 to 72 h after the overdose. The absorption phase in an overdose is highly variable because of carbamazepine's poor solubility, ability to significantly decrease gut motility, and to form pharmacobezors. One of the primary metabolites of carbamazepine is carbamazepine-10,11-epoxide (CBZE), which also has anticonvulsant activity. A minor pathway results in iminostilbene formation. Further hydrolysis and conjugation produce six other known metabolites including 10,11-dihydroxycarbamazepine. Protein binding is 75% for carbamazepine and 50% for CBZE. However, the percentage of protein binding may decrease in massive overdose due to saturable binding sites. The volume of distribution is 0.8–1.9 l kg^{-1}. The hydrolyzed and conjugated metabolites are eliminated through the kidneys, with only 1.2% free carbamazepine being found in the urine. Twenty-eight percent is eliminated unchanged in the feces. Carbamazepine induces drug metabolizing enzymes so that drug half-life is reduced in chronic use. The half-life in healthy adults ranges from 18 to 65 h in a single dose to 8–17 h during chronic administration. In newborns and children, the half-life is $\sim$9 h.

Mechanism of Toxicity

Carbamazepine is both an important anticonvulsant in therapeutic doses and a powerful proconvulsant in overdose. The therapeutic anticonvulsant mechanism is primarily related to blockade of presynaptic voltage-gated sodium channels. Blockade of the sodium channels is believed to inhibit the release of synaptic glutamate and possibly other neurotransmitters. Carbamazepine is also a powerful inhibitor of the muscurinic and nicotinic acetylcholine receptors, *N*-methyl-D-aspartate (NMDA) receptors and the central nervous system (CNS) adenosine receptors. In addition, carbamazepine is structurally related to the cyclic antidepressant impramine and in massive overdose may affect cardiac sodium channels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Carbamazepine is not commonly used in animals. Limited information on toxicity exits. Tachyarrhythmias, hypotension, and seizures have been seen.

Human

The primary and common toxic event involves the CNS. Cardiac conduction delays and ventricular arrhythmias can be seen but are infrequent. Sinus tachycardia and hypotension are more commonly seen. In the few deaths directly attributable to carbamazepine toxicity ventricular dysrhythmias have been the terminal event. Coma, seizures, and respiratory depression are commonly seen in adults at levels greater than 40 μg ml^{-1} (170 mmol l^{-1}). Status epilepticus has been reported. The incidence of serious toxicity is similar in adults and children. However, serum levels are less predictive in children.

Therefore, coma, seizures, and apnea may be seen at lower serum levels than in adults. Other manifestations of neurologic toxicity are nystagmus, ataxia, choreoathetoid movements, encephalopathy, absent corneal reflexes, decreased deep tendon reflexes, urinary retention, and dystonias. A cyclic clinical course can be seen, with a waxing and waning of symptoms. This may be due to the presence of a pharmacobezor in the gut or more commonly due to a decrease in gastrointestinal motility produced by the prominent anticholinergic effects of carbamazepine.

Chronic Toxicity (or Exposure)

Animal

Male albino rats given injections of carbamazepine over 3 months demonstrated decreased prostate weight and decreased sperm motility. These changes did not affect fertility. Rats born to mothers chronically fed carbamazepine during gestation demonstrated challenges with maintaining balance and had more difficulty lifting their hind legs than controls.

Human

Idiopathic hepatotoxicity has been reported as a rare manifestation of chronic therapy and is not dose related.

In Vitro Toxicity Data

Studies of carbamazepine on rat cerebellar granule cells have shown inhibition of NMDA-stimulated calcium entry in a rapid and reversible manner. These findings occurred in therapeutic concentrations of carbamazepine, which may help explain the antiseizure activity of carbamazepine. It is believed that the toxic cerebellar effects of carbamazepine may be due to this mechanism.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be used as appropriate. Activated charcoal effectively binds carbamazepine. Multiple dose activated charcoal ($0.5\,g\,kg^{-1}$ every 4 h) has been shown to decrease the half-life of carbamazepine. Generally, supportive measures are all that is required in carbamazepine overdose. Seizures initially should be managed with diazepam or lorazepam. However, persistent seizures may require advancement to phenobarbital or pentobarbital. Ventricular arrhythmias should be managed with lidocaine. The presence of persistently high serum levels or fluctuating elevated serum levels may suggest the presence of a pharmacobezor in the gut. Removal should be attempted, in the presence of an active bowel, with whole bowel irrigation using a polyethylene glycol-electrolyte solution.

Environmental Fate

No information is currently available on breakdown in soil groundwater or surface water.

See also: Diazepam; Lidocaine; Polyethylene Glycol.

Further Reading

Bridge TA, Norton RL, and Robertson WO (1994) Pediatric carbamazepine overdoses. *Pediatric Emergency Care* 10: 260–263.

Kasarskis EJ, Kuo CS, and Berger R (1992) Carbamazepine-induced cardiac dysfunction: Characterization of two distinct clinical syndromes. *Archives of Internal Medicine* 152: 186–191.

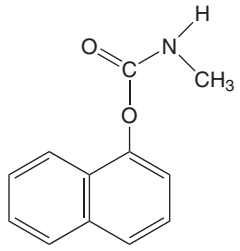
Spiller HA (2001) Management of carbamazepine overdose. *Pediatric Emergency Care* 17: 452–456.

Carbaryl

Paul R Harp

- Chemical Abstracts Service Registry Number: CAS 63-25-2
- Synonyms: 1-Naphthyl-*N*-methylcarbamate; Atoxan; Caprolin; Carbacide; Carbamine; Carbex; Carpolin; Cekubaryl; Denapon; Denopton; Devicarb; Dicarbam; Efaryl; Gamonil; Hexavin; Karbaspray; Karbatox; Karbosep; Kilex; Menaphtam; Monsur; Murvin; NAC; Panam; Pomex; Rayvon; Septene; Sevidol; Sevin; Sevinox; Tercyl; Toxan; Tricarnam; Vioxan; Vanisect; ENT 23969; UC 7744; OMS 29; SHA 056801

- Chemical/Pharmaceutical/Other Class: *N*-Methylcarbamate insecticide
- Chemical Structure:



Uses

Carbaryl is effective as both a contact and an ingested agent and is one of the most widely used broad-spectrum insecticides. Uses include control of a wide variety of pests on field crops, fruits, vegetables, nuts, ornamentals, turf, lawns, and domestic animals in agricultural, commercial, and residential environments. Liquid broadcast applications to residential lawns in the United States are to be voluntarily canceled, however. Formulations include dusts, wettable powders, granules, oils, aqueous suspensions, and baits.

Exposure Routes and Pathways

Due to extensive use, numerous types of applications, and the variety of formulations, human exposure may occur through all of the major pathways (dermal contact, ingestion, and inhalation). Dermal contact probably represents the pathway through which exposure most frequently occurs.

Toxicokinetics

The rate of dermal absorption of carbaryl in animal studies is dependent on the solvent used. Carbaryl can be hydrolyzed to 1-naphthol (the major metabolite) or hydroxylated to a naphthylmethylcarbamate, either of which may be conjugated with glucuronic acid or sulfate. Nonhydrolytic pathways also play a minor role in the biotransformation of carbaryl. In rats treated with carbaryl through oral gavage, the highest tissue levels of the pesticide were found in the liver, kidneys, and adipose tissue. Most mammals eliminate at least 75% of the original dose within 24–48 h. The route of elimination is generally urinary with small amounts of certain metabolites undergoing fecal elimination.

Mechanism of Toxicity

Carbaryl binds and inhibits acetylcholinesterase, the enzyme responsible for metabolizing the neurotransmitter acetylcholine and terminating its action at cholinergic synapses. Exposure to carbaryl results in synaptic accumulation of acetylcholine in both the central and peripheral nervous systems and hyperstimulation of muscarinic and nicotinic receptors leading to 'cholinergic crisis'. In contrast to the organophosphate anticholinesterases, acetylcholinesterase inhibition by the *N*-methylcarbamates is reversible with fairly rapid reactivation occurring through spontaneous decarbamoylation or via hydrolysis of the carbamate.

Acute and Short-Term Toxicity (or Exposure)

Animal

Signs of acute exposure in laboratory animals are similar to those described for humans and recovery from nonlethal exposures occurs rapidly. LD_{50} values for acute exposure in rats: 233–850 mg kg^{-1} (oral), >5000 mg kg^{-1} (dermal), and 0.005–0.023 mg l^{-1} (inhalation).

Human

The acute effects of exposure are due to cholinergic overstimulation and may include the SLUDGE syndrome (salivation, lacrimation, urination, diarrhea, gastrointestinal cramping, and emesis), respiratory depression, bronchospasms, increased bronchial secretions, pulmonary edema, blurred vision, miosis, headache, tremors, muscle fasciculations, convulsions, mental confusion, coma, and death (due to respiratory failure). Recovery from nonlethal exposures occurs very rapidly (usually within a few hours).

Chronic Toxicity (or Exposure)

Animal

Pigs receiving carbaryl in their diets developed incoordination, muscle contractions, and tremors cumulating in paraplegia. It is unclear if carbaryl or a metabolite, possibly unique to pigs, is responsible.

Human

Several cases of persistent neurophysiological or neurobehavioral effects have been reported following acute high-dose exposure to carbaryl. Studies of oral exposure in hogs also indicate possible neuropathological effects of carbaryl. The US Environment Protection Agency's Office of Pesticide Programs has classified carbaryl as likely to be a human carcinogen based on findings of hemangiosarcomas in mice. However, evaluation of the various

use scenarios indicated the noncancer effects of carbaryl generally presented a greater risk than did the carcinogenic potential.

In Vitro Toxicity Data

Carbaryl inhibited neurite outgrowth in N2a cells *in vitro*. Carbaryl also exhibited DNA-damaging activity in a human lymphoblastoid cell line and human liver HepG2 cells.

Clinical Management

Persons providing medical assistance should avoid contact with contaminated clothing. Contaminated clothing should be removed, bagged, and discarded. Contaminated leather garments such as shoes or gloves should be discarded. Exposed dermal areas should be cleaned thoroughly with soap and water. Exposed eyes should be flushed with copious amounts of clean water for at least 15 min. If necessary, use an endotracheal tube to maintain a clear airway, aspirate any secretions, and provide oxygen via mechanical ventilation.

If the patient is asymptomatic and can be treated soon after exposure, activated charcoal may be used to reduce absorption from the gastrointestinal tract. If potentially life-threatening quantities have been ingested, gastric lavage should be considered if it can be conducted within $\sim$1 h of exposure. Charcoal and/or catharsis are contraindicated in presence of severe vomiting or diarrhea. Muscarinic effects (i.e., SLUDGE) may be reduced by intravenous or intramuscular administration of atropine. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenobarbital may be helpful for recurrent seizures. Pralidoxime is indicated in cases of mixed exposure to both carbamates and organophosphorus compounds but is contraindicated in cases of carbamate-only exposure. Furosemide may be useful for pulmonary edema that continues after full atropinization. Metabolite analysis of a urine sample may allow confirmation of the intoxicating agent.

Environmental Fate

Carbaryl has low persistence in soil (half-life of 7–21 days). Degradation is primarily due to sunlight and microbial action. Carbaryl is bound to organic matter and can be transported in runoff. Carbaryl has been detected in groundwater. In surface water, carbaryl is degraded by hydrolysis and microbial processes. It has low volatility. The half-life in surface waters varies greatly with water pH.

Ecotoxicology

Carbaryl has low toxicity in birds. Reported LD_{50} values in mallard ducks, pheasants, quail, and pigeons were all greater than 1 g kg^{-1}. Carbaryl is only moderately toxic to aquatic organisms (LC_{50} values in rainbow trout and bluegill of 1.3 and 10 mg l^{-1}, respectively). Some accumulation of carbaryl in aquatic species can occur, for example, residues in fish were 140 times the concentration in water. Carbaryl shows less bioaccumulation in alkaline conditions. Carbaryl can be lethal to a variety of nontarget species including honey bees, earthworms, and beneficial insects.

Exposure Standards and Guidelines

The acute and chronic reference dose for carbaryl is 0.01 mg kg^{-1} day^{-1}, the acceptable daily intake is 0.01 mg kg^{-1} day^{-1}, and the permissible exposure limit is 5 mg m^{-3} (8 h).

See also: A-Esterases; Carbamate Pesticides; Cholinesterase Inhibition; Neurotoxicity; Pesticides.

Further Reading

Ecobichon DJ (2001) Toxic effects of pesticides. In: Klaassen CD (ed.) *Casarett and Doull's Toxicology*, 6th edn., pp. 763–810. New York: McGraw-Hill.

Relevant Websites

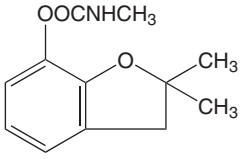
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.
http://www.infoventures.com – Carbaryl Pesticides Fact Sheet, US Forest Service.

Carbofuran

Xun Song

This article is a revision of the previous print edition article by Todd A Bartow and Paul W Ferguson, volume 1, pp. 221–222, © 1998, Elsevier Inc.

- Chemical Abstract Service Registry Number: CAS 1563-66-2
- Synonyms: 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl-*N*-methylcarbamate; Brifur®; Crisfuran®; Curaterr®; Furadan®; Pillarfuran®; Yaltox®; FMC 10242; Bay 70143; Chinufur; Niagra NIA-10242; OMS 864; NIOSH/RTECS FB 9450000; NA 2757; STCC 4921525
- Chemical/Pharmaceutical/Other Class: *N*-Methyl carbamate insecticide; Acaricide; Nematocide
- Chemical Structure:



Uses

Carbofuran is used as an agricultural insecticide on tobacco, corn, alfalfa, and other field crops.

Exposure Routes and Pathways

Exposure may occur through the oral, inhalation, and dermal routes.

Toxicokinetics

Carbofuran is well absorbed by oral and inhalation routes of exposure but poorly absorbed through intact skin. Approximately 75% of absorbed carbofuran is protein bound. Carbofuran is metabolized to yield 3-hydroxycarbofuran and 3-ketocarbofuran via oxidation, and to yield 3-hydroxy-7-phenol, 3-keto-7-phenol, and 7-phenol via hydrolysis. Most metabolites are in the form of glucuronide or sulfate conjugates, which are excreted in the urine. The half-life in the rat is 20 min for the parent compound and 64 min for the 3-hydroxycarbofuran metabolite.

Mechanism of Toxicity

Carbofuran is an inhibitor of acetylcholinesterase. Inhibition of acetylcholinesterase activity leads to an increase in acetylcholine at the nerve synapse resulting in excessive cholinergic stimulation. Following intravenous injection of $50\,\mu g\,kg^{-1}$ in rats, blood acetylcholinesterase activity was depressed by 83% within 2 min. With oral exposures, acetylcholinesterase activity was depressed by 37% within 15 min of ingestion. Recovery of acetylcholinesterase activity parallels carbofuran elimination.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values for carbofuran were 5.3–$13.2\,mg\,kg^{-1}$ in rats, $2\,mg\,kg^{-1}$ in mice, $19\,mg\,kg^{-1}$ in dogs, and $>400\,mg\,kg^{-1}$ in birds. The dermal LD_{50} values are greater than 1–$2\,g\,kg^{-1}$ in rabbits. The inhalation LC_{50} values were $85\,mg\,m^{-3}$ in rats and $52\,mg\,m^{-3}$ in dogs.

Human

Exposure to carbofuran may lead to cholinergic crisis with signs and symptoms including increased salivation, lacrimation, urinary incontinence, diarrhea, gastrointestinal cramping, and emesis (SLUDGE syndrome). The syndrome may be indistinguishable from that seen after organophosphate poisoning. Seizures, coma, diaphoresis, muscle weakness and fasciculation, bradycardia, and tachycardia may occur. Death may be due to severe bronchoconstriction and/or respiratory paralysis.

Workers in a pesticide plant in China exhibited dizziness, weakness, blurred vision, nausea and sweating, pallor, epigastric pain, vomiting, and tightness of the chest following carbofuran intoxication. Miosis was a common finding. Muscle fasciculations (gastrocnemius and orbicularis oculi) were noted in some workers. Blood cholinesterase inhibition was correlated with clinical signs. A pregnant woman was acutely poisoned by carbofuran ingestion. She recovered but the fetus died. Fetal liver, brain, and kidney all had carbofuran in concentrations similar to the maternal blood, indicating the ability of carbofuran to cross the human placenta and adversely affect the developing fetus.

Chronic Toxicity (or Exposure)

Animal

Long-term dietary exposure to carbofuran (50 ppm) led to significant decreases in cholinesterase activity in dogs and rats. No-observed-adverse-effect levels were 20 and 25 ppm in dogs and rats, respectively. High dosages ($5\,mg\,kg^{-1}\,day^{-1}$) for 2 years showed decreased body weight in rats and mice. Prolonged exposure to carbofuran can elicit signs of acute cholinergic toxicity.

Human

While prolonged exposures to carbofuran could lead to typical signs of cholinergic toxicity, recovery from cholinergic signs is rapid and residual chronic toxicity is not likely regardless of exposure.

In Vitro Toxicity Data

Carbofuran is not mutagenic in the Ames assay.

Clinical Management

Rescuers and medical personnel must take precautions to avoid becoming contaminated themselves during rescue and emergency treatment. Victims should be removed from the environment and 100% humidified supplemental oxygen should be administered with assisted ventilation as required. Patients with significant bronchorrhagia, pulmonary edema, convulsions, or coma may require endotracheal intubation and airway suctioning. Exposed skin and eyes should be flushed with copious amounts of water. Measures to decrease absorption may be beneficial soon after ingestion, but induced emesis should be avoided because of the potential for early development of coma or seizures. Atropine is antidotal for muscarinic symptoms and should be given in an initial dose of 2 mg and repeated every 15–30 min as required. The endpoint for atropinization is normalization of vital signs and drying of pulmonary secretions, not pupillary dilatation.

Administration of 2-PAM chloride (protopam and pralidoxime) is generally not recommended in carbamate poisoning since it has been shown to interfere with the efficacy of atropine. It was reported that the condition of patients suffering carbaryl-related poisoning deteriorated rapidly following the administration of 2-PAM. Seizure control with diazepam, phenobarbital, or phenytoin may be required. Cardiovascular support and intensive supportive care may be required in serious cases.

Environmental Fate

Carbofuran is soluble in water and moderately persistent in soil (half-life 30–120 days). Carbofuran is degraded by chemical, photochemical, and microbial processes. Hydrolysis is more rapid in alkaline conditions. Carbofuran breaks down in sunlight. Carbofuran has a high potential for leaching into groundwater. Carbofuran is mobile in sandy loam, silty clay, and silty loam soils. In surface water, carbofuran is subject to hydrolysis, particularly under alkaline conditions. Hydrolysis of carbofuran (half-lives) in water is 690, 8, and 1 weeks at pH values of 6, 7, and 8, respectively. As in soils, photodegradation and microbial transformation may also contribute to degradation. Carbofuran is not volatile and does not adsorb to sediment or particles.

Ecotoxicology

Carbofuran is highly toxic to birds. Carbofuran granules resemble grain seeds, thus the granular formulation can be highly toxic to birds. Predatory birds can be poisoned by prey that recently consumed carbofuran. The LD_{50} is 0.238–12 $mg\,kg^{-1}$ in a variety of bird species. Carbofuran is also highly toxic to many fish. The LC_{50} (96 h) is 0.24–0.38 $mg\,l^{-1}$ in bluegills and rainbow trout. Carbofuran has little potential for bioaccumulation. Carbofuran is toxic to bees except when used as a granular formulation.

Exposure Standards and Guidelines

The reference dose for carbofuran is $0.005\,mg\,kg^{-1}\,day^{-1}$, the acceptable daily intake is $0.002\,mg\,kg^{-1}\,day^{-1}$, and the threshold limit value (8 h) for carbofuran is $0.1\,mg\,m^{-3}$.

See also: Carbamate Pesticides; Cholinesterase Inhibition; Neurotoxicity; Pesticides.

Further Reading

Ecobichon DJ (2000) Carbamates. In: Spencer PS and Schaumburg HH (eds.) *Experimental and Clinical Neurotoxicology*, 2nd edn., pp. 289–298. New York: Oxford University Press.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Carbon Dioxide

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 124-38-9
- Synonyms: Carbon ice; Dry ice
- Chemical Formula: CO_2

Uses

Carbon dioxide is used in the synthesis of urea, for organic synthesis, and in the manufacture of dry ice, soft drinks, and fire extinguishers.

Mechanism of Toxicity

Carbon dioxide is a simple asphyxiant; that is, it causes toxicity by displacing oxygen from the breathing atmosphere primarily in enclosed spaces and results in hypoxia. It has been postulated that the cause of death in breathing high concentration of carbon dioxide is due to carbon dioxide poisoning and not hypoxia based on a study performed in dogs.

Acute and Short-Term Toxicity (or Exposure)

Animal

Extremely high concentrations (~40%) have resulted in death. At lethal concentrations effects have been seen in the central nervous system, lungs, liver, kidneys, and the myocardium in rats. Dogs exposed to 50% carbon dioxide for ~90 min or 80% for several minutes died from respiratory or cardiac failure.

Human

Carbon dioxide is a simple asphyxiant that displaces oxygen from the breathing atmosphere resulting in hypoxia. Four stages have been described (depending on the arterial oxygen saturation): (1) indifferent stage, 90% oxygen saturation; (2) compensatory stage, 82–90% oxygen saturation; (3) disturbance stage, 64–82% oxygen saturation; and (4) critical stage, 60–70% oxygen saturation or less.

Following exposure to asphyxiants, cardiovascular effects like tachycardia, arrhythmias, and ischemia are noted. Carbon dioxide exerts a direct toxic effect to the heart, resulting in diminished contractile force. It is also a vasodilator and the most potent cerebrovascular dilator known. Respiratory effects like hyperventilation, cyanosis, and pulmonary edema are also noted. Various neurologic effects like dizziness, headaches, sleepiness, and mental confusion can occur. Prolonged hypoxia may result in unconsciousness; seizures may be seen during serious cases of asphyxia. Gastrointestinal effects, like nausea and vomiting, may occur, but usually resolve within 24–48 h following termination of exposure. Decreased vision and increased intraocular pressure may be seen with inhalation of 10% carbon dioxide. Combined respiratory and metabolic acidosis was seen in a serious exposure to dry ice. The Lake Nyos disaster in August 1986 has been postulated to have resulted from the release of carbon dioxide from rising cold deep water producing a deadly cloud of gas. Cough, headache, fever, malaise, limb swelling, and unconsciousness were noted in the victims. Inhalation of carbon dioxide is teratogenic and has caused both male and female adverse reproductive effects in rodents. Increased fetal movements have been noted in humans following inhalation with 5% carbon dioxide in air.

The lowest lethal concentration (inhalation) for humans is 100 000 ppm for 1 min. Carbon dioxide concentrations of 20–30% can cause convulsions and coma within 1 min. Unconsciousness may occur when inhaling a concentration of 12% for 8–23 min. Inhalation of 6–10% causes dyspnea, headache, dizziness, sweating, and restlessness.

Chronic Toxicity (or Exposure)

Animal

Changes in body weight, nutrient metabolism, adrenal cortical activity, and blood chemistry were observed in guinea pigs following inhalation of 1.5% for up to 91 days. Rats on chronic exposure have had reversible tissue changes in the central nervous system, lungs, liver, kidneys, and muscle tissue of the heart.

Human

Carbon dioxide is an important component of the body and would not be expected to have a chronic toxicity. However, long-term exposures to levels as low as 0.5–1%, while being generally well tolerated, can alter the acid base and calcium–phosphorus balance resulting in metabolic acidosis and increased calcium deposits in soft tissues. Long-term exposures in the range of 1–2% can stress the adrenal cortex because of constant respiratory stimuli and this level of exposure is considered dangerous after several hours. Exposure to 2% for several hours produces

headache, breathing difficulty upon exertion, and deepened respiration. Fatalities have occurred with prolonged exposure to 15–30%.

Clinical Management

Victims should be moved immediately from the toxic atmosphere and receive 100% humidified supplemental oxygen with assisted ventilation as required. Patients with severe or prolonged exposure should be carefully evaluated for neurologic sequelae and provided with supportive treatment. Seizures may be controlled by administration of diazepam. If seizures cannot be controlled with diazepam or recur, phenytoin or phenobarbital should be administered. Rewarming has been indicated for frostbite. On ocular exposure, the eyes should be rinsed for at least 15 min.

Environmental Fate

The general concerns about greenhouse gases and climate changes are well known, though our ability to model the climate and the timing and magnitude of these effects is uncertain. The major greenhouse gases are carbon dioxide and methane, which together represent 92% of all US greenhouse gas emissions (carbon dioxide accounts for 82%). There is a clear trend of increasing concentrations of greenhouse gases in the atmosphere. The impact of further increases in concentrations of these gases will lead to ever-increasing warming of the climate, leading to a serious impact on human health and the environment. Many scientists believe that these impacts could include an increase in severe weather events such as hurricanes and floods, sea level rise, and increase in heat waves. These weather changes would trigger an increase in heat strokes, may cause a migration of tree and plant species, and initiate the penetration of airborne diseases in areas that do not currently experience these. Little attention has also been directed to investigating the possibility that escalating levels of carbon dioxide may serve as a selection pressure altering the genetic diversity of plant populations.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value – time-weighted average (TLV – TWA) is 5000 ppm and the ACGIH short-term exposure limit (STEL) is 30 000 ppm; the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) – TWA is 500 ppm (transitional limit) and 10 000 ppm (final rule limit), and the OSHA PEL – STEL is 30 000 ppm; the National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit is 10 000 ppm (TWA).

Miscellaneous

Carbon dioxide is a colorless and odorless gas. It has a molecular weight of 44.01 and specific gravity of 1.101 at $-37^{\circ}C$. It is incompatible with metals (e.g., aluminum peroxide, sodium peroxide, lithium peroxide, sodium, sodium carbide, titanium, and sodium–potassium alloy).

See also: Combustion Toxicology.

Further Reading

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Leaf D, Verolme HJ, and Hunt WF Jr. (2003) Overview of regulatory/policy/economic issues related to carbon dioxide. *Environment International* 29: 303–310.

Relevant Website

http://www.ccohs.ca – Health Effects of Carbon Dioxide Case from the Canadian Centre for Occupational Health and Safety.

Carbon Disulfide

Christopher H Day

This article is a revision of the previous print edition article by Linda Larsen, volume 1, pp. 223–224, © 1998, Elsevier Inc.

- Chemical Abstracts Services Registry Number: CAS 75-15-0
- Synonyms: Carbon bisulfide; Carbon sulfide; Dithiocarbonic anhydride; Sulfocarbonic anhydride
- Chemical/Pharmaceutical/Other Class: Solvent
- Chemical Formula: CS_2
- Chemical Structure: S : C : S

Uses

The primary use of carbon disulfide is in the manufacture of cellophane, carbon tetrachloride,

cellulose fibers, various dyes, and rayon. It is also used in the production of certain pesticides, paints and paint removers, enamels and enamel removers, rubber and rubber cement, tallows and waxes, preservatives, fumigants, and nematicides.

Exposure Routes and Pathways

Carbon disulfide is a liquid at room temperature, but quickly evaporates when exposed to air. The primary route of exposure to carbon disulfide is via inhalation of vapors. Exposure can also occur via ingestion, dermal, or eye contact. Most exposure occurs occupationally by individuals who come in contact with carbon disulfide under workplace conditions. Carbon disulfide is also produced under natural conditions by certain species of soil and sediment microorganisms, vegetation burned during forest and grass fires, and volcanic activity. On a global basis, it has been estimated that between 40% and 80% of carbon disulfide released into the environment is the result of these types of biogenic processes. Ambient air concentrations of carbon disulfide have been reported at $\sim$41 parts per trillion (ppt) in rural areas and 65 ppt in urban areas. The odor threshold for most people ranges from 0.02 to 0.1 ppm.

Toxicokinetics

Exposure to carbon disulfide via inhalation, ingestion, and dermal routes has been shown to result in rapid and pervasive absorbtion throughout the body. Carbon disulfide is metabolized to thiocarbamates by the liver and other target organs, such as the brain. Carbon disulfide is also metabolized by cytochrome P-450 to a short-lived oxygen intermediate. Most carbon disulfide metabolites are eliminated from the body in urine as dithiocarbamates. To a lesser extent, it is expelled from the body in the breath if it has not been metabolized. Small amounts may be eliminated from the body in saliva and sweat. Despite wide distribution throughout the body following exposure, carbon disulfide preferentially accumulates in organs such as the liver due to its lipophilicity.

Mechanism of Toxicity

The mechanisms of toxicity for carbon disulfide have not been definitively resolved. Although carbon disulfide is extensively metabolized in the human body, there is not a complete understanding of the metabolic pathways or products. However, two possible mechanisms have been forwarded to explain the neurotoxicity of carbon disulfide. One mechanism is associated with the formation of dithiocarbamates and potential derivatives, which inhibit dopamine-β-hydroxylase. The second mechanism suggested to result in neurotoxic effects associated with carbon disulfide exposure involves the formation of a form of vitamin B_6, of pyridoxamine, a dithiocarbamate derivative.

Acute and Short-Term Toxicity (or Exposure)

Animal

In general, acute studies with common test animals such as mice, rats, and rabbits indicate that carbon disulfide has low toxicity via the inhalation route, and is moderately toxic via the ingestion route.

Human

Acute toxicity observed in several early cases of inhalation exposure included a range of psychological effects (e.g., hallucinations; psychosis) following exposure to carbon disulfide concentrations ranging from approximately 1560 to 3125 mg m^{-3}. Short-term exposure to carbon disulfide vapors can cause headache, dizziness, blurred vision, disorientation, lethargy, damage to the cornea, retina, and optic nerve, and irritation of mucous membranes and the upper respiratory tract. Acute exposure to air concentrations that are well above occupational levels have been reported to cause significant neurological effects such as dyspnea, psychosis, and convulsions, and at exceedingly high concentrations (e.g., 15 625 mg m^{-3}) can result in coma and death.

Dermal exposure to high levels of carbon disulfide can result in redness and blistering of the skin, and if exposure to elevated levels continues long enough then second- and third-degree chemical burns are possible.

Chronic Toxicity (or Exposure)

Animal

Long-term exposure to carbon disulfide vapors has resulted in changes to liver enzymes in test animals.

Human

Long-term exposure to carbon disulfide via inhalation has been reported to result in neurological effects such as polyneuropathy, neurophysiologic changes, and general depression of nerve conduction velocities in humans. Cardiovascular effects that have been observed following chronic inhalation of carbon disulfide vapors by workers include generalized symptoms of heart disease, vascular

atherosclerotic changes, myocardial infarction, and increased incidents of angina.

There is evidence to suggest that carbon disulfide is a reproductive toxicant in humans. Menstrual disturbances in female workers and decreases in sperm count and libido in male workers have been reported following long-term inhalation exposure to carbon disulfide under workplace conditions.

Occupationally exposed individuals have also reported muscle aches and pain, lethargy, general fatigue, and headaches following long-term exposure to carbon disulfide vapors. Ocular effects that have been observed in people exposed in occupational settings include dot hemorrhages and microaneurysms.

Dermal exposure to fibers containing carbon disulfide by workers resulted in the development of blisters and eczema-like lesions on their hands.

In Vitro Toxicity Data

There are no studies available for carbon disulfide that provide strong evidence of genotoxicity.

Clinical Management

The victim of carbon disulfide exposure should be separated immediately from the source and placed in a fresh air environment, or provided with supplied air or oxygen as needed. Rescuers should carefully consider use of respiratory and dermal personal protective equipment due to the chemical, physical, and toxicological properties of carbon disulfide. In the case of exposure to liquid carbon disulfide, the rescuer must be aware of the potential for secondary exposure via direct contact with the victim's clothing. Once isolated from the source, the contaminated clothing should be removed and the skin flushed with water. Victims exhibiting symptoms of significant exposure, such as abnormal behavior, skin or eye irritation, or respiratory distress, should be transported to a medical facility for evaluation and monitoring.

Environmental Fate

Based on the physical and chemical properties of carbon disulfide, it is not expected to persist in the environment. Carbon disulfide has a high vapor pressure, relatively rapid oxidation rate, moderate solubility in water, and a low organic carbon partitioning coefficient (K_{oc}). Volatilization and photooxidation are the primary fate processes for carbon disulfide.

Ecotoxicology

The vast majority of carbon disulfide released into the environment is in the atmosphere. Therefore, terrestrial wildlife and birds in the vicinity of a release have the highest potential for primary exposure. Aquatic organisms would have a minimal exposure potential from an air release, but if the release is from a spill or an end-of-pipe discharge that empties into a water body, then the potential for aquatic organism exposure would be high.

Acute toxicity data are available for mammals, amphibians, fish, phytoplankton (e.g., algae), and zooplankton (e.g., daphnids). The lowest concentration reported to cause adverse effects in aquatic organisms is a 48 h LC_{50} of 2.1 mg l^{-1} for the daphnid, *Daphnia magna*. In mammalian test species, the lowest concentration reported to cause adverse effects is 690 mg m^{-3}, a 1 h LC_{50} for mice. These results suggest that carbon disulfide is moderately toxic to aquatic organisms and of low toxicity via inhalation to mammalian wildlife. No chronic ecotoxicity data could be located for carbon disulfide in the available literature.

Literature data for toxicological effects of carbon disulfide to avian or reptilian species are lacking at this time. There would be a great amount of uncertainty in attempting to quantify the potential for adverse effects to these taxa, or in the extrapolation of adverse effects from other taxa.

No experimentally derived bioconcentration or bioaccumulation factors were found in the available literature. Based on the moderate solubility and low octanol–water partitioning coefficient (K_{ow}), carbon disulfide is not expected to represent a significant

Table 1 Summary of exposure standards and guidelines for carbon disulfide

Agency	*Standards and guidelines* (ppm)	*Averaging time*
US EPA	RfC (0.2)	24 h a day for a lifetime
OSHA	PEL (20)	8 h a day over working lifetime
NIOSH	REL TWA (1)	10 h a day over working lifetime
NIOSH	IDLH (1 0 0)	NA
ACGIH	TLV TWA (10)	8 h a day over working lifetime

US EPA, United States Environmental Protection Agency; RfC, reference concentration; OSHA, Occupational Safety and Health Administration; NIOSH, National Institute for Occupational Safety and Health; ACGIH, American Conference of Governmental Industrial Hygienists; PEL, permissible exposure limit; REL, recommended exposure limit; TWA, time-weighted average; IDLH, immediately dangerous to life or health; TLV, threshold limit value; NA, not applicable.

concern to aquatic or terrestrial organisms via bioaccumulation or biomagnification.

Exposure Standards and Guidelines

Several agencies have established exposure standards or guidelines for carbon disulfide (summarized in **Table 1**). Generally, a standard or guideline represents the concentration that if met, will prevent an adverse effect from occurring at low exposure doses, and will therefore necessarily prevent the occurrence of more serious effects that are known to occur at higher doses. The chronic reference concentration (RfC) of 0.2 ppm ($0.7\,mg\,m^{-3}$) was set to prevent peripheral nervous system dysfunction in the general population over a lifetime of exposure. The ACGIH TLV of 10 ppm was set to prevent adverse effects to the cardiovascular system and central nervous system in workers exposed 8 h per day throughout their working lifetime.

See also: Hydrogen Sulfide; Neurotoxicity.

Relevant Websites

http://www.epa.gov – Website of the US Environmental Protection Agency. US Environmental Protection Agency (2004) *Integrated Risk Information System (IRIS) File: Carbon Disulfide*.

http://www.who.int – Website of the World Health Organization. World Health Organization (2002) *Concise International Chemical Assessment Document 46: Carbon Disulfide*. Geneva, Switzerland: WHO.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Carbon Disulfide.

Carbon Monoxide

Christine Stork and Deborah Anguish

This article is a revision of the previous print edition article by Daniel Steinmetz, volume 1, pp. 224–226, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 630-08-0
- Synonyms: Carbonic oxide
- Chemical/Pharmaceutical/Other Class: Inorganic compound of carbon and oxygen
- Chemical Formula: CO
- Chemical Structure: $C^{+}\equiv O^{-}$

Uses

Carbon monoxide is used in industries as a feedstock for the production of methanol, acrylates, phosgene, and ethylene. It is also used in metallurgy applications and in industrial fuels. A major source of carbon monoxide is the incomplete combustion of carbon-containing materials.

Exposure Routes and Pathways

Exposure to this colorless, odorless gas is via inhalation. Most exposures result from incomplete combustion, especially the emissions created by internal combustion engines. Other sources include the burning of wood, charcoal, or natural gas or propane for heating and cooking, and propane-powered indoor equipment such as fork lifts and ice rink resurfacers. Dermal and inhalation exposures to the paint stripper methylene chloride can cause carbon monoxide poisoning.

Toxicokinetics

Absorption of inhaled carbon monoxide occurs in the gas exchange region of the respiratory tract following inhalation. After absorption methylene chloride is metabolized in the liver to carbon monoxide. The half-life of carbon monoxide after exposure to methylene chloride can be prolonged due to continued absorption and metabolism. Most carbon monoxide binds reversibly to hemoglobin (Hb) in red blood cells; smaller amounts remain in solution or bind to cellular cytochromes. The absorption of the carbon monoxide molecule by Hb is a function of the alveolar partial pressures of carbon monoxide and oxygen, and the concentrations of carbon monoxide and oxygen in blood. Carbon monoxide's affinity for hemoglobin is 200–250 times greater than that of oxygen. Carboxyhemoglobin is completely dissociable, and carbon monoxide is liberated and eliminated through the lungs after exposure to carbon monoxide ceases. Small amounts are oxidized to carbon dioxide.

After binding to Hb to displace oxygen and form carboxyhemoglobin, carbon monoxide is transferred

rapidly throughout the body, where it produces asphyxia. The majority of the body burden exists as carboxyhemoglobin, bound to hemoglobin of red blood cells, while ~10% is present in extravascular space.

Carbon monoxide is eliminated via the lungs. Dissociation and excretion of carbon monoxide occur rapidly after cessation of exposure but slow as carboxyhemoglobin levels decrease. Cardiovascular injury can result from carboxymyoglobin formation and vasodilation from cellular effects of carbon monoxide. Clinical neurological effects and any delayed neurological sequelae can be attributed to asphyxia as well as lipid peroxidation, and hypotension, which induce ischemic-reperfusion injury.

Mechanism of Toxicity

As a result of hemoglobin's high relative affinity for carbon monoxide compared to oxygen and the resulting production of carboxyhemoglobin, decreased delivery of oxygen to tissues occurs, resulting in anemic hypoxia and metabolic and functional impairment. Carbon monoxide may also exert a toxic effect by binding to cellular cytochromes. Displacement of oxygen in the tissues ultimately results in anaerobic metabolism with subsequent buildup of metabolic acids.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals display similar toxicity to humans when exposed to carbon monoxide. Organ systems with large oxygen demands are affected initially and most profoundly.

Human

The effects of acute and chronic exposures to carbon monoxide have been well documented. The effects generally result from the hypoxic action exerted on the tissues. Among the earliest and most prominent effects are central nervous system disorders, such as headache and lightheadedness. At blood COHb levels approaching 30–40%, dizziness, incoordination, nausea, vomiting, and loss of consciousness may result. At still higher levels (>40% blood saturation), cardiovascular collapse, seizures, coma, and death may occur – usually attributed to cardiac dysrhyrhmias. Some studies have indicated that relatively small increments in carboxyhemoglobin levels may produce adverse cardiovascular effects, such as myocardial ischemia. Delayed neurological sequelae most likely involve lesions of the white matter. The absorption and elimination of carbon monoxide are slower in the fetal circulation than in the maternal circulation. Thus, the fetus may experience toxicity when the mother is at a low carbon monoxide level with no effects.

Chronic Toxicity (or Exposure)

Animal

Chronic, low-level carbon monoxide exposures produce decreased birth weights, cardiomegaly, EKG changes, and disruptions of cognitive function in several animal models. Rabbits exposed to carbon monoxide for 11 weeks demonstrated plaque formation in cardiac vessels indistinguishable from those seen from atherosclerotic heart disease.

Human

Humans are exposed to low levels of carbon monoxide every day from automobile traffic, from smoking, or being close to those who are cooking or heating with natural gas, or through occupational means. Toxicity is dose dependent. At doses that produce carboxyhemoglobin concentrations of <10%, no symptoms were evident in studies of humans, even during vigorous exercise. Higher doses produce more pronounced toxic effects. Epidemiologic evidence suggests that humans exposed to even moderate doses of carbon monoxide during pregnancy have lower birth weight children and have offspring who are at higher risk for sudden infant death syndrome.

Clinical Management

If carbon monoxide is inhaled, the victim must be removed from exposure and assisted in breathing as necessary. Methylene chloride should be washed well off the skin and the victim removed from the area to avoid continued absorption. Administration of oxygen in any carbon monoxide poisoned patient decreases recovery time significantly: The half-life of blood carboxyhemoglobin decreases from ~6 h in adults breathing air to less than 100 min when oxygen is administered. Hyperbaric oxygen accelerates the process of carboxyhemoglobin dissociation, decreasing the half-life of carbon monoxide to 23 min. Hyperbaric oxygen has recently been shown superior over normobaric oxygen therapy for preventing cognitive deficits in carbon monoxide poisoning.

See also: Blood; Combustion Toxicology; Methanol; Methylene Chloride; Phosgene.

Further Reading

Burney RE, Wu S, and Nemiroff MJ (1982) Mass carbon monoxide poisoning: Clinical effects and results of treatment in 184 victims. *Annals of Emergency Medicine* 11: 394–399.

Thom SR, Taber RL, and Mendiguren II (1995) Delayed neuropsychologic sequelae after carbon monoxide poisoning: Prevention by treatment with hyperbaric oxygen. *Annals of Emergency Medicine* 25: 474–480.

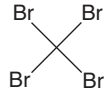
Relevant Website

http://www.inchem.org – Environmental Health Criteria (Number 213) for Carbon Monoxide (Second Edition) fron IPCS INCHEM.

Carbon Tetrabromide

Kashyap N Thakore and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 558-13-4
- Synonyms: Carbon bromide; Tetrabromide methane; Tetrabromo methane
- Chemical/Pharmaceutical/Other Class: Halomethane, oxidizing agent, solvent
- Chemical Formula: CBr_4
- Chemical Structure:



Uses

Carbon tetrabromide is used as an industrial solvent.

Exposure Routes and Pathways

The primary routes of entry are eye contact, skin contact, inhalation, and ingestion. When heated to decompose, carbon tetrabromide emits toxic fumes of Br^-.

Toxicokinetics

Carbon tetrabromide may be absorbed by dermal, inhalation, or oral routes. It is oxidatively metabolized by rat liver microsomes to electrophilic and potentially toxic metabolites. It is metabolized in the liver but causes primary effects on the kidneys. The electrophilic bromine derivatives formed can be excreted as such.

Mechanism of Toxicity

Carbon tetrabromide inhibits protein synthesis and causes lipid peroxidation, both of which may be involved in cell injury or death mediated by free radicals.

Acute and Short-Term Toxicity (or Exposure)

Animal

Carbon tetrabromide is poisonous by subcutaneous and intravenous routes and moderately toxic by ingestion. It causes kidney toxicity and is narcotic at high concentrations. LD_{50} values in mice are $298\,mg\,kg^{-1}$ (subcutaneous) and $56\,mg\,kg^{-1}$ (intravenous).

Human

Carbon tetrabromide is harmful by inhalation, ingestion, or skin absorption. It causes irritation to eyes, skin, mucous membranes, and the upper respiratory tract.

In occupational settings, technical measures should prevent any contact with the skin and mucous membranes. Workers exposed to carbon tetrabromide should wear personal protective equipment and their work should be carried out only in restricted areas. After use, clothing and equipment should be placed in an impervious container for decontamination or disposal. The American Conference of Governmental Industrial Hygienists threshold limit value for carbon tetrabromide is 0.1 ppm.

Chronic Toxicity (or Exposure)

No data are available to assess the mutagenic or genotoxic, carcinogenic, and teratogenic potential of this agent.

Clinical Management

In case of contact, eyes and skin should be flushed with water for 15–20 min. If inhaled, the victim should be removed to fresh air. If necessary, oxygen and artificial respiration should be administered. If the patient is in cardiac arrest, cardiopulmonary resuscitation should be performed. These life-supporting measures should be continued until medical assistance has arrived. An unconscious or

convulsing person should not be given liquids or induced to vomit.

Environmental Fate

Carbon tetrabromide is expected to have very high mobility in soil and volatilizes slowly from dry soil surface. Its biodegradation is expected to be slow and to exist solely as a vapor in the ambient atmosphere. It is not expected to adsorb to suspended solids and sediment in the water column. Its potential for bioconcentration in aquatic organisms is moderate.

Other Hazard

It is not flammable.

See also: Carbon Tetrachloride; Trihalomethanes.

Relevant Website

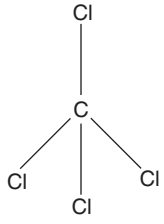
http://www.cdc.gov/niosh – NIOSH (2003) *Pocket Guide to Chemical Hazards*. Cincinnati, OH: National Institute for Occupational Safety and Health.

Carbon Tetrachloride

Thomas R Parker, Robert Howd, and Hierberto Robles

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 56-23-5
- Synonyms: Methane tetrachloride; Carbon tet; Carbon chloride; Tetrachloromethane; Perchloromethane; Tetrachlorocarbon; Carbona; Freon® 10; Halon® 104
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbon
- Chemical Structure: Tetrahedral structure with bond angles of 109.50°



Uses

The carcinogenic properties of carbon tetrachloride have caused a decline in the industrial use of this chemical. It was earlier used as a solvent for fats, oils, waxes, varnishes, lacquers, and resins and was often employed for cleaning equipment and machinery. The compound was also used as a refrigerant, as a fire extinguisher, a grain fumigant, and a dry cleaning agent; it was also used in veterinary medicine as an anthelmintic. The current use of carbon tetrachloride is limited to that of a chemical intermediate in the industrial production of a few chlorinated, organic chemicals.

Exposure Routes and Pathways

Exposure can occur via inhalation, ingestion, and dermal contact. Industrial exposures are anticipated to be the most common setting, with inhalation the most likely route.

Toxicokinetics

Due to its high lipid solubility, carbon tetrachloride is readily absorbed through inhalation and ingestion pathways, and to a lesser extent by dermal contact with the liquid form. Absorbed carbon tetrachloride tends to concentrate in body fat, liver, bone marrow, kidney, and brain. Laboratory experiments indicate that about half the absorbed dose is exhaled unchanged from the lungs. The remainder of the absorbed dose is metabolized primarily in the liver and eliminated in exhaled air and in urine and feces. Carbon tetrachloride is metabolized in the liver to a biologically active trichloromethyl radical. This radical can then undergo dimerization to hexachloroethane, reduction to chloroform, or bind to cellular macromolecules. An alternative metabolism pathway can transform carbon tetrachloride, via phosgene formation, to carbon monoxide and carbon dioxide.

Mechanism of Toxicity

Carbon tetrachloride is metabolized by cytochrome P-450 to the reactive metabolites trichloromethyl free radical and trichloromethylperoxy free radical. The trichloromethyl free radical may bind directly to cellular macromolecules such as lipids and proteins, and also to DNA, disrupting cell processes and breaking down membranes. The free radical can take part in *anaerobic* reactions, subsequently forming such toxic compounds as chloroform, hexachloroethane, and carbon monoxide. *Aerobic* biotransformation of the

•CCl_3 radical can yield trichloromethanol, a precursor to carbonyl chloride (phosgene). Since reactive metabolites are responsible for the bulk of the toxicity, tissues rich in CYP2E1, such as the liver and kidney, are the most sensitive toxicity targets for the compound. The unmetabolized fraction also produces some toxicity, and is associated with central nervous system (CNS) depression and irritation of the gastrointestinal tract.

Acute and Short-Term Toxicity (or Exposure)

Animal

Carbon tetrachloride produces systemic toxicity following short-term exposure via ingestion or inhalation. The major effects are CNS depression and hepatic and kidney damage. Symptoms of hepatic damage may appear after a delay of one or more days following acute exposure, while kidney damage develops within a few weeks. Pulmonary toxicity and respiratory disturbances have been observed in some cases. The acute oral toxicity of carbon tetrachloride to mice is relatively low, with a single dose median lethal dosage (LD_{50}) value of $13\,000\,mg\,kg^{-1}$. A rat study yielded higher acute lethality, with an LD_{50} of $\sim 8000\,mg\,kg^{-1}$.

Human

The early effect of acute carbon tetrachloride exposure by all routes is CNS depression, which can be accompanied by gastrointestinal effects such as nausea and vomiting. CNS depression can be followed by hepatic or renal injury. Hepatotoxic effects appear rapidly in humans; alterations in lipid metabolism in the liver may be observed 30 min following exposure, and histological changes within 1 h. Centrilobular necrosis, fatty degeneration, tender hepatomegaly, and jaundice are characteristic of the toxic lesions of the liver. Biological indicators of injury may include altered levels of serum enzymes such as SGOT.

The kidney is also a major target of carbon tetrachloride toxicity. The characteristic injuries observed are nephritis, nephrosis, and proteinuria. Delayed pulmonary edema and renal failure may follow hepatic damage. Renal failure is the most frequent cause of death in carbon tetrachloride poisonings.

Chronic Toxicity (or Exposure)

Animal

Chronic animal studies yielded results similar to shorter exposure durations. Rodents exposed to carbon tetrachloride in air for 6 months or longer were observed to have increased liver weights, total lipid increases, and hepatic fatty degeneration. Renal toxicity was also evident.

Chronic carbon tetrachloride exposures have produced liver tumors in several rodent species, with the tumor types including hepatocellular carcinoma and adenoma, and adrenal pheochromocytoma.

Human

Human health effects from longer-term human exposures to carbon tetrachloride generally resemble acute effects of liver and kidney damage. Consumption of alcohol and poorly controlled diabetes may increase the risk of harmful effects associated with carbon tetrachloride intoxication. The US EPA classifies carbon tetrachloride as a group B2, 'probable' human carcinogen.

In Vitro Toxicity Data

Almost all bacterial mutagenicity tests for carbon tetrachloride have been negative. Ames tests for reverse mutations using several strains of *Salmonella typhimurium*, with and without metabolic activation, were mostly negative. A weakly positive genotoxic response was reported in yeast. Negative or weak responses were observed in four studies examining unscheduled DNA synthesis.

Other studies indicate that carbon tetrachloride has the potential to form reactive intermediates that can covalently bind to DNA, which suggests genotoxicity.

Clinical Management

The victim should be removed from the contaminated environment and provided with supportive treatment. Care should be taken to maintain respiration by giving humidified oxygen through assisted ventilation, if necessary. Any contaminated clothing should be removed and the affected area should be washed with water and soap. Eyes exposed to the liquid should be irrigated with copious amounts of water. If liver and kidney damage is apparent, supportive therapy should be provided. Renal damage may be manifested by the appearance of polyuria, which might progress to oliguria and anuria. Hematuria and proteinuria may also be seen.

Environmental Fate

Carbon tetrachloride is highly volatile and does not easily break down in the environment. Most of the compound that is released to the environment accumulates in the atmosphere, where photodegradation

by shorter wavelength ultraviolet radiation appears to be the primary removal process. Absorption by the oceans and reactions with hydroxyl radical are likely lesser removal routes. The estimated half-life of atmospheric carbon tetrachloride is 30–100 years.

Ecotoxicology

Carbon tetrachloride is highly volatile and is relatively stable in the environment. Therefore, nearly all of the carbon tetrachloride produced is eventually emitted to the atmosphere. The chemical moves readily through soil and adsorbs only slightly to sediment. The estimated half-life of carbon tetrachloride in the atmosphere is 30–100 years. The hydrolysis half-life in water is estimated to be 7000 years at 25°C. Carbon tetrachloride has a low potential to bioconcentrate in animals. The logarithm of the bioconcentration factor in trout is 1.24.

Other Hazards

Carbon tetrachloride is nonflammable, and at one time was used as a fire extinguishing liquid. However, not only were the carbon tetrachloride vapors toxic, but also highly toxic phosgene gas was produced under fire conditions.

Exposure Standards and Guidelines

Cancer classifications of carbon tetrachloride by several groups are listed below:

- American Conference of Governmental Industrial Hygienists (ACGIH) Group A2 (suspected human carcinogen).
- US Environmental Protection Agency (EPA) Group B2 (probable human carcinogen).
- International Agency for Research on Cancer Group 2B (possibly carcinogenic to humans).

Drinking water standards are listed below:

- US EPA maximum contaminant level: 0.005 $mg\,l^{-1}$.
- Cal/EPA Public Health Goal: 0.005 $mg\,l^{-1}$.

Workplace standards (inhalation) are listed below

- Occupational Safety and Health Administration permissible exposure limit: 10 ppm (ceiling: 25 ppm, and 5 min maximum peak in any 4 h: 200 ppm).
- ACGIH threshold limit value – time-weighted average: 5 ppm.
- ACGIH threshold limit value – short-term exposure limit: 10 ppm.

See also: Alkyl Halides; Common Mechanism of Toxicity; Phosgene; Pollution, Soil; Pollution, Water.

Further Reading

Weber LW, Boll M, and Stampfl A (2003) Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. 33(2): 105–136.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Carbon Tetrachloride.
http://www.cdc.gov – NIOSH International Chemical Safety Cards.
http://ehp.niehs.nih.gov – NTP Tenth Report on Carcinogens (12/02).

Carbonyl Sulfide

Amy Merricle

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 463-58-1
- Synonyms: Carbon monoxide monosulfide; Carbon oxide sulfide; Carbon oxysulfide; RTECS/NIOSH FG6400000
- Chemical/Pharmaceutical/Other Class: Sulfides
- Chemical Formula: COS
- Chemical Structure: C–O–S

Uses

There are limited commercial uses of carbonyl sulfide. It is produced only in small quantities and used for small-scale experimental purposes and as a nonisolated, site-limited intermediate in the synthesis of organic sulfur compounds, thiocarbamate herbicides, and alkyl carbonates. Pesticide manufacturers

are believed to be the largest users of carbonyl sulfide. Similar to carbon disulfide, research conducted by the Australia Commonwealth Scientific and Industrial Research Organization's (CSIRO) Stored Grain Research Laboratory has shown carbonyl sulfide to be an effective soil fumigant for controlling insects on crops such as wheat, barley, oats, and peas, although carbonyl sulfide is not currently approved for this commercial use.

Exposure Routes and Pathways

Exposure occurs predominantly by the inhalation route, as most carbonyl sulfide released to the environment is released to the air. Occupational exposure to carbonyl sulfide may occur through inhalation or dermal contact during its production and use. The general population is exposed primarily from inhalation to ambient air. Carbonyl sulfide is released from natural sources such as deciduous and coniferous trees, volcanoes, salt marshes, and soils. Industrial sources of atmospheric carbonyl sulfide release include automobile exhaust, coal-fired power plants, biomass combustion, fish processing, combustion of refuse and plastics, petroleum manufacture, and manufacture of synthetic fibers, starch, and rubber. An estimated two-thirds of total carbonyl sulfide release worldwide is attributed to natural sources. Carbonyl sulfide can also be formed in the atmosphere through the chemical reaction of gas-phase carbon disulfide, and photochemically produced hydroxyl radicals. Carbonyl sulfide can be discharged to surface waters in the wastewater of viscose rayon plants. Drinking water normally does not contain carbonyl sulfide. Carbonyl sulfide is not expected to bioaccumulate in fish or other aquatic organisms; therefore, fish consumption is not considered a relevant route of exposure to this substance. A preliminary study of purgeable organic compounds in breast milk detected carbonyl sulfide in one of eight breast milk samples from nursing mothers living in urban centers in Pennsylvania, New Jersey, and Louisiana, suggesting the potential for exposure to breastfed infants. The source of the carbonyl sulfide in the breast milk sample was not discussed, and it may have been a metabolism by-product.

Toxicokinetics

Carbonyl sulfide is absorbed primarily in the lungs via the inhalation route, but can also be absorbed through the gastrointestinal tract and through the skin. Carbonyl sulfide is known to be absorbed into the blood, but transport and distribution is not fully understood. Studies of carbon disulfide metabolism using rat liver microsomes have demonstrated that carbonyl sulfide is a metabolic intermediate in the formation of carbon dioxide. Metabolism of carbonyl sulfide is mediated by the microsomal cytochrome P-450 monooxygenase system and is NADPH-dependant. Carbonyl sulfide is oxidized to atomic sulfur and carbon dioxide. The oxidative metabolism of carbonyl sulfide is a potential cause of toxicity due to the formation of highly reactive sulfur atoms. The atomic sulfur liberated in these reactions can be covalently bound to macromolecules or be oxidized to sulfate and excreted in urine. Carbonyl sulfide can be catalyzed by carbonic anhydrase to monothiocarbamate, which is spontaneously degraded to carbon dioxide and hydrogen sulfide. The hydrogen sulfide may be oxidized to sulfate or other still unknown metabolites. Monothiocarbamate can enter the urea cycle, forming thiourea, which is excreted in urine.

Mechanism of Toxicity

Toxicity from exposure to carbonyl sulfide is likely the result of the decomposition of carbonyl sulfide to carbon dioxide and hydrogen sulfide. Hydrogen sulfide inhibits respiration on the cellular level causing methemoglobinemia, which inhibits the cytochrome oxidase system causing cytotoxic anoxia. In one study, rats were treated with acetazolamide, an inhibitor of carbonic anhydrase. Test animals showed lower blood levels of hydrogen sulfide following exposure to carbonyl sulfide and exhibited decreased toxicity relative to rats that were not pretreated with acetazolamide. Hydrogen sulfide is believed to be primarily responsible for many of the reported adverse effects associated with exposure to carbonyl sulfide.

Acute and Short-Term Toxicity (or Exposure)

Animal

Exposure to carbonyl sulfide in animals produces serious nervous system effects with narcotic effects and acute respiratory failure at high concentrations. Acute inhalation exposure to carbonyl sulfide produced nervous system dysfunction and lower respiratory system irritation in rats. Rats exposed to carbonyl sulfide via inhalation for 4 h showed some central nervous system effects at 1062 and 1189 ppm. Results showed hypoactivity, lacrimation, breathing difficulties, cyanosis, bleeding from the nose, convulsions, tremors, and behavioral abnormalities, the most prominent of which, circling,

was demonstrated by approximately 50% of the 1062 ppm dose group survivors during the first 4 days postexposure. The lowest LC_{50} (95% confidence) was determined to be 1070 ppm for female rats.

As a follow-up to the LC_{50} study, a 2 week inhalation study was conducted. Results showed carbonyl sulfide toxicity for the high dose group (450 ppm), but only after at least 6 days of exposure. Females of the high exposure group weighed statistically less than controls after the second week of exposure. Signs of central nervous system dysfunction, ataxia, head tilting, circling, tremors, and convulsions were observed in 50% of the high dose group. Females in the mid (150 ppm) and the mid-high (250 ppm) dose groups had depressed red cell counts along with a slight depression in mean corpuscular volume. The authors concluded that no exposure-related effects occurred in animals exposed to carbonyl sulfide at 51 ppm for 11 days, 6 h per day.

Continuous exposure of rabbits to 50 ppm carbonyl sulfide for 1–7 weeks resulted in a slightly elevated mean serum cholesterol level, but had no significant effect on myocardial ultrastructure and did not show histopathological changes in lungs or coronary arteries.

Human

Carbonyl sulfide appears to elicit similar symptoms of poisoning as those seen from exposure to hydrogen sulfide, although produces less prominent initial warning signs, such as local irritation to the skin, eyes, and respiratory tract. Exposure to carbonyl sulfide may cause central and peripheral nervous system damage, damage to the respiratory tract, and ocular effects. Carbonyl sulfide exposure has also been associated with cardiovascular disease. Breathing high concentrations of carbonyl sulfide (greater than 1000 ppm) over a short time period may cause sudden unconsciousness, convulsions, coma, and fatal central respiratory paralysis. At low to moderately high vapor concentrations carbonyl sulfide can cause burning or redness of the eyes, painful conjunctivitis, photophobia, corneal opacity, headache, nausea, dizziness, confusion, cardiac arrhythmia, and pain and weakness in the extremities. Direct skin contact with carbonyl sulfide vapors may produce skin irritation and pain. Prolonged or repeated exposure to the skin may cause dermatitis. Gastrointestinal effects include profuse salivation, nausea, vomiting, and diarrhea. Central nervous system effects include giddiness, headache, vertigo, amnesia, confusion, and unconsciousness.

Chronic Toxicity (or Exposure)

Animal

No information was identified on the chronic reproductive, developmental, or carcinogenic effects of carbonyl sulfide in animals. However, carbonyl sulfide is the oxidation product of carbon disulfide, which has been shown by the National Institute of Health to be positive in the strain A mouse lung tumor bioassay. Significant increases in the incidence (tumor-bearing mouse) and frequency (tumors per mouse of lung adenomas) was observed in A/J mice.

Human

Chronic exposure to low concentrations of carbonyl sulfide may cause damage or irritation to the respiratory tract including symptoms of rhinitis, pharyngitis, bronchitis, and pneumonitis, and may cause pulmonary edema. Recovery depends upon the length of exposure and the dose. Residual effects during recovery may include coughing, slow pulse, and amnesia.

No information regarding the potential carcinogenicity or the developmental or reproductive toxicity of carbonyl sulfide in humans was identified. The Environmental Protection Agency has not classified carbonyl sulfide with respect to potential carcinogenicity.

In Vitro Toxicity Data

The National Toxicology Program found that carbonyl sulfide produced a weak positive response in the salmonella mutagenicity test. No further information regarding this test was identified.

Clinical Management

Following inhalation exposure, the victim should be moved to fresh air immediately. If the victim is not breathing, artificial respiration or cardiopulmonary resuscitation should be given, if necessary. If breathing is labored, the victim should be given oxygen. In case of ocular or dermal contact, the skin or eyes should be flushed with running water immediately. Soap and water may be used for washing exposed skin. If carbonyl sulfide is accidentally ingested, medical treatment should be sought immediately. Vomiting should not be induced. Further treatment is symptomatic. Rescuers must prevent exposure by wearing a self-contained breathing apparatus to rescue the victim.

Environmental Fate

Most of the releases of carbonyl sulfide to the environment are to air, where it is believed to have a long residence time. The half-life of carbonyl sulfide in the atmosphere is estimated to be ~2 years. It may be degraded in the atmosphere via a reaction with photochemically produced hydroxyl radicals or oxygen, direct photolysis, and other unknown processes related to the sulfur cycle. Sulfur dioxide, a greenhouse gas, is ultimately produced from these reactions. Carbonyl sulfide is relatively unreactive in the troposphere, but direct photolysis may occur in the stratosphere. Also, plants and soil microorganisms have been reported to remove carbonyl sulfide directly from the atmosphere. Plants are not expected to store carbonyl sulfide.

Carbonyl sulfide is extremely mobile in soils. If released to soil it will volatilize quickly to the atmosphere. It has a high solubility in water and will not readily adsorb to soil particles, sediment, or suspended organic matter. Therefore, carbonyl sulfide is expected to volatilize rapidly from soil and water or, depending upon volume, concentration, and site-specific characteristics (e.g., soil type, depth to groundwater, temperature, humidity), may be able to move rapidly through the ground and impact groundwater. Carbonyl sulfide may hydrolyzed in water to form hydrogen sulfide and carbon dioxide.

Ecotoxicology

Carbonyl sulfide is not expected to bioaccumulate in fish or other aquatic organisms. The United States Environmental Protection Agency reported that quantitative structure activity relationship estimates of acute toxicity for fish, daphnid, and algae are greater than $1000\,mg\,l^{-1}$.

Other Hazards

Carbonyl sulfide is a flammable gas, and may be explosive or spontaneously flammable in air under the right conditions. Vapors may ignite at distant ignition sources and flash back. When exposed to fire, humidity, or strong alkalis, carbonyl sulfide may form the toxic decomposition products carbon monoxide and hydrogen sulfide gas. In the presence of strong oxidizers, carbonyl sulfide presents a fire or explosion hazard. Carbonyl sulfide has a vapor density of 2.1 and is therefore heavier than air. Cylinders or tank cars containing carbonyl sulfide may rupture violently or rocket under fire conditions.

The National Fire Protection Agency (NFPA) flammable limits are as follows:

- lower – 12% by volume,
- upper – 29% by volume, and
- explosive limits is 12–29%.

Always refer to the Material Safety Data Sheet for information on proper handling and disposal.

Miscellaneous

The Clean Air Act (CAA) Amendments of 1990 list carbonyl sulfide as a hazardous air pollutant (HAP) generally known or suspected to cause serious health effects. Section 112(b) (1) of the CAA lists pollutants that are judged to be hazardous if emitted into the air. Carbonyl sulfide is included on this list. The statute calls for the identification of source categories that emit these HAPs, and the subsequent promotion of technology-based emission standards requiring compliance with maximum achievable control technology.

Section 112(r) of the CAA establishes a list of substances that, if present in a quantity in excess of a specific threshold, would require that the facility establish a risk management program to prevent chemical accidents, prepare a risk management plan, and submit the plan to the State and local emergency planning organizations. Carbonyl sulfide is regulated under CAA 112(r) because of its chemical property as a flammable gas.

Carbonyl sulfide may be regulated as a D003 hazardous waste under the Resource Conservation and Recovery Act when a solid waste containing this sulfide compound exhibits the characteristic of reactivity as stipulated in Title 40 of the Code of Federal Regulations, Section 261.23.

Carbonyl sulfide is also regulated under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Releases of substances on the CERCLA list of hazardous substances that are in excess of a specified reportable quantity must be reported to the United States Environmental Protection Agency's National Response Center. The reportable quantity for carbonyl sulfide is 100 pounds. Carbonyl sulfide is also subject to regulation under the Superfund Amendments and Reauthorization Act, Title III, Sections 311, 312, and 313, and Section 4 of the Toxic Substances Control Act.

See also: Carbon Disulfide; Hydrogen Sulfide; Pollution, Air.

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Carboxylesterases

Ramesh C Gupta

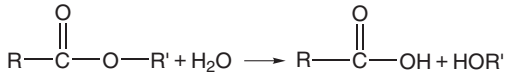
Carboxylesterases (CarbEs, EC 3.1.1.1; also known as aliesterases or tributyrinases) are a heterogeneous group of enzymes as they differ in substrate specificity. Despite the wide distribution of CarbEs in mammalian systems, most of their known substrates are foreign compounds that are not normally involved in intermediary metabolism. CarbEs hydrolyze xenobiotics containing an ester, thioester, or amide group, and thus play an important role in drug metabolism, carcinogenesis, and detoxification of many noxious chemicals present in our environment. The physiological function of CarbEs still remains obscure.

Physical, Chemical, and Biochemical Properties of CarbEs

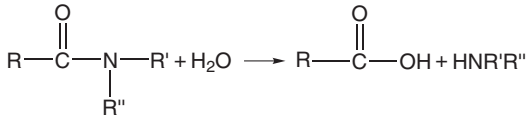
The mammalian hepatic, renal, and intestinal CarbEs consist of units with a molecular weight of ~60 000. Each unit bears one active site. The amino acid sequence around the active site of several CarbEs is Gly-Glu-Ser$^+$-Ala-Gly. The p*I* of hepatic CarbEs is usually in the range of pH 4.7–6.5 with the pH optimum in the range of pH 6–10. The behavior of hepatic microsomal and cytosolic CarbEs in *in vitro* and *in vivo* studies indicates that these enzymes are different. CarbE in hepatic microsomes consists of three isoenzymes (RH1, molecular weight 174 000, trimer, p*I* 6.0; RL1, molecular weight 61 000, monomer, p*I* 6.5; and RL2, molecular weight 61 000, monomer, p*I* 5.5), which differ considerably in terms of inducibility, substrate specificity, and immunological properties.

CarbEs can catalyze hydrolytic reactions of the following types:

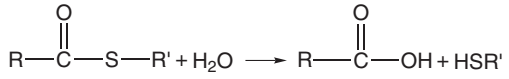
1. Carboxylester hydrolysis



2. Carboxylamide hydrolysis



3. Carboxythioester hydrolysis



The first two of these reactions are equally relevant for biotransformation process. Amides are often more stable to enzymatic hydrolysis than the corresponding esters with similar structures. For example, phenylacetate is hydrolyzed much faster than acetanilide. In addition, CarbE can hydrolyze therapeutically useful drug esters, such as chloramphenicol succinate, prednisolone succinate, procaine, and methylparaben.

Tissue Distribution of CarbEs

The activities of CarbEs have been localized and determined in almost all tissues, with the highest activity in liver. A substantial amount of the enzyme activity is present in heart, kidney, lungs, brain, skeletal muscles, testes, small intestine, pancreas, nasal mucosa, adipose tissue, and plasma. Normal values of CarbEs for some of the tissues, using tributyrin as the substrate, are given in **Table 1**. No significant variability in the

Table 1 Normal values of CarbE in different tissues of male Sprague–Dawley rats

Tissue	*CarbE activity (μmol tributyrin per gram per hour)*
Brain regions	
Cortex	68±2.6
Brainstem	68±2.3
Striatum	71±1.5
Hippocampus	72±3.1
Muscles	
Diaphragm	67±2.0
Heart	92±2.9
Liver	3014±6.0
Serum/plasma	30±2.0

Note: Values are mean ± SEM.

values of CarbEs has been found among discrete brain regions and among different fiber-dependent skeletal muscles. Studies have shown that CarbE activities of the liver, kidneys, brain, and intestinal mucosa are predominantly present in the microsomal fraction. The liver cytosolic CarbE activity is 1/20 of that present in the microsomes. The lowest CarbE activity is determined in plasma (1/70 of that in hepatic microsomes). At least some of the serum CarbE isoenzymes might originate from the liver.

Significance of CarbE Induction or Inhibition in Metabolism and Toxicity

As mentioned before, CarbEs have a limited physiological role *per se*, but their induction or inhibition by some drugs or xenobiotics can modify the metabolism and toxicity of their own or others to a great extent.

Induction of CarbEs

Oral or parenteral administration of phenobarbital can increase the cytosolic CarbE activity more than the microsomal activity, and the activity can remain elevated for 7 days after the last phenobarbital treatment. Phenobarbital treatment has no effect on the extrahepatic CarbE activity.

Parenteral administration of *p*,*p*′-DDT can augment hepatic CarbE for 14 days, probably due to the sequestration of the compound in adipose tissue. The hepatic CarbE activity can be increased up to threefold by phenobarbital and *p*,*p*′-DDT. Plasma CarbE activity is not altered by *p*,*p*′-DDT.

Hepatic and extrahepatic CarbE activities have been studied after the exposure of rats to polycyclic aromatic hydrocarbons. In dose- and time-dependent studies, benz(*a*)anthracene, benzo(*a*)pyrene, and 3-methylcholanthrene moderately induced the hepatic cytosolic and kidney microsomal CarbEs activities, while anthracene, phenanthrene, and chrysene had no effects on these enzymes. The hepatic microsomal and kidney cytosolic enzyme activities were not altered by the polycyclic aromatic hydrocarbons. The inducibility of hepatic cytosolic CarbE by the polycyclic aromatic hydrocarbons suggests that these compounds could be divided into two groups: benz(*a*)anthracene, benzo(*a*)pyrene, and 3-methylcholanthrene are moderate inducers, while anthracene, phenanthrene, and chrysene are noninducers. The commercial arochlors (i.e., polychlorinated biphenyls) increase hepatic CarbE by twofold.

From a toxicological point of view, the induction of CarbEs may protect against the toxic effects of an ester if the compound itself is directly responsible for the toxic action (e.g., the reduced toxicity of malathion or malaoxon following hexachlorobenzene exposure in rats). Conversely, the induction of CarbE may potentiate the toxic effects produced by the hydrolytic products of the compound (e.g., in the metabolism of allyl alcohol).

Inhibition of CarbEs

The most important inhibitors of CarbEs are organophosphorus insecticides (malathion, parathion, paraoxon, methyl parathion, EPN, and others), nerve agents (DFP, soman, sarin, tabun, and VX) and carbamate insecticides (carbofuran, carbaryl, aldicarb, propoxur, oxamyl, methomyl, and others). Organophosphorus toxicants inhibit CarbEs irreversibly by phosphorylation and carbamates inhibit CarbEs reversibly by carbamylation; similar to the basic mechanism (i.e., acylation of the active site):

$$\mathrm{EH} + \mathrm{AB} \underset{K_{-1}}{\overset{K_{+1}}{\leftrightarrow}} \mathrm{EHAB} \overset{K_{+2}}{\rightarrow} \mathrm{EA} + \mathrm{BH} \underset{\mathrm{H_2O}}{\overset{K_{+3}}{\rightarrow}} \mathrm{EH} + \underset{(+\mathrm{BH})}{\mathrm{AOH}}$$

where EH is the enzyme, AB is the inhibitor, EHAB is the enzyme–inhibitor complex, and EA is the acyl enzyme. In other words, organophosphates and carbamates inactivate CarbEs by rapid esterification of a serine residue in the active site. It is often followed by a slow hydrolysis of the new ester bond. Therefore, these compounds are not only inhibitors of CarbEs but also poor substrates. Other inhibitors of CarbEs include disulfiram (tetraethylthiuram disulfide) and glucocorticoids (dexamethasone).

Role of CarbEs in Organophosphate and Carbamate Poisoning

Depending on the involvement of one or more anticholinesterase agents, and their single or repeated exposure, CarbEs can have multiple roles, such as (1) a protective role by detoxifying organophosphates or

carbamates, (2) preinhibition of CarbEs as a major factor in potentiation of toxicity, and (3) role in tolerance development following repeated exposure to organophosphates.

Role of CarbEs in Detoxification of Organophosphates and Carbamates

The acute toxicity of organophosphates and carbamates is attributed to their effectiveness as inhibitors of AChE. During both acute and prolonged exposure to organophosphates and carbamates, the activities of other serine-containing esterases, such as CarbEs, are inhibited in both neuronal and non-neuronal tissues. Inhibition of CarbEs generally serves as a detoxifying mechanism by reducing the free concentration of AChE inhibitors. Low-level exposure to organophosphate or carbamate causes marked inhibition of CarbEs without inhibiting AChE, suggesting greater affinity of CarbEs than AChE to these inhibitors. CarbEs act like false targets or scavengers, which bind and thereby inactivate significant amounts of these inhibitors. In recent studies, one of the oximes, HI-6, reactivated CarbE activity, thereby providing additional protection against soman or possibly other organophosphate poisoning by acting as endogenous scavengers.

CarbEs, in addition to serving as nonspecific binding sites, can hydrolyze the carboxylester bond (esterolytic detoxification) in malathion-type organophosphates, carbamates, pyrethroids, and benzilate insecticides, and thereby reduce the free concentration of these insecticides. Therefore, CarbEs can detoxify organophosphates and carbamates by multiple mechanisms.

The diversity between the toxic effects of organophosphates, carbamates, or pyrethroids could partly be due to the existing variability in the levels of CarbE activity, which is related to species, strain, and gender differences. For example, rabbit liver CarbE is more sensitive to inhibition by malathion and isomalathion than pig liver CarbE; some strains of rats and mice have higher CarbE activity than others; and female rat plasma has higher CarbE activity than male rat plasma.

Role of CarbEs in Potentiation of Toxicity of Organophosphates or Carbamates

The toxicity of organophosphates or carbamates can be potentiated several-fold if the activity of CarbE is inhibited by pretreatment. Potentiation of malathion toxicity by EPN (*O*-ethyl-*O*-*p*-nitrophenyl phenylphosphonothionate) was reported about half a century ago. The mechanism responsible for potentiation by EPN of malathion toxicity has been explained on the basis of inhibition of the enzymatic hydrolysis of the carboxylester linkages of malathion. An impurity compound, *O*,*O*,*S*-trimethyl phosphorothioate, present in commercial formulations of malathion and phenthoate, potentiates the acute toxicity of malathion and phenthoate by inhibiting tissue CarbEs. One of the most susceptible animals is the dog.

Toxicity of organophosphates can be potentiated 15–20-fold in rats and mice by pretreatment with a metabolite of tri-*O*-cresylphosphate, CBDP (2-*O*-cresyl)-4*H*-1,3,2-benzodioxa-phosphorin-2-oxide), which is an irreversible inhibitor of CarbEs. In similar studies, tetraisopropylpyrophosphoramide (iso-OMPA), or mipafox, an organophosphate-irreversible inhibitor of CarbEs, potentiates three- to fivefold the toxicity of several OPs (soman, DFP, and methylparathion) and carbamates (carbofuran, aldicarb, propoxur, and carbaryl). Inhibition of CarbEs by CBDP, iso-OMPA, or mipafox pretreatment, particularly in plasma, liver, heart, brain, and skeletal muscles, is a major contributory factor in the potentiation of toxicity of organophosphates and carbamates. Thus, the toxicity of any drug, pesticide, or other type of agent that is normally detoxified by CarbEs, could be potentiated by pre-exposure to an organophosphorus or other carboxylesterase inhibitor.

Role of CarbEs in Organophosphate Tolerance Development

Daily exposure of rats, mice, or guinea pigs to certain organophosphates with sublethal doses can lead to severe toxicity during the first few days, but further exposure for 7–14 days can lead to development of tolerance. For example, daily dosing of DFP ($0.5\,mg\,kg^{-1}$, s.c.) produces severe anticholinesterase signs on day 5 (toxicity phase), but further administration results in tolerance development; because on day 14 rats are free of signs (tolerance phase). During tolerance, CarbE activity can recover up to 40% or more compared with the initial inhibition (day 5), suggesting renewed availability of nonspecific binding sites (CarbEs). The recovery of CarbE is probably due to *de novo* synthesis, since treatment with an inhibitor of protein synthesis, cycloheximide, abolishes tolerance development. In contrast to organophosphates, rats that were administered carbamates such as carbofuran or aldicarb daily for 3–4 weeks showed no development of tolerance to toxicity, probably due to lack of CarbE recovery. With organophosphorus toxicants, protection can be attenuated, toxicity can be potentiated, and tolerance can be abolished by preinhibition

of CarbEs with iso-OMPA, mipafox, or any other CarbE inhibitor against organophosphates.

See also: Biotransformation; Dithiocarbamates; Liver; Nerve Agents; Organophosphates; Pesticides.

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Carcinogen Classification Schemes

Michael A Kamrin

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Introduction

Several classification schemes have been developed for ranking the relative hazards to humans associated with chemicals that, by one or more criteria, may be considered to be potential carcinogens. The classification schemes are based on scientific judgments that typically take into account all the data available from *in vivo* animal bioassays, *in vitro* tests for genetic toxicity, human epidemiology, and structural relationships with other known carcinogens. Classification of a chemical as a carcinogen involves the consideration of many different factors. Classification schemes provide guidance on evaluating and weighting the available evidence and placing chemicals into defined categories that can be used to communicate the implications for risk. Factors usually taken into consideration in interpreting the results of an animal bioassay include the following:

- Adequacy of experimental design and conduct.
- Statistical significance of any increase in tumor incidence.
- Presence or absence of a dose–response relationship and correct dose selection.
- Nature of tumors (benign or malignant) and relevance of tumor type to humans.
- Historical control data (incidence and variability) for tumor type.
- Common (spontaneous) versus uncommon tumors.
- Number of organs/tissues with tumors.
- Mechanistic information.

Two commonly used classification schemes are the one developed by US Environmental Protection Agency (US EPA) and the one developed by the International Agency for Research on Cancer (IARC). The US EPA classification scheme is used as a tool for the regulation of chemicals under those laws it administers (e.g., FIFRA and TSCA) as well as by many state regulatory agencies. The IARC classification

scheme is commonly used in the European Community and is considered in certain US regulations and laws (e.g., OSHA Hazard Communication Standard). Other respected carcinogenic classification schemes include those developed by the National Institute of Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration (OSHA), National Toxicology Program (NTP), and American Conference of Government Industrial Hygienists (ACGIH). Each of these schemes is described in the following sections. It should be emphasized that these classification schemes are constantly evolving and that changes may occur over time.

US EPA Carcinogen Classifications

The US EPA carcinogen classifications are assigned to chemicals using the approach detailed in the *Guidelines for Carcinogens Risk Assessment* (51 FR 33992). The US EPA 'total-weight-of-evidence' scheme classifies potential carcinogens into five groups, A–E, that indicate the likelihood they are human carcinogens. These groups are described below.

- *Group A*: Human carcinogen – this is reserved for chemicals where there exists clear epidemiological evidence indicating an association between exposure to the chemical and cancer.
- *Group B*: Probable human carcinogen – this group is divided into two subgroups, B1 and B2. Group B1 indicates that there is 'sufficient' evidence to indicate that the material is an animal carcinogen and that there is 'limited' evidence of effects in humans. Group B2 indicates that although there is sufficient evidence in animals, the total weight of evidence for effects in humans is weaker or 'inadequate'.
- *Group C*: Possible human carcinogen – classification in this group indicates limited, often marginal evidence of carcinogenicity in animals and no evidence of any effects in humans.
- *Group D*: Not classifiable as to human carcinogenicity – this group is used for chemicals for which no data are available.
- *Group E*: Evidence of noncarcinogenicity for humans – this group is used for chemicals that show no evidence of any carcinogenicity in at least two adequately conducted animal tests with different species.

Proposed US EPA Classification Scheme

The US EPA classification scheme was published in the 1986 cancer guidelines (51 FR 33992). In April 1996, the US EPA proposed new cancer guidelines which differ substantially from the previous guidelines. The new guidelines recommend a narrative with descriptors that replace the previous letter designations. The narrative explains the kinds of evidence available and how they fit together in drawing conclusions, along with highlighting the significant issues and strengths and limitations of the data and conclusions. The descriptors have standardized definitions. The descriptors are not meant to replace an explanation of the nuances of the biological evidence but rather to summarize it. The use of descriptors within a narrative is intended to preserve the complexity (including the gray areas) that is an essential part of the hazard classification. Risk managers are instructed to consider the entire range of information included in the narrative rather than focusing simply on the descriptor.

Each category spans a wide variety of potential data sets and weights of evidence. The three proposed categories of descriptors for human carcinogenic potential are 'known/likely', 'cannot be determined', and 'not likely'.

Known/Likely

This category of descriptors is used when the available tumor effects and other key data are adequate to convincingly demonstrate carcinogenic potential for humans. It includes cases in which agents are known human carcinogens based on either epidemiologic evidence or a combination of epidemiologic and experimental evidence, demonstrating causality between human exposure and cancer; agents that should be treated as if they were known human carcinogens, based on a combination of epidemiologic data showing a plausible causal association (not demonstrating it definitively) and strong experimental evidence; and agents that are likely to produce cancer in humans due to the production or anticipated production of tumors by modes of action that are relevant or assumed to be relevant to human carcinogenicity.

Cannot Be Determined

This category of descriptors is used when available tumor effects or other key data are suggestive or conflicting or limited in quantity and, thus, are not adequate to convincingly demonstrate carcinogenic potential for humans. It includes cases in which agents' carcinogenic potential cannot be determined but there is suggestive evidence that raises concern for carcinogenic effects; agents whose carcinogenic potential cannot be determined because the existing evidence is composed of conflicting data (e.g., some

evidence is suggestive of carcinogenic effects, but other equally pertinent evidence does not confirm any concern), and agents whose carcinogenic potential cannot be determined because there are inadequate or no data to perform an assessment.

Not Likely

This category of descriptors is used when, in the absence of human data suggesting a potential for cancer effects, the experimental evidence is satisfactory for deciding that there is no basis for human hazard concern. It includes cases in which agents are not likely to be carcinogenic to humans because they have been evaluated in at least two well-conducted studies in two appropriate animal species without demonstrating carcinogenic effects; agents not likely to be carcinogenic to humans because they have been appropriately evaluated in animals and show only carcinogenic effects that have been shown not to be relevant to humans; agents not likely to be carcinogenic to humans when carcinogenicity is dose or route dependent (e.g., not likely below a certain dose range or not likely by a certain route of exposure); and agents not likely to be carcinogenic to humans based on extensive human experience that demonstrates lack of effect (e.g., phenobarbital).

In 1999, the EPA published additional draft materials relating to carcinogenicity classification. These materials provided additional guidance for evaluating weight of evidence and also proposed changing the three categories described in the 1996 document. The new categories are Carcinogenic to Humans; Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential; Data are Inadequate for an Assessment of Human Carcinogenic Potential; and Not Likely to Be Carcinogenic to Humans. None of these post-1986 guidelines have been finalized.

IARC Carcinogen Classifications

IARC is a department of the World Health Organization (WHO). The overall classification scheme developed by IARC is similar to that used by the US EPA (the US EPA scheme was initially developed based on an IARC scheme). Chemicals are classified into four groups with respect to their potential to cause cancer in humans. The classification reflects the strength of the evidence available from animal studies, epidemiology, and other relevant data. The IARC groups are outlined below.

- *Group 1*: The agent is carcinogenic to humans – this group is reserved for those chemicals or agents where there is 'sufficient evidence' of carcinogenicity in humans.
- *Group 2*: The agent is probably carcinogenic to humans – this group, like the US EPA group B, is divided into two subgroups, groups 2A and 2B, depending on the strength of the evidence available. Groups 2A and 2B indicate that the agent is 'probably' or 'possibly' carcinogenic to humans, respectively.
- *Group 3*: The agent is not classifiable as to its carcinogenicity to humans – this group is used for chemicals that do not fall into any of the other groups.
- *Group 4*: The agent is probably not carcinogenic to humans – this group is used for compounds where there exists evidence suggesting an absence of carcinogenic potential in humans.

NTP Carcinogen Classifications

The NTP is responsible for preparing *Reports on Carcinogens*. The *Reports on Carcinogens* are mandated by Public Law 95-662 and are for are informational purposes only. The listing of a substance in the annual report does not by itself establish that such a substance presents a risk to persons in their daily lives. Clause (I) in subparagraph (4) (A) of Section 301 (b) of the Public Health Service Act requires that a report be published which contains a list of all substances (1) 'which are either known to be carcinogens or may reasonably be anticipated to be carcinogens', and (2) to which a significant number of persons residing in the United States are exposed. The conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. As of the 2002 update, for the purpose of *Biennial Report on Carcinogens*, the classification scheme is outlined below.

- *Group 1*: Known to be human carcinogens – this group is reserved for those chemicals where there is sufficient evidence of carcinogenicity from studies in humans which indicate a causal relationship between exposure to the agent, substance, or mixture and human cancer.
- *Group 2*: Reasonably anticipated to be human carcinogens – this group, like the US EPA group B, is divided into two subgroups, groups 2A and 2B, depending on the strength of the evidence available.
- *Group 2A*: There is limited evidence of carcinogenicity from studies in humans which indicate that a causal interpretation is credible, but that alternative explanations, such as chance,

bias, or confounding, could not adequately be excluded.

- *Group 2B*: There is sufficient evidence of carcinogenicity from studies in experimental animals which indicate that there is an increased incidence of malignant tumors and/or combined benign and malignant tumors (a) in multiple species or at multiple tissue sites, (b) by multiple routes of exposure, or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or there is less than sufficient evidence of carcinogenicity in humans or laboratory animals. However, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous *Annual* or *Biennial Report on Carcinogens* as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

OSHA Carcinogen Classifications

The Occupational Safety and Health Act of 1970 provides the establishment of workplace standards for toxic materials or harmful physical agents

> which most adequately assures, to the extent feasible, on the basis of the best available evidence, that no employee will suffer material impairment of health or functional capacity even if such employee has regular exposure to the hazard dealt with by such standard for the period of his or her working life.

Potential occupational carcinogens regulated under OSHA are classified into two main categories based on the nature and extent of the available scientific evidence: category I potential carcinogens and category II potential carcinogens.

Category I Potential Carcinogens

A substance shall be identified, classified, and regulated as a category I potential carcinogen if, upon scientific evaluation, the secretary determines that the substance meets the definition of a potential occupational carcinogen in (1) humans, or (2) a single mammalian species in a long-term bioassay in which the results are in concordance with some other scientifically evaluated evidence of a potential carcinogenic hazard, or (3) a single mammalian species in an adequately conducted long-term bioassay, in appropriate circumstances in which the secretary determines the requirement for concordance is not necessary. Evidence of concordance is any of the following: positive results from independent testing in the same or other species, positive results in short-term tests, or induction of tumors at injection or implantation.

Category II Potential Carcinogens

A substance shall be identified, classified, and regulated as a category II potential carcinogen if, upon scientific evaluation, the secretary determines that (1) the substance meets the criteria set forth for category I, but the evidence is found by the secretary to be only 'suggestive'; or (2) the substance meets the criteria set forth for category I in a single mammalian species without evidence of concordance.

NIOSH Carcinogen Classifications

Acting under the authority of the Occupational Safety and Health Act of 1970 (Public Law 91-596), the NIOSH develops and periodically revises recommended exposure limits (RELs) for hazardous substances or conditions in the workplace. These recommendations are then published and transmitted to OSHA for use in promulgating legal standards. NIOSH may identify numerous chemicals that it believes should be treated as occupational carcinogens even though OSHA has not yet identified them as such. Generally, where OSHA has adopted the NIOSH recommendations as OSHA standards, the OSHA PELs and NIOSH RELs are equal. In cases in which the NIOSH recommendations have not been formally adopted by OSHA, the NIOSH RELs may be different from the OSHA PELs. For example, the NIOSH exposure limit for trichloroethylene (25 ppm) differs from the OSHA exposure limit (50 ppm).

The NIOSH classification scheme is one of the simplest carcinogen classification schemes; it combines all carcinogens into one category. Within this single category, NIOSH narratively describes the site of the cancer and whether the effect was seen in humans or animals. In determining carcinogenicity, NIOSH uses a classification scheme outlined in 29 CFR 1990.103, which states in part:

> Potential occupational carcinogen means any substance, or combination or mixture of substances, which causes an increased incidence of benign and/or malignant neoplasms, or a substantial decrease in the latency period between exposure and onset of neoplasms in humans or in one or more experimental mammalian species as the result of any oral, respiratory, or dermal exposure, or any other exposure which results in the induction of tumors at a site other than the site of administration. This definition also includes any substance

which is metabolized into one or more potential occupational carcinogens by mammals.

The NIOSH thresholds for carcinogens were not designed to be protective of 100% of the population. NIOSH usually recommends that occupational exposures to carcinogens be limited to the lowest feasible concentration. This perhaps is the reason that the NIOSH exposure limit for vinyl chloride is the lowest reliably detectable concentration and the OSHA exposure limit is 1 ppm.

ACGIH Carcinogen Classifications

ACGIH classifies substances associated with industrial processes that are recognized to have carcinogenic or cocarcinogenic potential. In general, the stated classification is intended to provide a practical guideline for the industrial hygiene professional to assist in control of exposures in the workplace. The classification and threshold limit values (TLVs) are not mandated by federal or state regulations, although the ACGIH classifications and values may be considered when standards are adopted by the regulatory agencies. Currently, five categories of carcinogens have been designated by the TLV Committee to recognize the qualitative differences in research results or other data. These five categories are outlined below.

- *A1*: Confirmed human carcinogen – the agent is carcinogenic to humans based on the weight of evidence from epidemiologic studies of exposed humans, and/or convincing clinical evidence in exposed humans.
- *A2*: Suspected human carcinogen – the agent is carcinogenic in experimental animals at dose levels, by route(s) of administration, at site(s), of histologic types(s), or by mechanism(s) that are considered relevant to worker exposure. Available epidemiologic studies are conflicting or insufficient to confirm an increased risk of cancer in exposed humans.
- *A3*: Animal carcinogen – the agent is carcinogenic in experimental animals at a relatively high dose, by route(s) of administration, at site(s), of histologic types(s), or by mechanism(s) that are not considered relevant to worker exposure. Available epidemiologic studies do not confirm an increased risk of cancer in exposed humans. Available evidence suggests that the agent is not likely to cause cancer in humans except under uncommon or unlikely routes or levels of exposure.
- *A4*: Not classifiable as a human carcinogen – there are inadequate data on which to classify the agent in terms of its carcinogenicity in humans and/or animals.
- *A5*: Not suspected as a human carcinogen – the agent is not suspected to be a human carcinogen on the basis of properly conducted epidemiologic studies in humans. These studies have sufficiently long follow-up, reliable exposure histories, sufficiently high dose, and adequate statistical power to conclude that exposure to the agent does not convey a significant risk of cancer to humans. Evidence suggesting a lack of carcinogenicity in experimental animals will be considered if it is supported by other relevant data.

Substances for which no human or experimental animal carcinogenic data have been reported are assigned no carcinogen designation by the ACGIH.

See also: American Conference of Governmental Industrial Hygienists; Carcinogenesis; Dose–Response Relationship; Epidemiology; Federal Insecticide, Fungicide, and Rodenticide Act, US; International Agency for Research on Cancer; Levels of Effect in Toxicological Assessment; National Institute for Occupational Safety and Health; National Toxicology Program; Occupational Safety and Health Act, US; Occupational Safety and Health Administration; Risk Assessment, Human Health; Toxic Substances Control Act, US; Toxicity Testing, Carcinogenesis.

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http://www.osha.gov – Occupational Safety and Health Administration.
http://www.acgih.org – American Conference of Governmental Industrial Hygienists.
http://www.cdc.gov – National Institute for Occupational Safety and Health.
http://www.iarc.fr – International Agency for Research on Cancer.
http://ntp-server.niehs.nih.gov – National Toxicology Program.

Carcinogen–DNA Adduct Formation and DNA Repair

Ainsley Weston and Miriam C Poirier

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Definition

Carcinogen–DNA adducts are addition products formed by covalent binding of all or part of a carcinogen molecule to chemical moieties in DNA; adducts are formed when an activated chemical species (electrophilic, positively charged metabolite) binds covalently to negatively charged moieties in DNA.

Importance of DNA Adduct Formation in the Process of Carcinogenesis

Carcinogen–DNA adducts of exogenous genotoxic chemical carcinogens may induce errors in DNA sequence (mutations). Subsequent transcription on a damaged template may result in the formation of abnormal proteins or the absence of protein. DNA adduct formation and mutagenesis are considered to bring about changes in gene expression that produce clonal expansions of cells lacking in growth control (tumors). A substantial period of time is required for a tumor to become evident, and DNA damage is considered to be necessary but not sufficient for tumorigenesis, since other events must also take place. DNA adduct levels, measured at any point in time, reflect tissue-specific rates of damage processing that include DNA adduct formation and removal (DNA repair), DNA adduct instability, tissue turnover and other events. In experimental model systems dose–response associations have been observed for DNA adduct formation, mutagenesis, and tumorigenesis. Reductions in tumor incidences have been observed when DNA adduct levels have been lowered, either by DNA repair processes or by administration of chemopreventive agents that inhibit DNA adduct formation with no change in dose.

Biotransformation of Carcinogenic Chemicals to Species that Modify DNA

Exogenous carcinogenic chemicals that form DNA adducts can be direct acting if they are highly reactive. Examples are the nitrosoureas, some nitrosamines, ethylene oxide, and ozone. However, most are inert like the polycyclic aromatic hydrocarbons (PAHs) and require biotransformation (metabolic activation). Biotransformation consists of metabolic alteration by families of enzymes that convert a small fraction of the initial dose to highly reactive intermediate metabolites able to 'modify' (become bound to) DNA, thus accomplishing the first essential step (initiation) in the carcinogenic process. Exogenous carcinogens that require metabolic activation include some plant and fungal products (aflatoxins, ochratoxins, hydrazines), pyrolysis products from cooking (heterocyclic amines, PAHs), industrial combustion products (aromatic amines, PAHs, nitro-PAHs, benzene, vinyl chloride, nitrosamines, ethylene oxide), urban pollution contaminants (PAHs, nitro-PAHs, aromatic amines) and components of tobacco (tobacco-specific nitrosamines) and tobacco smoke (PAHs, nitrosamines, and aromatic amines). The metabolic processes that lead to DNA adduct formation for several classes of genotoxic chemical carcinogens, including the PAHs, the aromatic amines, the heterocyclic amines, some fungal products and oxyradical damage, are described briefly.

The PAHs, which include the human carcinogen benzo[*a*]pyrene (BP) (**Figure 1**), are composed of variable numbers of fused benzene rings and are chemically unreactive, as well as insoluble in water. These compounds are ubiquitous environmental contaminants found in cigarette smoke and products of partial combustion, and are produced by many industrial processes. They are metabolized to simple epoxides by cytochrome P-450, hydrated through the action of epoxide hydrolase and subjected again to epoxidation (cytochrome P-450) to form unstable dihydrodiol-epoxides. The unstable metabolites spontaneously convert to positively charged, highly reactive free radicals (carbocations, the ultimate

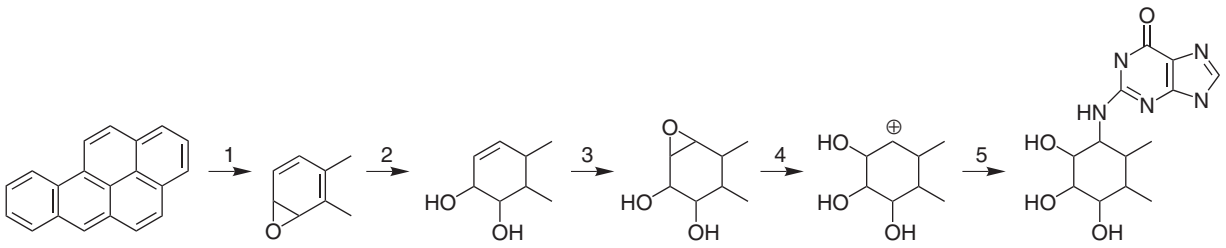

Figure 1 Metabolic activation of benzo[*a*]pyrene (a representative PAH). The parent hydrocarbons are chemically inert and require metabolic activation before they can exert their biological effects. Cytochrome P-450 enzymes (principally CYP1A1) catalyze the formation of simple arene oxides from the parent hydrocarbons (1). The arene oxides are converted to dihydrodiols by the action of epoxide hydrolase (2). The resulting dihydrodiols are further oxidized by cytochrome P-450 enzymes (principally CYP3A4) at the site of the olefinic double bond (3). Vicinal diol-epoxides are high unstable and the arene-ring opening is spontaneous yielding a highly reactive carbocation (4). The electrophilic carbocationic species can form a covalent bond with the exocyclic amino group of deoxyguanosine (5). The resulting polycyclic aromatic hydrocarbon–DNA adduct lies in the minor groove of the double helix.

carcinogenic forms), which bind covalently to DNA and protein. The metabolic scheme for BP is shown in **Figure 1** and the structure of the major DNA adduct, between BP and deoxyguanosine is shown in **Figure 2a**.

Aromatic amines are characterized by the presence of benzene rings and an exocyclic nitrogen. A prototypical aromatic amine, 4-aminobiphenyl (4-ABP), found in tobacco smoke and industrial exhaust, has been implicated in human bladder cancer. The presence of the amino group, that can be either acetylated or nonacetylated, contributes to the complexity of aromatic amine metabolism. Activation of aromatic amines proceeds by *N*-oxidation with sulfotransferase catalysis, resulting in the formation of acetylated (**Figure 2b**), and nonacetylated (**Figure 2c**), guanine adducts. **Figure 2b** and **c** shows guanine adducts of the carcinogen *N*-2-acetylaminofluorene.

Heterocyclic amines, for example, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), are formed from the pyrolysis ($>150^{\circ}C$) of amino acids, creatinine, and glucose that occurs during cooking of meat and fish. They are known as food mutagens and their metabolism, largely influenced by the amine moiety, is similar to that of the aromatic amines. They undergo cytochrome P-450-induced *N*-hydroxylation (CYP1A2). *N*-hydroxylation metabolites of some heterocyclic amines (2-amino-3-methyl-imidazole-[4,5-*f*]quinoline; IQ) can react directly with DNA, while others require further enzymic *O*-esterification. The major guanine adduct of PhIP is shown in **Figure 2d**.

Fungal mycotoxins, including aflatoxin B_1 derived from *Aspergillus flavus*, contaminate cereals, grains, and nuts, and aflatoxin B_1 ingestion is correlated with a high incidence of liver cancer in animal models and humans. Aflatoxins are heterocyclic and contain several endocyclic oxygen molecules. They are activated by simple epoxidation (cytochrome P-450) across the olefinic double bond at the 8,9-position, giving rise to a carbocation. However, some addition products with DNA are unstable and lead to nonmutagenic depurination. The major aflatoxin-guanine adduct is shown in **Figure 2e**.

Oxyradicals (reactive oxygen species), formed as a result of endogenous processes or exposure to exogenous chemicals, can cause oxidation of DNA. Two common examples of oxyradical damage found in DNA include thymine glycol and 8-hydroxydeoxyguanosine adducts (**Figure 2h**). Probably the most common endogenous oxyradical exposure is to $O_2^{\bullet -}$ (superoxide anion) and H_2O_2 (hydrogen peroxide). This occurs when O_2 is reduced for the production of energy, and although most of the electrons are contained, there is some leakage. Other endogenous sources of oxyradicals include reactions of $O_2^{\bullet -}$ with Fe^{3+} or NO to form unstable intermediates (e.g., ONOOH) that are powerful, direct-acting oxidants or that yield hydroxyl radical. The mechanism involving NO is also the basis for inflammation, and can represent a normal response to infection. Exposure to organic peroxides, catechol, hydroxyquinone and 4-nitroquinoline-*N*-oxide among others, leads to oxyradical damage. Moreover, cells can be stimulated to produce peroxisomes by treatment with certain drugs and plasticizers. The role of oxyradical DNA damage in chemical carcinogenesis is currently unclear, although the mutagenic potential of these adducts has been amply demonstrated in experimental systems.

Measurement of Carcinogen–DNA Adducts as Human Exposure Dosimeters

The promise of human DNA adduct biomonitoring is the application of a human biomarker that is directly correlated with cancer risk for cancer prevention or

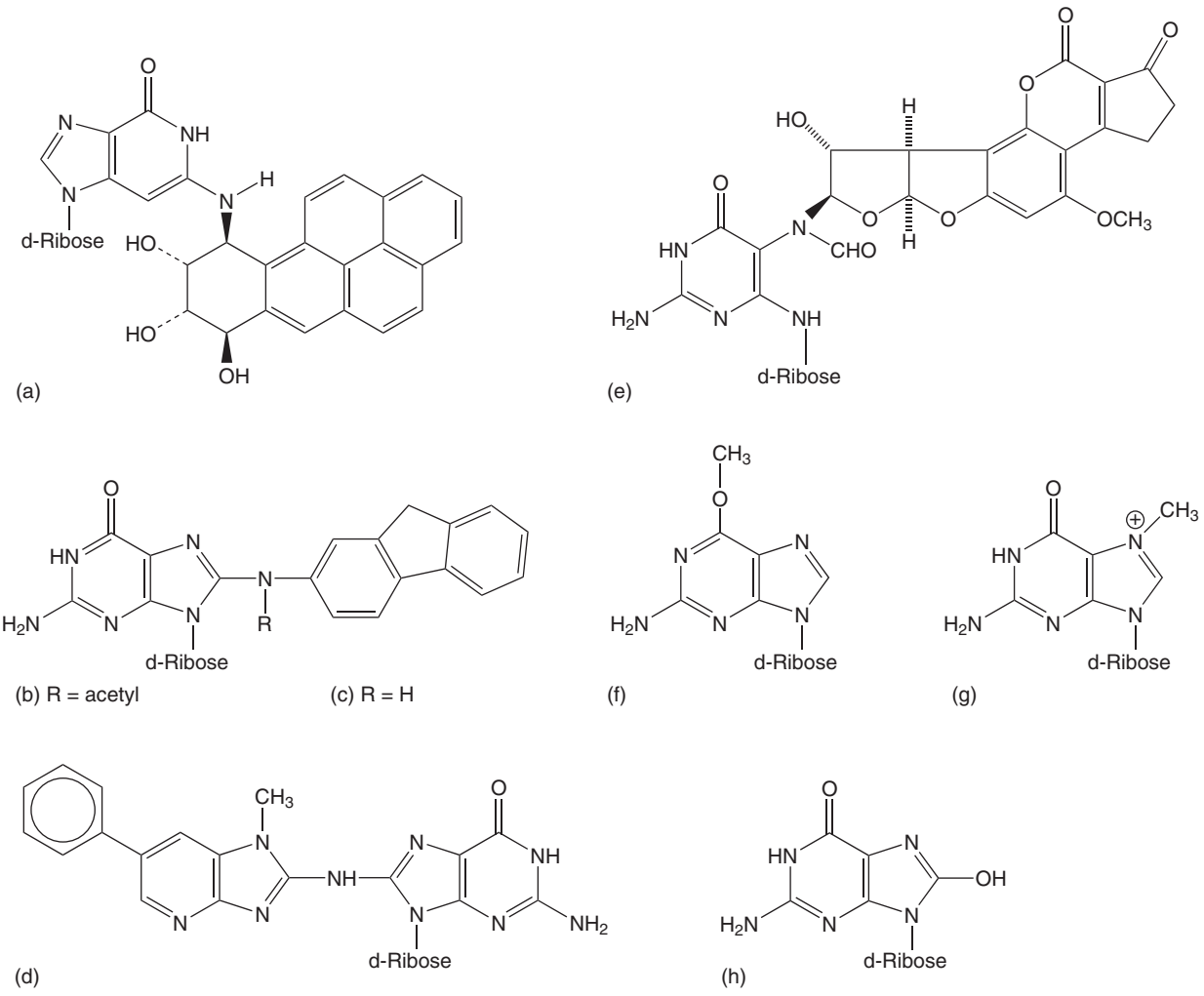

Figure 2 Molecular structures of carcinogen adducts of deoxyguanosine: (a) (7R)-N^2-(10-{7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene}-yl)-deoxyguanosine, formed when benzo[*a*]pyrene-7,8-diol 9,10-epoxide reacts with the exocyclic amino group of deoxyguanosine; (b) *N*-(deoxyguanosin-8-yl)-2-(acetylamino)fluorene, formed when *N*-hydroxylacetylaminofluorene reacts with the C8 position of the imidazole ring; (c) *N*-(deoxyguanosin-8-yl)-2-(amino)fluorene, formed when *N*-hydroxylaminofluorene reacts with the C8 position of the pyrimidine ring structure; (d) *N*-(deoxyguanosin-8-yl)-2-amino-1-methyl-6-phenylimidazo-[4,5*b*]-pyridine, formed when the *N*-hydroxylamine metabolite of 2-amino-1-methyl-6-phenylimidazo-[4,5*b*]-pyridine (PHIP), a glutamic acid pyrolysate, reacts with deoxyguanosine; (e) ring-opened form of *N*-(deoxyguanosin-7-yl)-9-hydroxyaflatoxin B_1, formed following the reaction of the 8,9-epoxide metabolite of aflatoxin B_1 at the N7 position of deoxyguanosine; (f) O^6-methyldeoxyguanosine, formed when an alkyl radical (CH_3^+), derived from an alkylating agent, reacts at the O^6-position of deoxyguanosine; (g) N7-methyldeoxyguanosine, formed when an alkyl radical (CH_3^+), derived from an alkylating agent, reacts at the N7-position of deoxyguanosine; (h) 8-hydroxydeoxyguanosine, formed through exogenous or endogenous oxy-radical damage (H_2O_2, $^{\bullet}OH$, $O_2^{\bullet -}$) at the C8 position of deoxyguanosine.

intervention. This field has expanded exponentially since the 1980s, a progress made possible by the development of highly sensitive methods for the detection of DNA adducts in human tissue. The most widely used methods include immunoassays and immunohistochemistry, ^{32}P-postlabeling, fluorescence and phosphorescence spectroscopy, and gas chromatography/mass spectrometry. Detection limits for quantitative assays are typically in the range of 1 adduct in 10^9 nucleotides. However, accelerator mass spectrometry, a highly sophisticated but less accessible method, has a detection limit of $\sim$1 adduct in 10^{12} nucleotides.

When used without preparative procedures, the most commonly used techniques are typically unable to provide quantitation of individual adducts and chemical characterization of a specific adduct. This is because humans are exposed to complex mixtures of chemical carcinogens, and human DNA will contain multiple DNA adducts induced by different xenobiotic agents. The development of preparative strategies for sample purification that can be applied

prior to the ultimate DNA adduct quantitation has made possible chemical characterization of specific DNA adducts in human tissues. The combination of preparative methods (immunoaffinity chromatography, high-performance liquid chromatography or other chromatography) with immunoassays, ^{32}P-postlabeling or synchronous fluorescence spectrometry has made possible the identification of specific DNA adduct structures. In addition, chemical derivatization approaches have facilitated the various novel permutations of gas chromatography/mass spectrometry that have become widely applied for the determination of specific human DNA adducts.

The majority of studies designed to monitor DNA adducts in human tissues fall into the category of exposure documentation and have concentrated on environmental and occupational exposures to agents for which precise dosimetry is difficult or impossible. Many studies have shown decreases in DNA adduct levels (qualitative dosimetry) in groups of subjects removed from exposure by virtue of location or season. Quantitative dosimetry for human DNA adduct formation has been established with medicinal (cisplatin, procarbazine, dacarbazine, and 8-methoxypsoralen) and dietary (aflatoxins) exposures where dosimetry can be established accurately. A major goal of carcinogen dosimetry is the application of human DNA adduct formation data within epidemiologic study designs to predict human cancer risk. This goal has been achieved in two prospective nested case–control studies and several case–control studies. In the prospective studies which involved lung cancers in smokers and liver cancers in individuals exposed to aflatoxins, relative risks for cancer were increased three- to sevenfold in individuals with elevated DNA adduct levels. In the case–control studies elevated DNA adduct levels (odds ratios 2.3–16.2) were found in the cases compared to the controls. Whereas the epidemiologic studies investigating the relationship between human DNA adduct levels and cancer risk will take many years, these early studies appear to support the data from experimental models that has shown that DNA adduct formation is necessary but not sufficient for tumorigenesis.

Biological Repair of Adduct Damage in DNA (DNA Repair)

Toxicological damage to DNA can alter its chemical structure in many different ways. Covalent addition products may be formed with activated, bulky aromatic compounds or smaller alkyl-species (**Figure 2f** and **g**). Oxyradical formation, often the by-product of normal metabolic processes, dimerization and deamination also modifies the chemical structure of DNA, and single- and double-strand breaks (DSBs) can occur. All of these types of DNA damage may lead to permanent changes in DNA sequence, and some have been associated with the development of disease (e.g., cancer, progeria, Cockayne's syndrome, retinal dystrophy, thalassemia, xeroderma pigmentosum, and birth defects) and normal aging.

A series of metabolic pathways has evolved to counteract DNA damage through removal of the lesions. Mechanisms of DNA repair are complex, generally requiring the products of several genes to act in concert to accomplish restoration of DNA structure. Cell cycle restriction point genes are responsible for conducting the whole DNA repair process. These complexes usually comprise a damage sensor, a damage eliminator, a polymerase or patch synthesizer, and a ligase. However, more than 150 genes are known to participate in DNA repair and some contribute to more than one pathway. For example, genes from multiple pathways are assembled in the BRCA-1-associated genome surveillance complex that constitutes a sensory apparatus for detection and binding to damaged DNA.

There are six general mechanisms of DNA repair: direct repair (DR), nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination repair (HRR), and nonhomologous end joining (NHEJ).

Direct DNA Repair

In contrast to the general scheme outlined above, DR employs only a single suicide enzyme. An alkyltransferase commutes an alkyl group from an alkylated base (O^6-methyldeoxyguanosine) to a cysteine residue in its own active site. Because there is no strand scission, there is also no need for patch synthesis or ligation. In this suicidal process one adduct consumes one molecule of enzyme.

Nucleotide Excision Repair and Base Excision Repair

Both of these processes require multiple enzymes acting either sequentially or, as indicated above, as molecular complexes. The hallmarks of both NER and BER are strand scission, removal of a segment of DNA containing the adducted base, 5′ to 3′-oriented DNA patch synthesis through the action of a polymerase, using the intact strand as a template, and ligation of the free ends. The distinctions between these two mechanisms are the proteins involved and the types of adducts that are repaired. Bulky adducts, like those of aromatic compounds or

malondialdehydes (the result of lipid metabolism), cause DNA distortions that are relatively large and are repaired by NER. In NER the structural distortion is recognized by products of sensory genes (e.g., *XPA*, *XPC*, or *XPE*) and excised by endonucleases (e.g., XPF, XPG, or FEN), the patch is synthesized by a polymerase (polΔ or polε) and ligated (DNA ligase I or DNA ligase III). Further, there are two types of NER, global genomic repair (GGR) and transcription-coupled repair (TCR), which are characterized by different sets of genes. The repair patch generated for TCR is limited to the transcribed strand of transcriptionally active genes and involves multiple (15–30) nucleotides. The repair patch for GGR is limited to a single nucleotide, but GGR can occur on either strand in both transcriptionally active and inactive regions of the genome.

Small adducts, like 3-methyladeneine, 5-hydroxuracil and 5-hydroxymethlyuracil, are repaired by BER. In BER a lesion is detected and removed by a glycosylase (e.g., hOgg1 or UDG) creating an apurinic site (AP). Subsequently an endonuclease degrades the damaged strand (e.g., APEI, FEN1) a patch is synthesized by a polymerase (polβ) and ligation occurs (DNA ligase I or DNA ligase III). The patch size in BER can either be short (one nucleotide) or long (two to 10 nucleotides). Short BER is polβ-dependent and ligation is accomplished by DNA ligase III, whereas long BER is associated with proliferating cell nuclear antigen (PCNA) and ligation is accomplished by DNA ligase I. Oxidative damage may be repaired by either NER or BER.

Mismatch Repair

Nucleotide mismatches occur when DNA repair processes insert an inappropriate but unmodified, conventional base opposite a noncomplementary partner. These may be transitions (purine to purine or pyrimidine to pyrimidine: G–T or A–C) or transversions (purine to pyrimidine or pyrimidine to purine: C–C, T–T, C–T; A–A, G–G, A–G). For example, in post-replication 'repair', a DNA damage tolerance mechanism that leaves a gap in response to replication on a damaged template, the polymerase always inserts an adenine in the gap. In addition, deamination of cytosine results in thymidine. Both NER and MMR feature degradation of a relatively large portion of the damaged strand, followed by 5′ to 3′ patch synthesis using the undamaged strand as a template, and ligation to complete the repair. The DNA mismatch is recognized by a repair protein complex (either MSH1–MSH2–MSH6–PMS1 or MSH1–MSH2–MSH6–PMS2) that simultaneously anchors to both the mismatch and the closest unmethylated adenine in the GATC recognition sequence. The entire sequence between the mismatch and the GATC recognition sequence is eroded, and PCNA is recruited to act as a sliding clamp and support the action of a DNA polymerase (polΔ or polε) in replication of the repair patch. A ligase (DNA ligase I) subsequently complexes with polymerase (polΔ) to complete the repair function.

Mismatch DNA repair is critical in the maintenance of a stable genome. Inheritance of mutations in genes involved in mismatch DNA repair (*MSH1, MSH2, MSH6, PMS1, PMS2*) can predispose to cancers of the brain, endometrium, ovaries, and bowel. Somatic mutations in these genes may also contribute to the mutator phenotype.

Homologous Recombination Repair

Double-strand breaks can be caused by ionizing radiation, oxidative stress and mechanical stress (e.g., when a topoisomerase encounters a bulky adduct during DNA replication). There are two distinct mechanisms for repairing DSBs, HRR and NHEJ. Several sensors of HRR that trigger the process include ATM, RAD3-related ATM, and DNA-protein kinases (Chk2). Homologous recombination involves a number of DNA repair proteins (RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54, XRCC2, XRCC3, BRCA1, and BRCA2). First, a nibrin (NBS1) complex with RAD50/MRE11 (also known as MRN complex) brings about simultaneous recision of both strands, which is thought to be 5′ to 3′. RAD51 and its paralogs (accessory proteins – RAD51B, C, D) prepare the single-stranded DNA segments for sister chromatid exchange and invasion of the homologous duplex. Polymerization occurs using the undamaged homologous duplex DNA sequences as a template. The process is completed by either through resolution of Holliday junctions by the action of an endonuclease and strand sealing by a ligase, or disengagement of the Holliday junctions, DNA pairing and gap filling in the damaged homolog. The first scenario would give an equal opportunity for crossover events and noncrossover events to occur. However, there is now evidence to suggest that HRR is more often accomplished without a crossover event, a mechanism that is more conservative in reducing genomic alterations.

Nonhomologous End-Joining

The other major repair pathway involved in DNA DSB repair is NHEJ. While this pathway brings about DSB repair, it is also involved in immunological diversification by re-ligating the products of recombinase (RAG1 and RAG2) cleavage. Unlike

HRR, NHEJ is independent of a genetic DNA sequence homolog because repair occurs without copying an undamaged template. Components of the NHEJ pathway include XRCC4, ligase IV, KU70, and DNA protein kinase. In addition, if ligation of two blunt ends cannot restore the original sequence, a deletion mutation will result. Therefore, this mechanism is sometimes referred to as 'illegitimate'. In the presence of single-stranded over-hanging segments, some degradation may occur to create a blunt end before ligation occurs (ligase IV). Alternatively, recruitment of polμ or polλ by the XRCC4-ligase IV complex may result in gap filling.

See also: Cytochrome P-450; Polycyclic Aromatic Amines; Polycyclic Aromatic Hydrocarbons (PAHs).

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Carcinogenesis

David E Malarkey and Robert R Maronpot

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Overview

Cancer, or neoplasia, which occurs in one of every four individuals and results in the death of one of every five individuals in the United States, is a complex disease with multiple causes. Many intrinsic and extrinsic factors influence the development of cancer. Intrinsic or host factors include age, sex, genetic constitution, immune system function, metabolism, hormone levels, and nutritional status. Extrinsic factors include substances eaten, drunk, or smoked; workplace and environmental (air, water, and soil) exposures; natural and medical radiation exposure; sexual behavior; and elements of lifestyle such as social and cultural environment, personal behavior, and habits. Intrinsic and extrinsic factors can interact with one another to influence the development of cancer. Because of the physical and emotional suffering associated with cancer and the immense cost to the nation in lost production and income and medical and research expenditures, considerable effort continues to be exerted to understand this complex disease so that strategies can be developed to decrease or prevent its occurrence. Current regulatory guidelines have been crafted to reduce the probability of developing cancer by lowering human exposure to agents identified as potentially capable of causing cancer.

During the past 40 years of cancer research, much information has been generated indicating that

cancer is a multistep, progressive disease. Support for this contention is derived from research on epidemiology and population genetics, morphological and clinical study of neoplasms, as well as experimental investigations in animals. Structural studies of biopsy and autopsy tissue samples from humans and animals, particularly experimental animal models of carcinogenesis, have provided important information about this multistep process at the phenotypic level. More recently, molecular biological analyses have confirmed the principles that neoplasms arise from the clonal expansion of a single cell and that during its evolution into a neoplastic mass, it accumulates nonlethal genetic damage, particularly in genes that regulate growth and DNA repair processes. The process of carcinogenesis may take months in experimental laboratory animals and years in humans. Identification of this process early in its evolution enhances the likelihood that intervention strategies such as surgical removal of a benign neoplasm may result in termination of the disease and clinical cure. By the time a neoplasm has progressed to the malignant stage and spread throughout the body, even heroic radiation and chemotherapy combined with surgery are unlikely to result in clinical cure. The process of carcinogenesis may be depicted schematically as in **Figure 1** with the various steps along the pathway from normalcy to malignancy characterized by morphological and/or clinical features. It is here that the disciplines of clinical oncology, molecular biology and pathology are utilized to define the location of the specific neoplasm in this progressive cascade.

Nomenclature of Cancer (Neoplasia)

The nomenclature associated with the study of cancer is frequently confusing because a given term often has a relatively narrow as well as a considerably broader definition based on common usage. Carcinogenesis, for example, is narrowly defined as the production of carcinoma but is more commonly used in the broadest possible sense to indicate generation of neoplasms which are new and typically abnormal growths, generally uncontrolled and becoming progressively more serious with time. Neoplasia, meaning 'new growth' and often used synonymously with carcinogenesis, refers to the process of development of neoplasms. Two important terms which relate to the clinical behavior and growth characteristics of neoplasms are (1) benign and (2) malignant, characteristic features of which are listed in **Table 1**. Basically, benign neoplasms are slow-growing, localized growths frequently amenable to surgical removal with a low probability of recurrence. Malignant neoplasms have a more aggressive growth, are locally invasive, sometimes metastasize (spread to distant sites), and are difficult to remove surgically.

Two terms that have both a narrow and a broad definition are (1) tumor and (2) cancer. Tumor

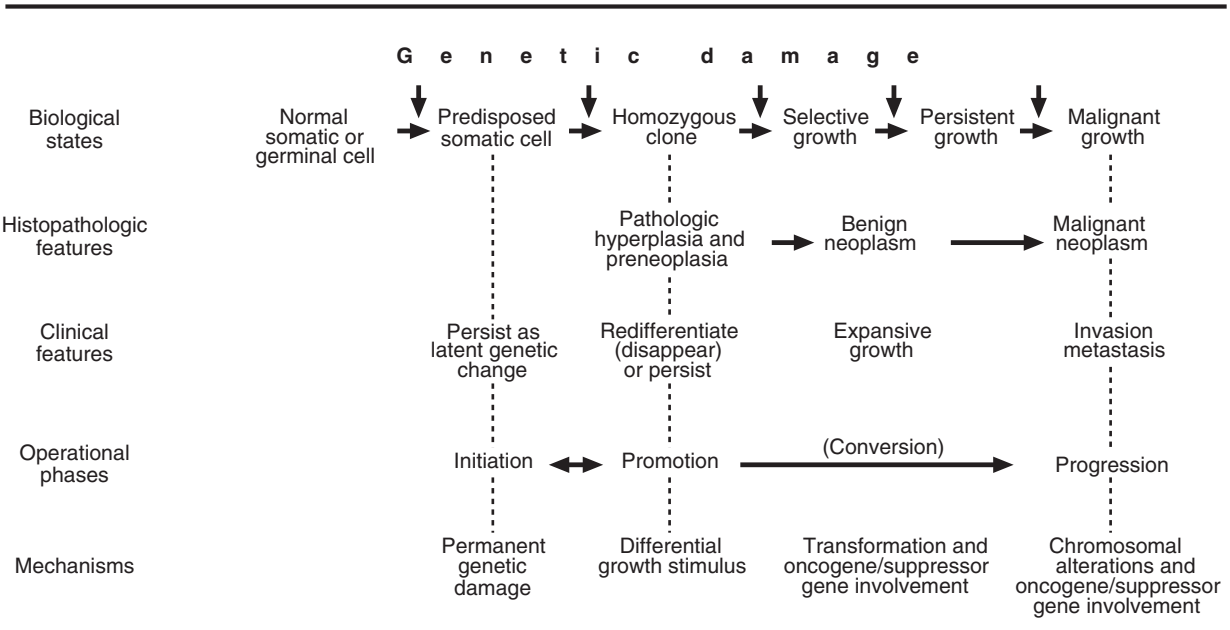

Figure 1 Process of carcinogenesis depicted schematically showing the postulate pathway in which accumulation of genetic damage leads to malignant neoplasia.

Table 1 Comparative features of benign and malignant neoplasms

Effect	*Benign*	*Malignant*
General effect on host	Little; not generally lethal	Will usually kill the host if not treated
Injury to host	Usually negligible but may compress or obstruct vital tissue	Can kill the host by destruction of vital tissue
Growth rate	Slow	Rapid (but slower than tissue repair); growth escapes normal control mechanisms
Extent of growth	Encapsulated; remains localized at site of origin	Infiltrates or invades and spreads to distant sites
Mode of growth	Typically grows by expansion and displaces surrounding tissues	Invades and destroys surrounding tissues
Microscopic features	Cells and structures formed by cells resemble normal tissues; may be encapsulated	Anaplastic, dysplastic, and pleomorphic; may be associated with hemorrhage, necrosis, and inflammation
Cytologic features	Mitoses rare; nucleus normal in staining and shape; nucleolus not conspicuous	Mitoses may be numerous and abnormal; nucleus often enlarged, irregular in shape, and hyperchromatic; nucleolus hyperchromatic and enlarged
Radiation sensitivity	Radiation sensitivity similar to that of normal tissues; rarely treated with radiation	Radiation sensitivity increased in approximate proportion to the degree of malignancy; frequently treated with radiation

broadly refers to any tissue enlargement or swelling, however it is often used synonymously with the term neoplasm. A cancer generally refers to a malignant neoplasm. Unfortunately, the layperson and the professional frequently use tumor and cancer interchangeably alike without qualifying whether it is a benign or malignant process. In other words, if it is said that an individual has a tumor, that individual may have a benign neoplasm (most often the case) but could have a malignant neoplasm if the term 'tumor' is being used loosely. If an individual is said to have a cancer, that usually means the individual has a malignant neoplasm but, here again, loose use of the term 'cancer' might include any neoplasm, including a benign one. Scientists contribute to the confusion by sometimes indicating that an agent may cause cancer, meaning either benign or malignant neoplasia. Alternatively, they may indicate that an agent is tumorigenic, which could mean that it causes tumors but frequently means that it may also cause malignant neoplasms (cancers). Common and uncritical usage of these terms is so ingrained that attempts to standardize nomenclature have been largely unsuccessful. The least ambiguous terms are 'benign neoplasm' and 'malignant neoplasm'.

Most neoplasms are classified and named based on (1) the cell or tissue of origin and (2) benign or malignant growth characteristics. There are two basic cell types from which neoplasms may originate: mesenchymal cells and epithelial cells (**Figure 2**). Mesenchymal pertains to mesenchyma (embryonic connective tissue in the mesoderm) from which adult tissues such as connective tissue, blood and lymphatic vessels, and muscles and bones are formed. Epithelial cells line the internal and external surfaces of the body and form many of the major organs such as liver and lungs. Most epithelial tissues are derived from the embryonic germ layers referred to as entoderm and ectoderm.

There are general guidelines used in naming neoplasms. A benign epithelial neoplasm originating within a glandular tissue is called an 'adenoma', having the prefix 'adeno' to designate that the origin is one of many glandular tissues and the suffix 'oma' to indicate a swelling or tissue enlargement. One or more qualifiers may be added to the name to indicate the tissue of origin and various morphological features as in hepatocellular (liver cell) adenoma, thyroid follicular (forming follicles) adenoma, or renal (kidney) tubular cell adenoma. An adenoma with morphological features resembling finger-like or warty projections would be called a papillary adenoma; with cystic spaces, a cystadenoma; with both of these features, a papillary cystadenoma. Benign mesenchymal neoplasms also utilize the 'oma' suffix in their name, as in meningioma, hemangioma, and fibroma. The prefix for mesenchymal neoplasms usually identifies the specific tissue of origin such as meninges (meningioma), blood vessels (hemangioma), or fibrous connective tissue (fibroma). Nomenclature for several benign neoplasms is presented in **Table 2**.

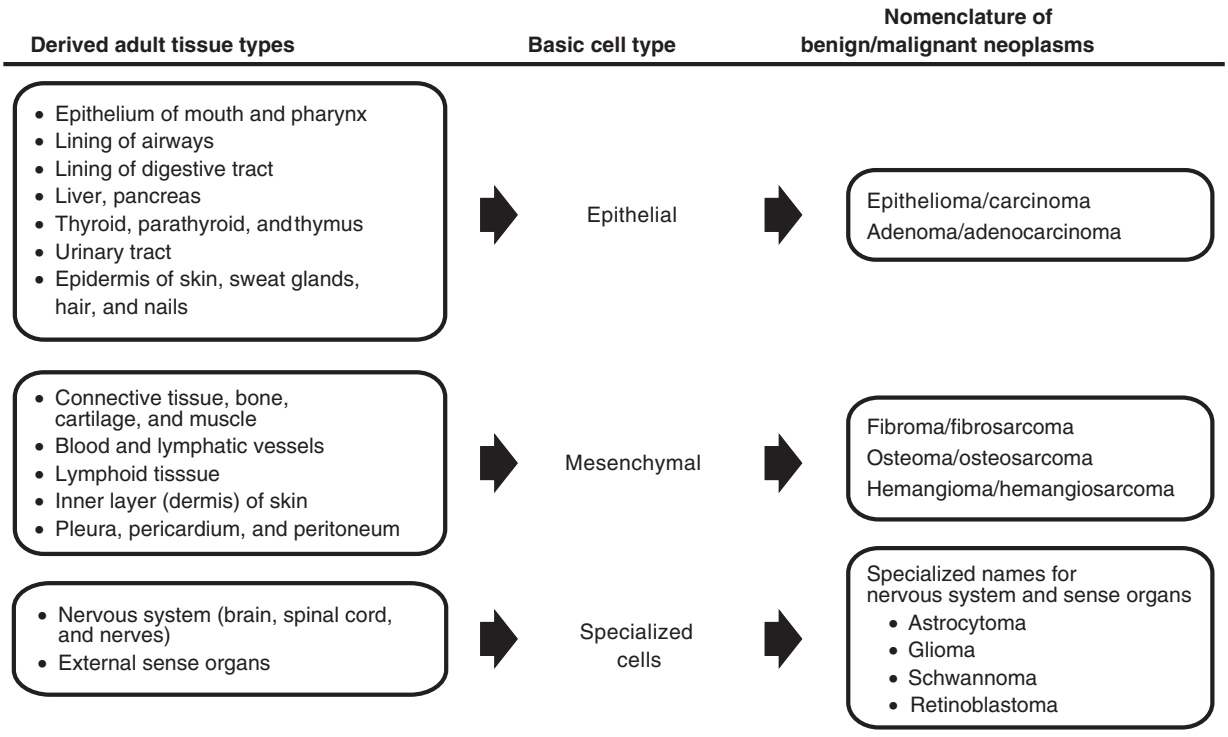

Figure 2 Tissue types associated with neoplasm names.

Malignant epithelial neoplasms are typically called 'carcinomas' and qualified by histogenetic origin. Thus, malignant skin neoplasms are called epidermal carcinomas if they arise in the superficial layers or epidermis of the skin. If they are composed predominantly of squamous cells, they are called squamous cell carcinomas; if chiefly basal cells, basal cell carcinomas. Malignant mesenchymal neoplasms are called 'sarcomas'. Examples of the latter include fibrosarcoma, a malignant neoplasm of the connective tissue; osteosarcoma, a malignant bone neoplasm; and leiomyosarcoma, a malignant neoplasm of the smooth muscle tissue. The nomenclature for several malignant neoplasms is presented in **Table 2**.

Much of the general confusion surrounding the nomenclature of neoplasms results from numerous exceptions and permutations in the general histogenetic and clinical guidelines for naming neoplasms. Many of these exceptions are deeply ingrained in traditional pathology practice, and attempts at standardization have been largely unsuccessful. Examples are thymoma, lymphoma, melanoma, and neuroblastoma – neoplasms which are generally regarded as malignant despite their benign-sounding names and should more properly be called malignant thymoma or thymic sarcoma, malignant lymphoma or lymphosarcoma, malignant melanoma or melanosarcoma, and malignant neuroblastoma, respectively. Other neoplasms are named for their physical attributes such as pheochromocytoma (dark-colored neoplasms typically arising in the adrenal medulla). In addition, some neoplasms were originally named for the person first describing the lesion, and examples such as Hodgkin's disease of lymphoid tissue and Wilms' kidney tumor have persisted to this day. Neoplasms composed of mixtures of cells are named accordingly; examples include fibroadenoma, adenosquamous carcinoma, and carcinosarcoma. To complicate matters further there are several tissue alterations that are not neoplasms but have names suggesting that they are: hamartomas (a disorganized aggregate of normal tissue components thought to represent faulty differentiation during embryonic development) and choristomas (focal collections of normal tissue found at an abnormal site such as islands of pancreatic cells in the wall of the stomach). There are also instances in which a neoplasm is histologically considered malignant but clinically benign, such as in basal cell carcinoma of the skin. In addition, localized overgrowths of normal tissue components such as skin tags and vocal cord polyps are clinically recognized as tumors but are not truly neoplastic.

For brief definitions of various terms associated with carcinogenesis, refer to the glossary at the end of this entry.

Table 2 Selected nomenclature of neoplasia

Tissue	*Benign neoplasia*	*Malignant neoplasia*
Epithelium		
Squamous	Squamous cell papilloma	Squamous cell carcinoma
Transitional	Transitional cell papilloma	Transition cell carcinoma
Glandular		
Liver cell	Hepatocellular adenoma	Hepatocellular carcinoma
Islet cell	Islet cell adenoma	Islet cell adenocarcinoma
Connective tissue		
Adult fibrous	Fibroma	Fibrosarcoma
Embryonic	Myxoma	Myxosarcoma
Cartilage	Chondroma	Chondrosarcoma
Bone	Osteoma	Osteosarcoma
Fat	Lipoma	Liposarcoma
Muscle		
Smooth	Leiomyoma	Leiomyosarcoma
Skeletal	Rhabdomyoma	Rhabdomyosarcoma
Cardiac	Rhabdomyoma	Rhabdomyosarcoma
Endothelium		
Lymph	Lymphangioma	Lymphangiosarcoma
Blood	Hemangioma	Hemangiosarcoma
Lymphoreticular		
Thymus	Not recognized	Thymoma
Lymph nodes	Not recognized	Lymphosarcoma (malignant lymphoma)
Hematopoietic		
Bone marrow	Not recognized	Leukemia
Neural tissue		
Nerve sheath	Neurilemmoma	Neurilemmosarcoma
Astrocytes	Not recognized	Astrocytoma

Note: -oma, swelling; sarc-, malignant neoplasm of mesenchymal origin; carcin-, malignant neoplasm of epithelial origin.

Tissue Changes Associated with Carcinogenesis

Quantitative – Hyperplasia and Preneoplasia

Proliferative lesions, which may be classified morphologically as hyperplasia, preneoplasia, benign neoplasia, or malignant neoplasia, represent a continuum of change with considerable overlap rather than discrete morphologic entities (**Figure 3**). The definitive classification of a given lesion as preneoplasia, benign neoplasia, or malignant neoplasia represents a judgment based on the experience of the diagnostic pathologist and familiarity with the species and tissue in question. These lesions are recognized by their microscopic appearance and effect on surrounding tissues and typically are a localized proliferation or hyperplasia of a specific cell type. Most neoplasms are believed to be derived from the clonal proliferation of a single initiated cell. Usually at some point early in the clonal expansion, the differentially proliferating cells become phenotypically distinguishable from the surrounding normal tissue. Although such lesions may not yet have sufficient characteristics to qualify as neoplasms, their recognition early in the process of carcinogenesis has led many to regard them as 'preneoplastic'.

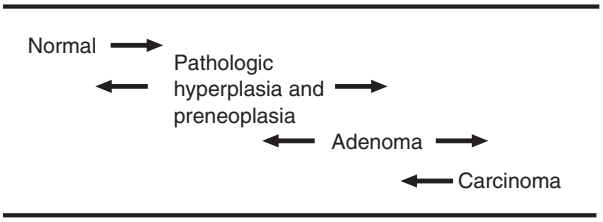

Figure 3 Morphologic continuum of carcinogenesis.

There is considerable confusion regarding the significance of hyperplasia in the neoplastic process. Hyperplasia is an increase in the number of cells per unit of tissue, typically limited in amount and terminating when the stimulus that evoked it is removed. Different cell types have varying capacities to undergo hyperplasia in response to physiological or pathological stimuli. One of the most difficult judgments, even for the experienced pathologist, is whether an observed hyperplasia is part of the process of cancer development or merely an adaptive or physiologic response not likely to progress to neoplasia. The tissue affected, whether the hyperplasia is diffuse or nodular, the age of the affected individual, the proximate cause of the hyperplastic response, and the growth pattern of the hyperplastic tissues, influences this judgment.

Preneoplasia is a form of hyperplasia (an absolute increase in the number of cells in a tissue). Although not all neoplasms exhibit a preneoplastic change recognizable by the pathologist, in those instances in which presumptive alterations are observed, their occurrence documents that there is a response to tissue insult. Examples of presumptive preneoplastic lesions are presented in **Table 3**. In those experimental models of carcinogenesis in which preneoplasia is observed, it precedes the occurrence of benign neoplasia. An important feature of preneoplastic lesions is their propensity for reversibility. In some instances a preneoplastic lesion represents the clonal expansion of a cell that has sustained genetic damage so that benign neoplasms arise within the preneoplastic lesion, presumably when one of the preneoplastic cells sustains additional genetic damage, giving it a growth advantage. In other situations, the antecedent change is a localized polyclonal cellular proliferation historically associated with subsequent development of a neoplasm in the same tissue.

A classical example is alcoholic cirrhosis, which in the case of chronic alcohol abuse, leads to multiple, polyclonal areas of liver cell hyperplasia and an increased risk for development of hepatocellular neoplasia. In both preneoplasia and certain forms of hyperplasia, the antecedent lesions typically have a higher rate of cell proliferation than the surrounding normal cells and, thus, these cells are at increased risk to sustain additional genetic damage and progress to the next stage in the carcinogenic process.

A benign neoplasm is generally a localized expansive growth that compresses adjacent normal tissue but is usually not immediately life threatening unless it physically interferes with normal function, for example, by blocking the intestinal tract or compressing vital areas in the brain. Controversy regarding the significance of benign neoplasia with respect to the development of malignancy is similar to that associated with preneoplastic lesions. A benign neoplasm, the clonal expansion of cells that have sustained some degree of genetic damage, is further along the spectrum of changes that precede the development of malignant neoplasia. In experimental carcinogenesis animal models, malignant neoplasia is not infrequently observed arising from or within a benign neoplasm. Features of benign neoplasms are listed in **Table 1**.

Malignant neoplasms are rapidly growing, locally invasive tissue proliferations that destroy surrounding tissues and are thus life threatening. They also have the malicious feature of spreading to distant sites in the body via the blood and lymphatic system. Although malignancy develops with greater frequency in association with (1) pathologic hyperplasia and preneoplasia, (2) qualitative alterations in cells, and (3) benign neoplasia than in association with normal tissues, these changes are not necessary precursors to malignancy. *In situ* carcinomas are malignant neoplasms that originate without evidence of antecedent benign tissue alteration. When precursor lesions are present prior to or concomitant with malignant neoplasia, it is probable that the malignancy is a consequence of the same or similar factors that produced the precursor lesions. Characteristics of malignant neoplasms are listed in **Table 1**.

Table 3 Examples of presumptive preneoplastic lesions

Tissue	*Presumptive preneoplastic lesion*
Mammary gland	Hyperplastic alveolar nodules
	Atypical epithelial hyperplasia
	Lobular hyperplasia
	Intraductal hyperplasia
	Hyperplastic terminal duct
Liver	Foci of cellular alteration
	Hepatocellular hyperplasia
	Oval cell proliferation
	Cholangiofibrosis
Kidney	Atypical tubular dilation
	Atypical tubular hyperplasia
Skin	Increase in dark basal keratinocytes
	Focal hyperplasia/hyperkeratosis
Pancreas	Foci of acinar cell alteration
	Hyperplastic nodules
	Atypical acinar cell nodules
Colon	Aberrant crypt foci

Qualitative – Metaplasia, Dysplasia, and Anaplasia

In addition to quantitative increases in certain cells, several qualitative cytological features help allow the morphologic classification of the spectrum of proliferative lesions that may be observed in the process of carcinogenesis. Three frequently used qualitative cytological features are metaplasia, dysplasia, and anaplasia.

Metaplasia is the reversible substitution of one type of fully differentiated cell for another within a given tissue. A classic example is the replacement of the normal ciliated columnar epithelial cells in the respiratory tract airways by squamous epithelium (**Figure 4**) in situations in which there is chronic irritation from certain components of inhaled tobacco smoke. While the squamous epithelium is believed to provide functional protection against the irritant properties of the smoke, the loss of the ciliated columnar epithelium results in reduction of the functional capacity of the lungs to clear particulates from the respiratory tract. When the irritation is removed, the squamous epithelium is replaced by normal ciliated columnar epithelium.

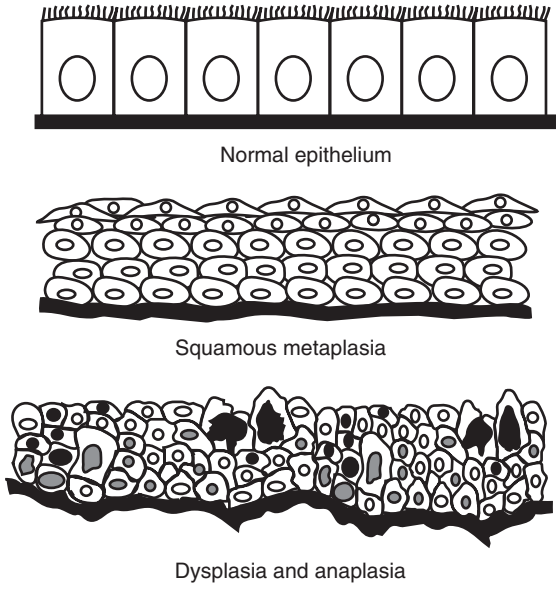

Figure 4 Qualitative changes in epithelial tissues.

Dysplasia is defined as abnormal growth of a tissue with respect to shape, size, and the organization of component cells. Normal cell-to-cell orientations are disorganized or disrupted, and the cells themselves vary in size and shape (**Figure 4**). When present, dysplasia may be associated with chronic irritation, occur with metaplasia, and be seen in neoplastic transformation. It is a change that is a hallmark of increased risk for development of neoplasia. Like metaplasia, dysplasia is a potentially reversible tissue alteration. It is also considered in some circumstances as a preneoplastic change.

Anaplasia is a qualitative alteration of cellular differentiation. Anaplastic cells are typically undifferentiated and may bear little, if any, resemblance to mature cells. This feature is considered a hallmark of malignancy.

Staging and Grading of Cancers

In human oncology the experience from collective years of observation of the outcome of many cancers has strengthened the predicitivity of histological grades and clinical staging in prognostication. The purpose of grading and staging a neoplasm is to predict its biological behavior and to help establish an appropriate therapeutic regimen. Grading is a subjective evaluation of morphologic characteristics based on the extent of cellular anaplasia and the degree of proliferation evident from microscopic evaluation. Generally, neoplasms with a high degree of anaplasia, associated specific morphologic patterns of growth, and evidence of numerous mitoses, some of which may be abnormal, are given a high grade of malignancy. Most grading schemes categorize neoplasms into one of three or four grades of increasing malignancy.

Staging of a cancer, which is independent of grading, is an index of the extent to which a cancer has spread in the body. It also provides information regarding the patient's clinical prognosis, and usually influences the choice of appropriate therapy more than grading. Criteria used for staging neoplasms include the size of the primary neoplasm, the degree to which there is invasion of surrounding normal tissues, whether the cancer has spread to local lymph nodes, and the presence of spread to distant sites in the body. Thus, it is apparent that staging will have a large influence on the therapeutic approach. A small and localized breast cancer would most likely be treated by surgical excision and possibly radiation therapy, whereas a large, infiltrative breast cancer would more likely be treated by mastectomy. If the cancer has spread to lymph nodes or distant sites, more aggressive therapy is implemented.

The ultimate fate of cells or proliferative tissue masses is influenced by the amount of sustained genetic damage. Cells with minimal DNA damage may persist in a latent form, indistinguishable from surrounding normal cells. If such a latent cell sustains additional damage even long after the initial insult, it may then progress further along the pathway to malignancy (see **Figure 1**). As additional genetic damage occurs, the altered cell population expands and eventually leads to irreversible uncontrolled growth that may or may not be corrected by aggressive medical intervention.

Molecular Basis of Cancer

Multistep Genetic Model of Carcinogenesis

Genetically, the multistage process involves the activation of growth-enhancing protooncogenes, inactivation of the recessive growth-inhibitory tumor suppressor genes as well as epigenetic events that alter gene expression and processes such as those involved in cell death, DNA repair, and methylation (**Table 4**). Cancer cells frequently contain mutations in multiple genes as well as large chromosomal abnormalities. Since their discovery $\sim$25 years ago, more than 100 protooncogenes and $\sim$15 tumor suppressor genes have been identified. Protooncogenes were first discovered in cancer-causing animal viruses that carried them. Intense study of these viruses, particularly by Varmus and Bishop in the 1970s, resulted in the discovery that endogenous animal genes had been picked up by virus ancestors and incorporated into the viral genome. Soon thereafter a number of these protooncogenes were identified in both the animal and human genome and later found to play a role in cancer development.

Table 4 Genetic and epigenetic events involved in cancer development

Proto-oncogenes (growth-enhancing)	
Growth factors	PDGF-B, FGF, sis
Growth factor receptors	EGFR, CSF
Signal transduction	*ras*, *abl*
Nuclear regulatory proteins	*myc*, *fos*
Cell cycle regulators	Cyclins and cdks
Tumor suppressor genes (growth-inhibiting)	
Cell surface molecules	TGF-βR
Regulate signal transduction	NF1
DNA repair, cell cycle	p53, Rb, BRCA1
Apoptosis genes	Bcl-2, Bcl-x, Bax, bad, Bcl-xS
DNA repair genes	HNPCC, XP
Epigenetic events	Methylation

A widely accepted multistep model of carcinogenesis proposed by Fearon and Vogelstein in 1990 serves as the framework for studies in carcinogenesis (**Figure 5**). By studying multiple benign and malignant colonic neoplasms from individuals with multiple tumors it was found that benign neoplasms harbored mutations in genes such as APC, *ras*, and p53, and that there were frequently multiple mutations per neoplasm, particularly of the malignant neoplasms. The model describes a progressive acquisition of mutations and it is believed the total accumulation of mutations (at least five to seven) rather than the order is important in the carcinogenic process. New evidence has been published to further refine this model. Recently it has been proposed that some neoplasms are dependent on the continued activation or overexpression of a particular oncogene for maintaining malignant behavior. Others have found that some neoplasms are 'hypersensitive' to the inhibitory effects of specific tumor suppressor genes. These findings suggest that the multistage process of carcinogenesis is not simply a summation of individual effects of cancer genes but that some individual cancer genes can override the others (referred to by some as the 'Achilles heel of cancer') and they offer new strategies for the prevention and therapy of cancer.

Oncogenes

Among the estimated 25 000 genes in the mammalian genome, there are ~100 genes that are classified as oncogenes because activation of these genes appears to be an essential event for the development of many, if not all, cancers. In fact, oncogenes were first discovered by studying genetic alterations in cancers. The term oncogene activation indicates a quantitative or qualitative alteration in the expression or function of the oncogene. The term oncogene is unfortunate since the unaltered (nonactivated) oncogene (usually referred to as a protooncogene) actually serves an essential function in the mammalian genome. That protooncogenes are highly conserved in evolution is evidenced by structurally and functionally similar genes in yeast, earthworms, animals, and humans. The highly conserved nature of protooncogenes is believed to be related to their essential function in normal tissue growth and differentiation. Since their normal function is to control how a tissue grows and develops, it is apparent that, if they do not function appropriately, abnormal growth and development may occur. When a primary manifestation of such abnormal growth was observed to be neoplasia, these protooncogenes were named oncogenes. This nomenclature has persisted despite the ultimate discovery that the unaltered

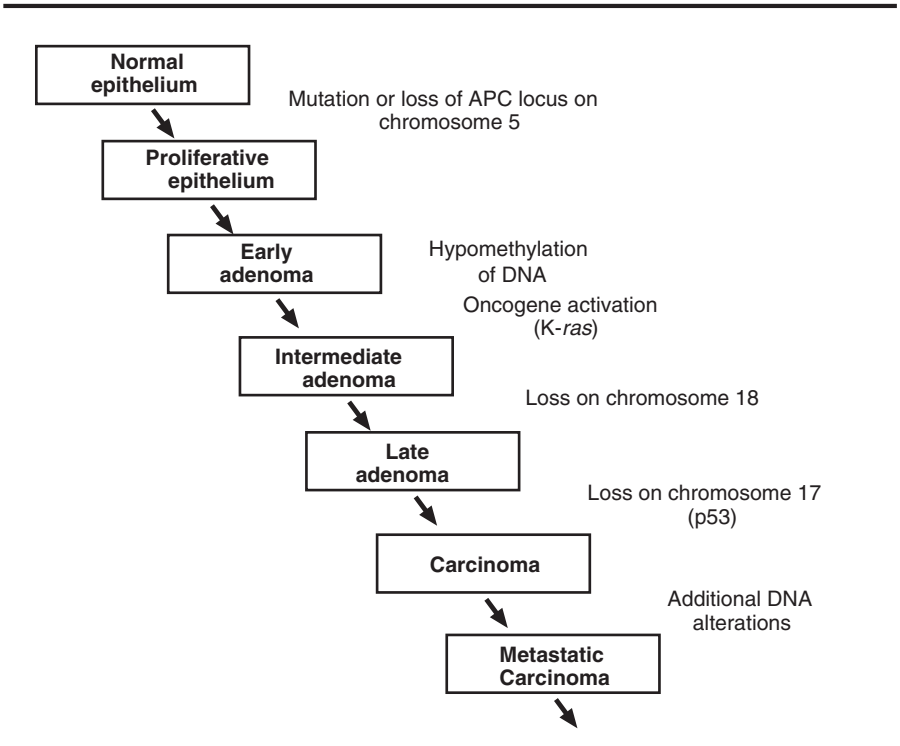

Figure 5 Multistep aspects of human colon carcinogenesis.

forms of these genes are normal components of the genome.

The appearance (phenotype) and function of a tissue is a consequence of which genes are actively producing their programmed product, typically a protein, which in turn affects the structure and function of the cells comprising a given tissue. All somatic cells in the body inherit a complete complement of maternal and paternal genes. The reason that some cells form liver and produce products such as albumin while other cells form kidney tubules that function to excrete substances from the body is a consequence of which genes are expressed in those cells. In liver cells, several critical genes that are important in kidney function are not expressed and vice versa. Specific gene expression and its effect upon tissue phenotype and function are modulated by several intrinsic and extrinsic factors (**Figure 6**). Since a primary function of many oncogenes is to control cell growth, proliferation, and differentiation, inappropriate expression of these genes would be expected to influence abnormally tissue proliferation and growth. Oncogene activation is a consequence of inappropriate or excessive expression of a proto-oncogene.

Oncogenes can be activated by several different mechanisms (e.g., retroviral transduction, chromosomal translocation, gene amplification, point mutation, promoter/enhancer insertion, or decreased methylation of promoters). Once activated an oncogene will either be inappropriately expressed (e.g., production of an altered message and protein) or overexpressed (e.g., production of too much of a normal message and protein). Either situation may contribute to the neoplastic process by influencing cellular proliferation and differentiation. Examples of activated or amplified oncogenes detected in human and animal neoplasms are listed in **Tables 5** and **6**. For some cancers the frequency of oncogene activation is relatively high, while for other cancers the activation of known oncogenes is uncommon. Identification of specific alterations in oncogenes in certain cancers represents a first step in determining the molecular basis of cancer and could eventually lead to the development of molecular intervention and therapeutic strategies. Experimental evidence indicates that oncogene activation can be an early critical event in carcinogenesis, and experimental studies with known chemical carcinogens show that they produce specific alterations in certain oncogenes reflecting the manner in which the carcinogen chemically affects DNA.

Tumor Suppressor Genes

Tumor suppressor genes, originally called anti-oncogenes, function to suppress the development of cancerous growth. While oncogenes must be activated to be effective, tumor suppressor genes must be inactivated or lost for cancer to develop. It has been shown that loss or mutation of both paternal and maternal copies, that is, in both alleles, of a

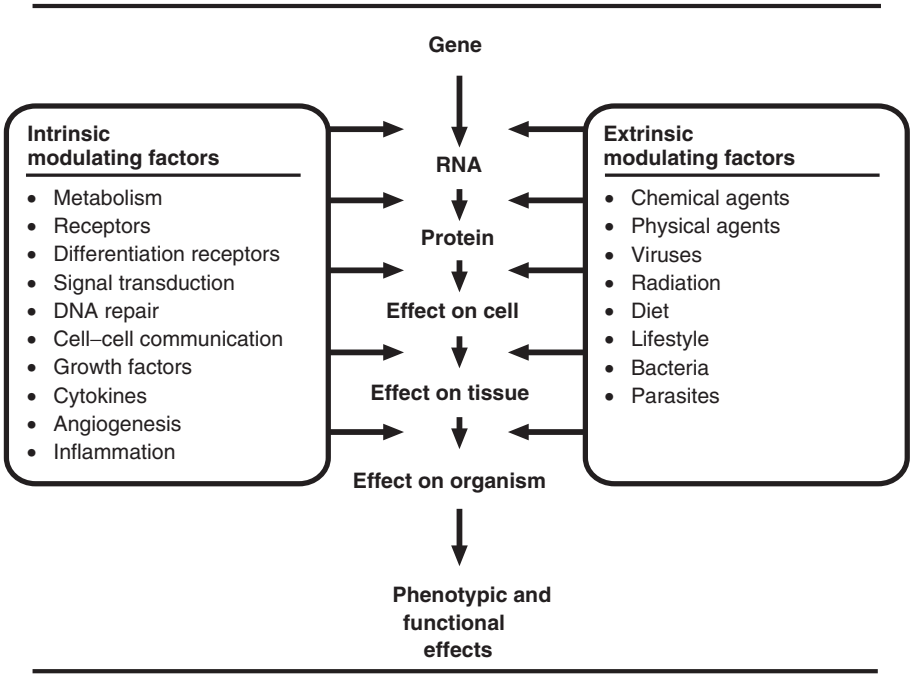

Figure 6 Intrinsic and extrinsic factors modulating specific gene expression and its effect on tissue phenotype and function.

Table 5 Examples of human neoplasms associated with activated or amplified oncogenes

Oncogene	*Type of human neoplasia*
H-RAS	Squamous cell carcinoma
	Urinary bladder carcinoma
	Lung carcinoma
	Acute myelogenous leukemia
K-RAS	Lung adenocarcinoma
	Colon carcinoma
	Ovarian carcinoma
	Gastric carcinoma
	Renal cell carcinoma
	Acute myelogenous leukemia
	Pancreatic ductal adenocarcinoma
N-RAS	Acute myelogenous leukemia
	Chronic myelogenous leukemia
ABL	Chronic myelogenous leukemia
ERBB$_2$	Breast carcinoma
	Salivary gland adenocarcinoma
MYC	Small cell carcinoma of the lung
	Burkitt's lymphoma
N-MYC	Neuroblastoma

Table 6 Examples of animals neoplasms associated with activated oncogenes

Oncogene	*Type of animal neoplasia*
H-ras	Hepatocellular adenoma and carcinoma
	Harderian gland adenoma
	Mammary carcinoma
	Skin squamous cell carcinoma
K-ras	Lung adenoma and adenocarcinoma
	Pancreatic carcinoma
	Hepatocellular carcinoma
N-ras	Leukemia
	Lymphosarcoma
Raf	Fibrosarcoma
neu (erbB$_2$)	Neuroblastoma
Abl	Lymphosarcoma
c-myc	Leukemia
	Lymphosarcoma

tumor suppressor gene must occur to ablate their effect of suppressing cancer formation. A well-known and extensively studied tumor suppressor gene is the retinoblastoma gene (*RB-1*). In hereditary retinoblastoma an affected child is born with deletions of portions of one allele of chromosome 13 containing the *RB-1* gene. If a second event leading to a loss or alteration of the remaining *RB-1* allele occurs while retinal cells are undergoing growth during development, the ocular neoplasm, retinoblastoma, frequently present in both eyes, will occur early in life. Loss or alteration of both copies of this tumor suppressor gene is sufficient to cause retinoblastoma. Although named for the disease in which it was discovered, alterations in the *RB-1* gene have been detected in breast, lung, prostate, and bone cancers.

Acquisition of Mutations

The rate of mutation has been intensely studied in the carcinogenic process. Mutations in cellular DNA can arise during normal cell replication by infidelity in DNA replication (mispairing) as well as by chromosomal deletions, amplifications, or rearrangements. Considering mispairing in nucleotide bases alone, it is estimated that spontaneous mispairing during normal cell replication can occur with a frequency of $\sim 1.4 \times 10^{-10}$ nucleotide bases per cell division. Since there are $\sim 10^{16}$ cell divisions per human lifespan and 2×10^9 nucleotide base pairs per genome, a total of 2.8×10^{15} mispairings could occur in a lifetime $((1.4 \times 10^{-10}) \times (2 \times 10^9) \times 10^{16})$. If each mispair led to a mutation that resulted in a cancer, a typical human would have billions of cancers in one average lifetime. Since such estimates of cancer frequency are clearly in excess of what is observed, it is necessary to postulate that events in addition to a single mutation are necessary for most cancers to occur and that many mispairings are repaired or fatal to the cell. There are efficient mechanisms to repair DNA damage, thereby precluding successive accumulation of critical mutations. Cell proliferation is also critical for 'fixing' DNA damage since, without production of daughter cells from a damaged mother cell, there would be no inheritance of DNA damage. The cell has relatively efficient mechanisms to repair damage provided there is time prior to cell division. If a tissue is proliferating rapidly, cell division could occur before the cell has time to mend damaged DNA. While all of the above underscore the importance of cell proliferation in carcinogenesis, neoplasia does not occur exclusively or necessarily at higher frequency in tissues that have a rapid intrinsic rate of cell proliferation. Consequently, other important mechanistic factors influence the complex process of carcinogenesis.

In 1994, Loeb *et al.* proposed that neoplastic cells likely have a higher mutation rate than normal cells ($\sim 2 \times 10^{-7}$ per gene per cell division) and thereby increase the likelihood of neoplastic cells acquiring further mutations conducive to neoplastic growth features. This is referred to as the 'mutator phenotype' (**Figure 7**). It suggests that early mutation in stability genes (i.e., DNA repair, mismatch repair, DNA replication, or chromosome maintenance) will lead to the mutator phenotype and further mutations contribute to the subsequent invasive and metastatic properties of the neoplastic growth. Others argue that the mutation rate is similar between neoplastic

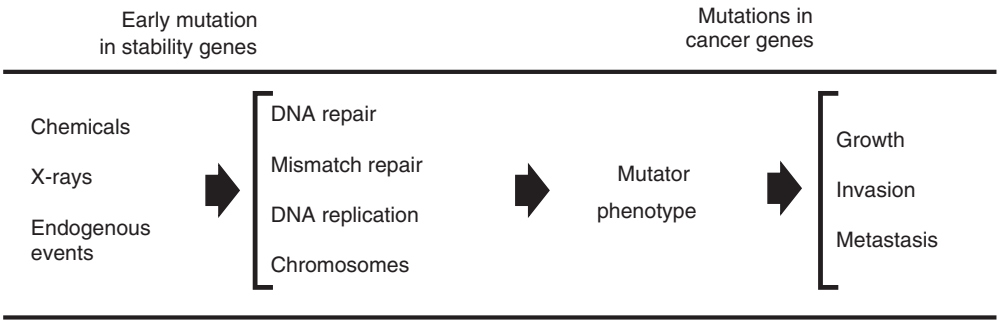

Figure 7 Mutator phenotype model.

and normal cells and that it is the higher rate of cell proliferation in neoplasms that gives them more opportunity to accumulate mutations. The healthy debates continue to feed our quest to prevent and cure the neoplastic process.

Growth Factors, Hormones, and Signal Transduction

While alterations in cellular DNA are critical in carcinogenesis, some cancer-causing agents, particularly those that are not genotoxic, play a major role in cancer development by indirectly influencing gene expression and growth control by altering signal transduction. While the pivotal role of hormones in the orchestration of tissue growth and development has been appreciated for decades, the recent discovery of polypeptide growth factors has added to our knowledge of the complex constellation of control mechanisms that affect normal cellular growth. Both hormones and growth factors bind to specific cellular receptors and thereby trigger a cascade of intracellular reactions that seem to be associated ultimately with cellular proliferation. This cascade of intracellular reactions is sometimes referred to as signal transduction, the process whereby a stimulus external to the cell triggers a cascade of intracellular biochemical reactions that ultimately lead to expression of specific genes. A simplified depiction of the interaction of hormones and growth factors with cellular signal transduction is presented in **Figure 8**. This concept is perhaps best exemplified by the process whereby a normal hormone stimulates a tissue to grow. An example is breast development and milk production in response to the hormone prolactin. In this example, prolactin binds to a specific prolactin receptor on the external surface of the cell, which, in turn, triggers a biochemical change inside the cell membrane via molecules that are attached to the external receptor and pass through the cell membrane. This in turn triggers a long chain of biochemical reactions ultimately resulting in a signal to specific genes in the cellular DNA so that they become active. The specific genes, in this example, initiate a program that causes breast cells to divide and secrete milk. The signal transduction pathways in mammalian cells are highly interactive with numerous positive (signal-sending) and negative (signal-blocking) feedback loops. An appropriate balance between the positive and negative feedback loops is necessary for the proper functional response to the initial stimulus.

Some forms of cancer development are believed to be facilitated by perturbations in one or more places in the signal transduction pathway. Thus, exposure to certain agents may potentially affect the balance of positive and negative feedback loops in the signal transduction pathway and make cells more susceptible to stimuli that promote growth. An example is the nongenotoxic skin tumor promoter, phorbol ester, which activates protein kinase C, a multifunctional element in the signal transduction pathway that mediates many critical cellular regulatory processes. Treatment of initiated mouse skin with phorbol ester activates protein kinase C, resulting in the development of benign and malignant skin neoplasms. The complexity and pivotal importance of the signal transduction pathways help explain why multiple types of agents influence carcinogenesis, why multiple steps are involved in the carcinogenic process, and why different cancers are so heterogeneous. Signal transduction involves shifts in intracellular ion fluxes for elements such as sodium, potassium, and calcium. It also often involves activation of protein kinase C, an enzyme that phosphorylates many proteins that may be important in producing a mitogenic response. Part of the signal transduction cascade involves increased expression of cyclic adenosine monophosphate, now recognized as a mitogenic signal, and increased expression of one or more cellular protooncogenes. Current research results demonstrate that increasing numbers

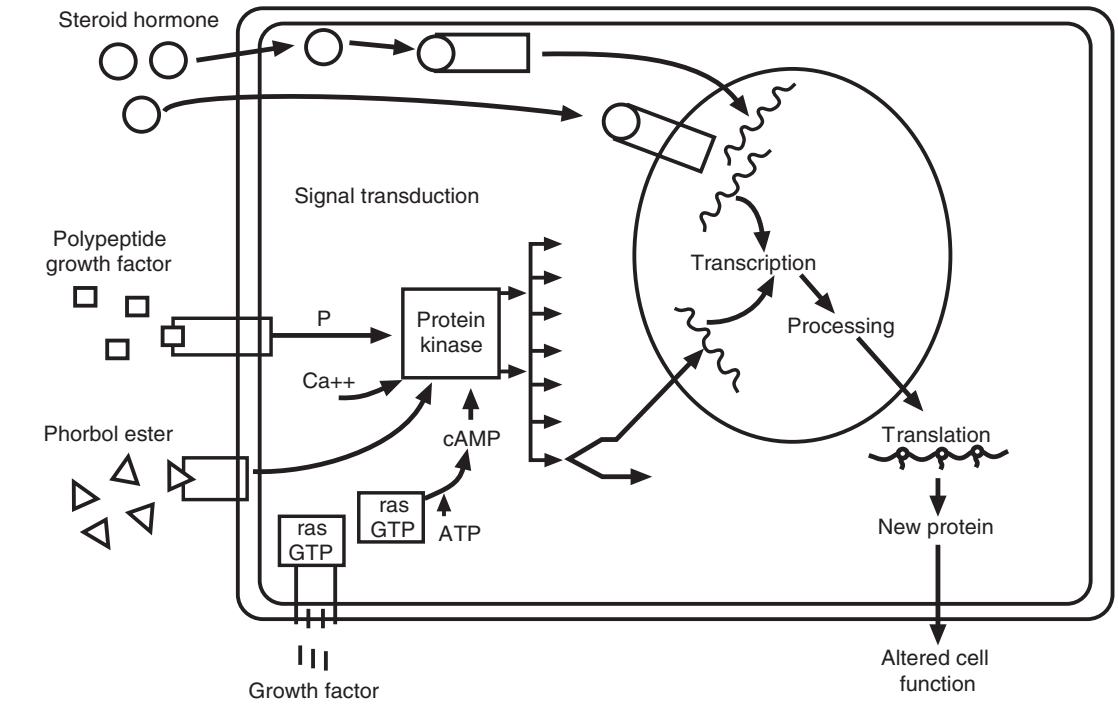

Figure 8 Simplified depiction of the interaction of hormones and growth factors with cellular signal transduction.

of protooncogenes and growth factors are integral parts of the signal transduction pathway and, when altered, influence development of cancer by subverting signal transduction.

Telomeres and Telomerase

Telomerase activation appears to be a critical component of the immortalization process in neoplastic cells and it may provide the basis for new therapeutic targets. Telomeres are specialized structures at the ends of chromosomes and telomerase is the enzyme that maintains the length of the telomeres. During each round of cell division there is a loss of a small number of nucleotides causing progressive erosion of genetic material at the end of each chromosome. As the normal cell divides the telomeres shorten and telomerase is inactive. After a certain number of divisions the shortened telomeres signal the cell to cease dividing and the cells become 'senescent' or perhaps will die by apoptosis. Germ cells and some neoplastic cells have sustained function of the telomerase enzyme which helps maintain lengthening of the telomeres and promote continued replication. Tumors having an increased telomerase activity suggest a direct effect, but it is only part of the story. For example, p53 is activated by telomerase and in the absence of p53 these cells fail to undergo apoptosis and go on to proliferate.

Heredity and Cancer: Family Cancer Syndromes

That certain cancers occur in greater frequency within families represents primary empirical evidence for susceptibility based on some hereditary element. Some genetic predispositions exist for cancers of unknown etiology, while interactions between genetic susceptibility and environmental factors are probably responsible for a large proportion of human cancers. Hereditary predispositions include DNA repair deficiencies, inability to detoxify carcinogens, and germline loss or mutations of critical genes. Examples of genetic predispositions to cancer are listed in **Table 7** and include neurofibromatosis, retinoblastoma, breast cancer, and adenomatosis of the colon. In many of these instances, one event in the carcinogenic process is believed to be an inherited germline mutation in the DNA. Another inherited anomaly, an inability to repair ultraviolet light-induced DNA damage in individuals with the condition xeroderma pigmentosum, is associated with sensitivity to sunlight and a high incidence of skin

Table 7 Examples of genetic predisposition to cancer development in humans

Genetic predisposition	*Associated cancer*
Germline deletion on chromosome 13	Retinoblastoma
	Osteosarcoma
Germline deletion on chromosome 11	Renal nephroblastoma (Wilms' tumor)
	Hepatoblastoma
	Rhabdomyosarcoma
	Adrenal carcinoma
Germline mutation in BRCA1 or BRCA2	Breast or ovarian cancer
Li–Fraumeni syndrome	Soft tissue sarcomas in children
	Breast cancer in mothers
Von Hippel Lindau disease	Hemangiomas in the brain and retina
Von Recklinghausen's disease	Fibrosarcoma
	Neuroma
	Pheochromocytoma
Familial dysplastic nevi	Malignant melanoma
Xeroderma pigmentosa – defective ability to repair damaged DNA	Cutaneous squamous cell carcinoma
Ataxia-telangiectasia	Leukemia
	Malignant lymphoma
	Stomach carcinoma
Familial adenomatous polyposis	Colon adenocarcinoma

neoplasia. However, the majority of genetic damage associated with carcinogenesis is acquired either *in utero* or from environmental and/or lifestyle factors to which individuals are exposed. Even for those individuals with a hereditary predisposition to neoplasia, additional DNA damage is necessary to lead ultimately to its development. Environmental factors that may increase the risk of cancer development in genetically predisposed individuals include exposure to radiation and agents that stimulate cellular proliferation. Experimental systems in which to study genetic susceptibility to cancer are critically needed to assess the role of gene–environmental interaction in the development of human cancer.

For some cancers in genetically predisposed individuals, the data are consistent with an association between malignant neoplasia and biallelic genetic alteration and this is supported by studies of tumor suppressor genes which prevent the development of neoplasia. Alteration or loss of a single tumor suppressor gene allele is usually insufficient to permit the development of neoplasia. In other words, the remaining functional tumor suppressor gene copy is sufficient to prevent the development of neoplasia; if it is lost or altered, however, neoplasia can develop. This situation occurs in hereditary childhood retinoblastoma, a malignant neoplasm of the retinal cells of the eye. Susceptible individuals inherit a partial loss of one copy (one allele) of chromosome 13, where the retinoblastoma tumor suppressor gene (*RB-1*) is located, and acquire an alteration or loss of the remaining *RB-1* allele during early development. The affected child subsequently develops retinoblastoma, often within the first 2 years of life.

The Immune System and Cancer

The proper functioning of the immune system is evidenced by recovery from common childhood diseases such as mumps and chicken pox. A properly functioning immune system recognizes the foreignness of the agents responsible for these diseases, responds to the infection, eliminates the foreign agents, and confers long-term immunity to subsequent infection by the same or similar agents. It has been proposed that cancer cells are recognized as foreign and that the immune system functions to eliminate such cells from the body before they are transformed into large, malignant neoplasms. This process involves elaboration of antibodies that bind to the cancer cells and activate a process whereby the cancer cells are killed. In addition, specific cells of the immune system, such as cytotoxic T lymphocytes, natural killer cells, and macrophages, have a mechanism for recognizing foreign cells and eliminating them from the body. The process of immune surveillance and removal of cancer cells is facilitated when the cancer cells express surface antigens that are recognized as foreign. Exposure to agents that depress the normal functioning of the immune system can lead indirectly to neoplasia by permitting early persistence and development of recently emergent cancer cells. Once a neoplasm has reached a critical size and growth rate, it may not be possible for even a properly functional immune system to effectively eliminate the neoplastic cells.

Operational Phases and Theoretical Aspects of Carcinogenesis

In addition to being complex, the process of carcinogenesis is typically prolonged, requiring a significant portion of the lifespan to become clinically apparent. While perturbations in cellular DNA are essential to carcinogenesis, they alone are not sufficient to cause cancer in all cases. Thus, in some experimental situations, a few minutes of exposure to a carcinogen is sufficient to result ultimately in cancer, whereas in other situations, exposure to the same carcinogen will not result in cancer unless there is additional experimental manipulation. Smokers illustrate this principle since many, but not all,

ultimately develop lung cancer. In other experimental studies, simultaneous administration of a carcinogen and a second agent may enhance, reduce, or block the carcinogenic process depending on the agent employed. These and other carcinogenesis studies have elucidated some of the mechanisms and factors that influence carcinogenesis, delimited some of the specific stages in the multistep process, and continually reminded us of the complexity of this disease process.

Multistep experimental models of carcinogenesis are useful in defining events in the neoplastic process; provide the foundations for current operational descriptions and hypotheses of the biological mechanisms of carcinogenesis (see **Figure 1**); are available for many organ systems including the skin, liver, urinary bladder, lung, intestine, mammary gland, and pancreas; and frequently are derived from studies of the effects of chemical agents on laboratory animals. The operational phases of carcinogenesis include initiation, promotion, and progression.

Table 8 Salient features of initiation and promotion of neoplasia

Initiators/initiation
- Effect is irreversible
- Only one exposure may suffice
- Multiple exposures may be additive
- Cannot identify initiated cells
- Agents are considered carcinogens
- Agents are usually mutagenic
- No measurable threshold dose
- Must be administered before the promoter
- Does not result in neoplasia unless promoter is subsequently applied
- Number of initiated cells dependent on dose of initiator

Promoters/promotion
- Nonadditive
- Agents not capable of initiation
- Modulated by diet, hormones, environment, and other factors
- Measurable threshold dose
- Measurable maximal response
- Agents not considered carcinogens but may be cocarcinogens
- Must be administered after the initiator
- Agents are usually not mutagenic
- Prolonged exposure is usually required

Initiation

During the initiation phase of chemical carcinogenesis, a chemical agent or carcinogen interacts with a cell to produce an irreversible change that may ultimately be manifested by a capacity for autonomous growth. The initiated cell appears normal, and the capacity for autonomous growth may remain latent for weeks, months, or years. Initiation implies alteration of the affected cell's DNA at one or more sites, a mutational event that is by definition hereditary. Direct-acting carcinogens interact directly with cellular DNA to produce the damage while indirect-acting carcinogens must be metabolized by the cell to produce a chemical species that interacts with cellular DNA. The majority of damaged cells have the ability to repair the damaged DNA over a period of days or weeks; however, if a cell undergoes cell division with its attendant DNA replication prior to repair of the DNA damage, the DNA alteration becomes 'fixed', is no longer reparable, and is inherited by all subsequent daughter cells. The operational phase of initiation is relatively short and may occur within hours or days. In contrast, the progression of an initiated cell to a fully malignant neoplasm is a prolonged process requiring months in animals and years in humans. Based on a large body of evidence that most initiators are mutagenic or genotoxic, a battery of short-term mutagenicity tests in bacteria and cell culture systems has evolved to identify chemicals with genotoxic properties. Once identified, such chemicals should be rigorously regulated to prevent human exposure. This approach is considered prudent because of the irreversible and hereditary nature of the changes that occur during initiation. Indeed, it is generally believed that even a single molecule of a mutagenic substance is potentially sufficient to damage DNA irreversibly. Thus, for practical purposes there is no threshold or safe level of exposure to a mutagenic agent. Salient features of initiation are listed in **Table 8**.

Initiators interact with host cellular macromolecules and nucleic acids in specific patterns. The majority of known initiators have both initiating and promoting (see below) activity and can thus induce neoplasms rapidly and in high yield when there is repeated or high-level exposure. When given at sufficiently low single doses, an initiated cell requires subsequent promotion for the development of any neoplasia. Thus, the dose of an initiator is a critical determinant of its carcinogenic potential.

Promotion

Promotion is classically considered that portion of the multistep carcinogenic process in which specific agents, known as promoters, enhance the development of neoplasms by providing initiated cells with a selective growth advantage over the surrounding normal cells. The characteristic features of promotion are listed in **Table 8**. By definition, a promoter is given at some time after chemically induced or

fortuitous initiation and the experimental doses of promoting agent are insufficient to produce cancer without prior initiation. When classical promoters are administered at sufficiently high doses and for prolonged intervals, neoplasia can occur without evidence of prior initiation. Under these conditions, a promoting agent must be considered a complete carcinogen unless fortuitous initiation from background radiation, dietary contaminants, environmental toxins, etc., is believed to have occurred. However, under experimental conditions commonly employed in short- and medium-term initiation-promotion experiments, neoplasia does not typically occur in animals that are not previously initiated.

The temporal sequence of promoter administration is critical to the operational definition of promotion. The agent must be administered after initiation and cause enhancement of the neoplastic process to be considered a promoter. If an agent is given simultaneously with an initiator and results in enhancement of development of neoplasms, it is regarded as a cocarcinogen rather than a promoter. While some promoters are cocarcinogenic (e.g., phorbol esters), not all promoters (e.g., phenobarbital and phenol) possess cocarcinogenicity and, conversely, not all cocarcinogens are promoters. Under these same conditions of simultaneous administration, a diminution in the neoplasm response is considered evidence of anticarcinogenic activity. Several rodent liver tumor promoters, which are active when administered after a variety of initiators, prevent or delay the development of liver neoplasms when added to diets along with an active carcinogen. Finally, reversing the order of administration by giving a known promoter prior to an initiator may prevent the expression of carcinogenic activity on the part of the initiator.

While upper and lower thresholds have been demonstrated experimentally for promoters, some consider that, in an absolute sense, it is statistically impossible to prove or disprove the existence of thresholds for promoters for much the same reasons that this cannot be done for initiators. One can never be certain that an apparent no-effect level would, indeed, be without effect if a sufficiently large enough number of animals were used. Promoters include agents such as drugs, plant products, and hormones that do not directly interact with host cellular DNA (are not genotoxic) but somehow influence the expression of genetic information encoded in the cellular DNA. Experimental evidence suggests that regulation of gene expression is unique to the nature of the promoting agent administered. Some promoters are believed to produce their effect by interaction with receptors in the cell membrane, cytoplasm, or nucleus (e.g., hormones, dioxin, phorbol ester, and polychlorinated biphenyls). Alternatively, promoting agents may exert their effect through their molecular orientation at cellular interfaces. Other promoters may selectively stimulate DNA synthesis and enhance cell proliferation in initiated cells, thereby giving them a selective growth advantage over surrounding normal cells.

Promoters appear to have a relatively high tissue specificity. Thus, phenobarbital functions as a promoter for rodent liver neoplasia but not urinary bladder neoplasia. Saccharin, on the other hand, promotes urinary bladder neoplasia but not liver neoplasia in the rat. Similarly, 12-*o*-tetradecanoylphorbol-13-acetate (phorbol ester) is a potent skin and forestomach neoplasm promoter in the laboratory rodent but has no appreciable activity in the liver. Other agents, such as the antioxidants 3-*t*-butyl-4-methoxyphenol and 2,6-di-*t*-butyl-4-methoxyphenol, may act as promoters in one organ and antipromoters in another and have no effect in a third organ. Thus, the practical definition of a promoter must include the designation of the susceptible tissue.

Tumor promotion may be modulated by several factors such as age, sex, diet, and hormone balance. The correlation of increased rates of breast cancer in women following a 'Western' lifestyle has implicated meat and fat consumption as playing an important role in breast cancer development. Experimental demonstration of the role of a high-fat diet in the promotion of mammary cancer in rats exposed to the mammary carcinogen dimethylbenzanthracene has been documented. Similarly, bile acids, as modulated by fat consumption, are known promoters of rat liver carcinogenesis and human colorectal cancer. Age- and sex-associated modulations in hormonal levels of estrogens, progesterone, and androgens have been implicated as potential promoters of breast cancer on the basis of epidemiological studies in humans. Experimental studies have repeatedly shown that these hormones, in addition to pituitary prolactin, serve to promote mammary cancer in rats initiated with mammary carcinogens.

Progression

Progression is that part of the multistep neoplastic process associated with the development of an initiated cell into a biologically malignant cell population. In common usage progression is frequently used to signify the stages whereby a benign proliferation becomes malignant or, alternatively, where a neoplasm develops from a low grade to a high grade of malignancy. During progression neoplasms show

increased invasiveness; develop the ability to metastasize; and then biochemical, metabolic, and morphologic characteristics are altered.

Expression of tumor cell heterogeneity, an important characteristic of tumor progression, includes production of antigenic and protein product variants, ability to elaborate angiogenesis factors, emergence of chromosomal variants, development of metastatic capability, alterations in metabolism, and a decrease in sensitivity to radiation. The development of intraneoplastic diversity may result from increasing genetic damage. Alternatively, the heterogeneity observed in tumor progression may be generated by epigenetic, regulatory mechanisms that are a part of the process of promotion. More than likely, genetic and nongenetic events subsequent to initiation operate in a nonmutually exclusive manner during progression, possibly in an ordered cascade of latter events superimposed upon earlier events.

The most plausible mechanism of progression invokes the notion that, during the process of tumor growth, there is a selection that favors enhanced growth of a subpopulation of the neoplastic cells. In support of this mechanism is increased phenotypic heterogeneity observed in malignant but not benign neoplastic proliferations. Presumably, a variety of subpopulations arises, and it is only a matter of time before the emergence of a subpopulation with more malignant biological characteristics or at least an accelerated growth advantage. This can be observed occasionally during experimental hepatocarcinogenesis when a phenotypically distinguishable carcinoma can be observed arising within an existing adenoma.

Distinction between tumor promotion and tumor progression is not readily discernible in the routine histopathologic evaluation of neoplasms and may be somewhat academic because promotion may be considered part of the process of progression. In both situations the critical event is accentuated growth. What is believed to distinguish progression from promotion is the presence of structural genomic alterations in the former and their absence in the latter. Both structural genomic changes and biochemical changes associated with tumor progression cannot be defined by conventional histopathology. Established and emerging technologies centered around histochemistry, immunocytochemistry, *in situ* hybridization, identification of activated oncogenes, loss of tumor suppressor genes, gene expression, proteomic and metabolomic profiling and discovery offer promise to distinguish various stages of progression in the evolution from benign to malignant neoplasms.

Exogenous Factors Influencing Carcinogenesis

Important exogenous factors that contribute to induction of cancer include natural and synthetic chemicals, environmental exposures to ultraviolet and medical radiation, diet and lifestyle, and infectious agents, such as viruses, parasites, and bacteria. Evidence for a causal association between exogenous factors and neoplasia is derived from studies of epidemiology, occupationally common cancers, and animal models.

Chemical and Physical Agents and Lifestyle Factors

Many chemicals that cause cancer interact directly with and alter DNA or are metabolized to chemical derivatives capable of doing so. Exposure to carcinogens can occur in certain occupational settings. Associations of human hepatic angiosarcomas with workplace exposure to vinyl chloride, pulmonary mesotheliomas with exposure to asbestos fibers, and leukemia with benzene are well-known examples. Exposure to other carcinogenic agents may occur in the diet or as a consequence of certain lifestyle practices, such as cigarette smoking associated with pulmonary cancer and high animal fat diets linked to breast and colon cancer. Strong associations have been made between exposure of light-skinned individuals to ultraviolet radiation and skin cancer. Exposure to occupational ionizing radiation, X-rays, and medical use of radioisotopes has also been associated with human neoplasia. Examples include leukemias in radiologists and atom bomb victims, lung cancer in uranium mineworkers, and thyroid and breast cancer following diagnostic or therapeutic use of radiation.

Infectious Agents and Inflammation

Viral, parasitic, and bacterial infections have been linked to cancer (**Table 9**). DNA viruses such as Epstein–Barr, hepatitis B, hepatitis C, papillomaviruses, and Kaposi sarcoma herpes virus and RNA viruses such as human T-cell leukemia virus type I and human immunodeficiency virus have been implicated in causing cancer in humans and are listed as 'known-to-cause-cancer' in humans by the International Agency for Research on Cancer (IARC). In man, the liver fluke, *Opisthorchis viverrini,* is associated with the development of cholangiocarcinomas of the liver and the blood fluke, *Schistosoma haematobium*, with carcinoma of the urinary bladder. There is evidence that chronic *Helicobacter pylori* infection of the stomach in man is not only related to

Table 9 Viruses causally related to or strongly associated with animal and human neoplasia

Virus	*Type of neoplasm*	*Species*
DNA viruses		
Myxoma	Myxoma	Rabbit
	Myxomatosis	Rabbit
Herpes	Lymphosarcoma	Chicken
		Monkey
		Rabbit
Herpes simplex 2	Cervical carcinoma	Human
Papillomaviruses	Papillomas	Cow
		Rabbit
		Horse
		Dog
Human papillomavirus	Warts	Human
	Epidermoid carcinoma	
	Cervical carcinoma	
Woodchuck hepatitis virus	Hepatocellular carcinoma	Woodchuck
Hepatitis B virus	Hepatocellular carcinoma	Human
RNA retroviruses		
Human T cell leukemia virus (HTLV-I and -II)	T cell lymphoma	Human
Avian erythroblastosis virus	Leukemia	Chicken
	Sarcoma	
Abelson leukemia virus	Leukemia	Mouse
Hervey sarcoma virus	Sarcoma	Rat
	Leukemia	
Feline sarcoma virus	Sarcoma	Cat

gastrointestinal ulcers, but also may be linked to gastric carcinoma or lymphoma development.

For oncogenic viruses, the viral or host genes generally drive the neoplastic process while with some agents there appears to be an association of chronic inflammation and nitric oxide (NO) production in the development of cancer. When DNA viruses infect cells, the viral DNA inserts itself wholly or partially into the genome of the infected cell. It appears that such integration of viral DNA into the mammalian genome is sometimes sufficient to cause neoplastic transformation of the infected cell, which is accompanied by the production of new proteins essential for the neoplastic process. RNA viruses associated with neoplasia are chiefly represented by the retroviruses. RNA viruses possess an enzyme called reverse transcriptase, which is capable of forming a DNA copy of the viral RNA when the virus infects a host cell. This DNA ultimately inserts itself into the host genome in much the same way as DNA viruses do, possibly resulting in the development of neoplasia.

The role of inflammation in cancer development is being intensely studied. There are a number of chronic inflammatory conditions, infectious and noninfectious, in man and animals associated with an increasing risk of cancer and there are many investigators examining the role of NO and oxygen radical damage to DNA or other cellular processes such as cell proliferation and apoptosis. NO induces p53, prevents apoptosis in cells such as endothelium, promotes angiogenesis, and inhibits DNA-repair activities – all processes that might provide a selective advantage to neoplastic cell growth.

Identification of Carcinogenic Agents

There are two methods utilized to identify potential human carcinogens, the most direct of which is based on retrospective epidemiological studies in human populations using existing historical records associated with known cases of neoplasia. These records include death certificates where cause of death is indicated; hospital records; responses to questionnaires that document environmental or work-associated exposure to potential carcinogenic agents; and studies of neoplasia in culturally, ethnically, or religiously distinctive human populations. Association of cigarette smoking with lung cancer and exposure to asbestos with mesotheliomas was the result of such retrospective epidemiological work. Prospective epidemiological studies identify a given population of individuals who agree to be monitored for several years to permit identification of potential carcinogenic factors associated with neoplasms which may occur.

Another method used to identify potential human carcinogens involves testing known chemicals and agents in experimental animals. Such tests have been referred to as animal bioassays and are typically conducted using rats and mice exposed to high doses of the suspect agent for a large portion (typically 2 years) of their lifespan. If such agents are observed to produce neoplasia in the experimental animals, the agent is regarded as a potential human carcinogen. In countries throughout the world, legal requirements mandate that all new chemical agents and drugs be tested in animal bioassays to determine whether they cause cancer in the test animals. Additionally, since the mid-1960s in the United States, the National Cancer Institute and currently the National Toxicology Program have collectively conducted animal bioassays on more than 500 chemical agents to assess their potential to cause cancer.

Interpretation of results from human epidemiological studies and animal bioassays to identify carcinogenic agents has often proved difficult and

controversial. Humans are rarely exposed to only one potential cancer-causing agent in their lifetime, and the amount and duration of that exposure may be difficult or impossible to quantify rigorously. Many years may intervene between exposure to a potential carcinogen and ultimate development of neoplasia, making accurate assessment of cause and effect almost impossible. Despite such limitations, epidemiological studies that clearly show an association between a given chemical exposure or lifestyle habit with an enhanced rate of a specific cancer are regarded as the most relevant method for identification of human carcinogens. While animal bioassays have proved useful for the identification of agents that can cause cancer in the laboratory rodent, they only identify an agent as potentially hazardous to human health. Additional facts and factors must be considered in classifying such an agent as a likely human carcinogen.

The current approach for assessing the scientific relevance of either epidemiological or animal bioassay results to human health risk involves a 'weight-of-evidence' procedure in which national and international panels of expert scientists from several disciplines examine all available information on the suspect agent in making their assessment. Included in this analysis are the strength of the epidemiological evidence, the dose–response curve of the animal response, comparative species metabolism and ability to extrapolate between species, likely mechanism of cancer induction for the agent in question, the genotoxicity of the agent, the amount of the agent in the environment, and the number of people potentially exposed to the agent. Based on this type of analysis, so far 88 agents have been classified as known human carcinogens by the International Agency for Research on Cancer (some of which are in **Table 10**) and 64 more agents have been designated as probable human carcinogens. The 10th US Health and Human Services *Annual Report on Carcinogens* lists 49 known human carcinogens and 174 substances that are reasonably anticipated to be human carcinogens.

Table 10 Some selected agents or mixtures for which there is sufficient evidence of carcinogenicity in humans

Organic compounds
2-Napthylamine
4-Aminobiphenyl
Aflatoxin B_1
Analgesics containing phenacetin
Azathioprine
Benzene
Benzidine
Betel quid with tobacco
Bis(chloromethyl)ether
Chlorambucil
Chlonaphazine
Chloromethyl methyl ether
Cyclophosphamide
Diethylstilbesterol
Melphalan
Methyl-CCNU
MOPP
(and other combined therapies)
Mustard gas
Myleran
Thiotepa
Tobacco products and tobacco smoke
Treosulfan
Vinyl chloride
Soots, tars, and oils
Coal tar pitches
Coal tars
Mineral oils,
untreated and mildly treated
Shale oils
Soots
Hormones
Diethylstilbesterol
Estrogens
Oral contraceptives
Metals
Arsenic compounds
Chromium compounds
Nickel and nickel compounds
Fibers
Asbestos
Erionite
Talc-containing asbestos fibers
Other
8-Methoxypsoralen + UV radiation
Viruses
Epstein-Barr
Hepatitis B & C
Papillomaviruses
T-cell leukemia virus type I
Parasites
Opisthorchis virerrini
Schistosoma haematobium

Molecular Epidemiology of Cancer

The molecular epidemiology of cancer is the study of molecular alterations, primarily mutations, in investigating the causative agents of cancer as well as identifying individual cancer risk. The possibility of identifying cancer-causing agents based on the occurrence of predictable molecular alterations that are found in the neoplasm is intriguing. It is based on the hypothesis that there are carcinogen-specific patterns of mutations that reflect direct interactions of

carcinogens with cancer genes. For example, lung and colon cancers from people who smoke tend to have a specific mutation in the *ras* oncogene or p53 tumor suppressor gene (i.e., mostly a G–T nucleotide base substitution) and that this mutation is likely due to the direct interaction of the carcinogen in smoke benzo(*a*)pyrene with DNA. Such chemical-specific mutational profiles (or 'molecular signatures') have been used to support a causal association between particular genetic events in tumors and a specific carcinogen such as neoplasms associated with exposure to radon, aflatoxin B_1, vinyl chloride, and the nitrosamines (**Tables 11** and **12**). The strongest evidence for linkage between a cancer-causing agent and a specific type of neoplasm is that of the CC–TT double base changes observed in skin neoplasms of man and animals. This mutation is consistent with the predicted UV-induced damage of dipyrimidine dimers. In liver tumors from persons living in geographic areas with a high exposure to aflatoxin B_1 there is a frequent mutation at the third nucleotide pair of codon 249 in the p53 gene, suggesting the mutation is chemical-specific and imparts a specific growth or survival advantage to the mutated liver cells.

Table 11 Molecular signatures of malignant human cancers

Exposure	*Neoplasm type*	*Predominant mutation (nucleotide base changes)*
Cigarette smoke	Lung carcinoma	K-*ras*, codons 12 and 13 (G–T)
	Colon	K-*ras*, codons 12 and 13
	Lung carcinoma	p53, multiple codons (G–T)
Radon	Lung carcinoma	p53, codon 249 (G–T)
Aflatoxin B_1	Hepatocellular carcinoma	p53, codon 249 (G–T)
Ultraviolet light	Skin carcinoma	p53, dipyrimidine sites (CC–TT)
Vinly chloride	Hepatic angiosarcoma	p53, codon 249 (A–T)
	Hepatocellular carcinoma	K-*ras*, codons 12 and 13 (G–A)

Table 12 Molecular signatures of malignant rodent cancers

Exposure	*Neoplasm type (species)*	*Predominant mutation (nucleotide base changes)*
Methylnitrosourea	Mammary carcinoma (R)	K-*ras*, codon 12 (G–C)
Aflatoxin B_1	Lung carcinomas (M)	K-*ras*, codon 12 (G–C)
Diethylnitrosamine	HCC (M)	H-*ras*, codon 61 (A–G)
Ultraviolet light	Skin carcinoma (M)	p53, dipyrimidine sites (CC–TT)
Vinly chloride	HCC (R)	H-*ras*, codon 61 (A–T)

R, rat; M, mouse. HCC, hepatocellular carcinoma.

Animal studies have confirmed that there are certainly chemical-specific mutational profiles in neoplasms; however, there are many examples where the mutational profile varies by strain (**Table 13**), species, dose, or dosing regiment. For example, diethylnitrosamine, a strong, cross-species hepatocarcinogen, will induce liver neoplasms in mice, rats, and rainbow trout, but the frequency and type of *ras* mutation in the neoplasm varies widely, and the mutations are not simply a reflection of direct DNA interaction (**Table 14**). In some studies, *in vitro* mutation assays were poor predictors of liver tumor mutation profiles in the mouse. In this complex process, carcinogens might also be influencing events such as DNA repair, oxidative DNA damage, methylation, cell death, proliferation, and/or a hypermutable state.

Molecular epidemiologic studies aimed at identifying an individual's risk of developing cancer have found that persons with germline mutations in cancer genes (i.e., BRCA1 or BRCA2) or variations (polymorphisms) of carcinogen-metabolizing enzyme activities (i.e., cytochrome P-450s or glutathione-*S*-transferases) or DNA repair capacities can be at increased risk of developing neoplasia in their

Table 13 Sensitivity to liver tumor development and H-*ras* codon 61 mutations in spontaneous hepatocellular tumors of various strains of mice

Sensitivity	*Strain*	*Frequency*		*Codon 61 mutation (normal = CAA)*		
				AAA	*CGA*	*CTA*
High	C3H	23/89	(26%)	17	3	3
Intermediate	B6C3F1	183/333	(56%)[a]	106	50	21
	CD-1	9/36	(25%)	8	1	0
Low	C57BL	5/34	(15%)[a]	0	1	4

[a] Occasional mutations in other codons of H- and K-*ras*.

Adapted from Maronpot RR, Fox T, Malarkey DE, and Goldsworthy TL (1995) Mutations in the *ras* protooncogene: Clues to etiology and molecular pathogenesis of mouse liver tumors. *Toxicology* 101: 125–156.

Table 14 Species and strain comparisons of mutational profiles induced by diethylinitrosamine (DEN)

Animal	*Frequency of ras mutations*		*Type*	*Nucleotide base substitutions*				
				C–A	*A–G*	*A–T*	*A–C*	*G–A*
CD-1 mouse	13/25	(52%)	H- and N-*ras*	12	1	0	0	0
C3H mouse	54/114	(26%)	H-*ras*	28	24	2	0	0
B6C3F1 mouse	63/239	(26%)	H-*ras*	16	32	15	0	0
C57BL mouse	2/59	(2%)	H-*ras*	0	1	0	1	0
F344 rat	0/19	(0%)	K-*ras*	0	0	0	0	0
Rainbow trout	6/7	(86%)	K-*ras*	0	0	0	0	6

Data adapted partly from Maronpot RR, Fox T, Malarkey DE, and Goldsworthy TL (1995) Mutations in the *ras* protooncogene: Clues to etiology and molecular pathogenesis of mouse liver tumors. *Toxicology* 101: 125–156.

lifetime. High-throughput analyses to examine single nucleotide polymorphisms (SNPs) are being used to search for biomarkers of cancer risk in individuals and some of this information is being used to help people take preventive measures to decrease their risk of developing cancer.

Summary and Conclusions

All of life is a balancing act of good versus evil and production versus destruction. Similar balancing factors are evident in carcinogenesis where regulatory mechanisms for tissue proliferation are balanced against those for cellular differentiation. It is well established that carcinogenesis requires the accumulation of multiple alterations in the genome of the affected (cancer) cells. At the genetic level, two opposing classes of genes, oncogenes, and tumor suppressor genes, have been implicated in the carcinogenic process. In addition, the development of cancer is influenced by host factors such as age, sex, diet, nutrition, general health status, and inherited predispositions for cancer and by complex positive and negative intracellular signaling mechanisms. Treatment of cancer is based on our understanding of the mechanistic underpinnings of the carcinogenic process and attempts to shift the balance of critical factors in favor of patient survival. The probability of developing cancer is directly proportional to the intensity, route, and duration of exposure to cancer-causing factors as well as genetic susceptibility. Public health strategies are based on the premise that reduction or prevention of exposure to cancer-causing factors will decrease the incidence of cancer.

See also: Carcinogen Classification Schemes; Carcinogen–DNA Adduct Formation and DNA Repair; Cell Proliferation; Chromosome Aberrations; Epidemiology; Immune System; International Agency for Research on Cancer; Mechanisms of Toxicity; Molecular Toxicology–Recombinant DNA Technology; Mouse Lymphoma Assay; Radiation Toxicology, Ionizing and Nonionizing; Skin; Toxicity Testing, Carcinogenesis.

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Glossary

Adenomatosis	a condition in which numerous adenomatous growths develop in a tissue.
Allele	one of the two gene pairs situated at the same location on a chromosome; one allele is inherited from the mother and the other from the father; characteristics such as being short or tall or having blue eyes versus brown eyes are determined by the expression of inherited alleles.

Anaplasia	lack of normal organizational or structural differentiation of a tissue; anaplastic cells are typically poorly differentiated.
Angiogenesis	the development of blood vessels.
Benign	a classification of anticipated biological behavior of neoplasms in which the prognosis for survival is good; benign neoplasms grow slowly, remain localized, and usually cause little harm to the host.
Biallelic damage	damage to both maternal and paternal copies of a gene.
Cancer	generally refers to a malignant neoplasm.
Carcinogenesis	the process of development of cancer or neoplasms.
Choristoma	a mass or collection of well-differentiated cells from one organ found within another organ, for example, adrenal tissue present in the lung.
Clonal	pertaining to a clone; a line of cells descended from a single cell.
Cocarcinogen	an agent that has no inherent carcinogenic activity by itself but is capable of augmenting neoplasm formation when given simultaneously with a genotoxic carcinogen.
DNA	abbreviation for deoxyribonucleic acid; the basic building block of genetic material in all organisms except RNA viruses.
Dysplasia	disordered tissue formation characterized by changes in size, shape, and orientational relationships of adult types of cells; primarily seen in epithelial cells.
Epithelial cell	cells which line the internal and external surfaces of the body and form the bulk of many of the major organs of the body; they are formed from the embryonic germ layers known as entoderm and ectoderm.
Gene	the basic biological unit of heredity which is located on a chromosome.
Genome	the total complement of genes present in the set of chromosomes characteristic of a given organism.
Genotoxic	toxic to DNA; an agent or process that interacts with cellular DNA either directly or after metabolic transformation; mutagens are genotoxic agents.
Grade (grading)	a subjective evaluation of the morphologic characteristics of a neoplasm based on the degree of anaplasia and proliferation evident from microscopic examination as a measure of biological outcome or degree of malignancy.
Growth factors	agents that contribute to and stimulate tissue growth.
Hamartoma	a localized overgrowth of differentiated cells that have an altered growth pattern in relation to the tissue in which they are found, for example, a nodule of disorganized striated muscle fibers within a normal skeletal muscle.
Hepatocarcinogenesis	the development of liver cancer.
Heterozygous	having different alleles at a specific position on a chromosome.
Histogenetic	pertaining to the origin, formation, or development of tissues from undifferentiated embryonic germ cell layers.
Homeostatic	pertaining to the natural state of equilibrium of the normal internal environment of the body; maintained by complex positive and negative feedback control mechanisms.
Homozygous	having identical alleles at a specific position on a chromosome.
Hyperplasia	a numerical increase in the number of normal-appearing cells within a tissue or organ.
Immune system	a primary defense system in the body capable of attacking and potentially destroying cancer cells; consists of lymphoid and related tissues from which cells are recruited to produce antibodies or to directly attack cancer cells.
Initiation	the first operational phase of the process of carcinogenesis during which a cell sustains a heritable alteration in DNA.
Malignant	a classification of anticipated biological behavior of neoplasms in which the prognosis for survival is poor; malignant neoplasms grow rapidly, invade, destroy tissue, and are usually fatal.
Mesenchymal cell	cells derived from embryonic mesoderm, which constitute the supporting structure of tissue such as connective tissue, blood vessels, muscles, and bones.
Metaplasia	the substitution of one type of fully differentiated cells for the fully differentiated cell type normally present in a given tissue.

Metastasize	the spreading of neoplastic cells from a primary site of origin to a distant, noncontiguous site where their growth occurs.
Mitogenic	stimulating cell proliferation or division; causes mitosis.
Mutation	a structural alteration in DNA that is hereditary and may give rise to an altered phenotype.
Neoplasia	the process of the development of neoplasms; essentially synonymous with carcinogenesis.
Neoplasm	new and typically abnormal growth which is generally uncontrolled and becomes progressively more serious with time.
Neurofibromatosis	a hereditary condition of the nervous system and other tissues of the body characterized by development of numerous neoplasms (neurofibromas) distributed over the entire body.
Nucleotide	a biochemical component of DNA that consists of a purine or pyrimidine base, a ribose or deoxyribose sugar, and a phosphate group; a basic building block of DNA.
Oncogene	a so-called cancer gene because alterations in its structure or expression are typically associated with neoplasms; an activated form of a protooncogene.
Oncogene activation	the process whereby a protooncogene is altered such that it stimulates enhanced cellular growth; several different mechanisms can lead to such activation.
Oncology	the study of neoplasia or carcinogenesis.
Phenotype	the physical appearance, biochemical makeup, and physiological behavior of an individual.
Preneoplasia	refers to the recognizable structural changes in a tissue that are sometimes antecedent to the development of neo-plasia; the presence of preneoplasia indicates an increased probability for the development of neoplasia.
Progression	an operational phase of carcinogenesis associated with the development of an initiated cell into a fully malignant neoplasm; sometimes used in a more limited sense to refer to the change from a benign neoplasm to a fully malignant neoplasm.
Promotion	an operational phase of carcinogenesis in which there is enhancement of neoplasm formation when an agent (the promoter) is administered after exposure to a genotoxic carcinogen.
Protooncogene	a normal cellular structural gene that, when activated by mutations, amplifications, rearrangements, or viral transduction, functions as an oncogene and is associated with neoplasia; regulates normal processes related to cell growth and differentiation.
Retinoblastoma	an ocular neoplasm arising from germ cells of the retina.
Retroviruses	a large group of RNA viruses.
Stage (staging)	a subjective assessment of the extent to which a neoplasm has spread in the body and, thus, an indication of the patient's clinical prognosis.
Threshold	the level of an agent below which no physiological, biochemical, or pathological effect can be measured.
Tumor	any tissue enlargement or swelling; frequently used as equivalent to a benign neoplasm.
Tumor suppressor gene	a gene that normally functions to suppress uncontrolled tissue growth.
Weight of evidence	an approach for assessing the potential carcinogenic risk of an agent by considering all available information relative to the biological action of the agent.

Carcinogenicity Toxicity Testing *See* Toxicity Testing, Carcinogenesis.

Cardiovascular System

Arthur Penn and Gleeson Murphy

Introduction

The central tenet of toxicology was stated by the sixteenth-century physician Paracelsus: "All substances are poisons. There is none that is not a poison. The right dose differentiates a poison and a remedy." The scope of this chapter includes the toxic effects of several classes of chemicals on the human cardiovascular system (CVS). These include drugs (including those commonly abused, as well as therapeutic), pesticides, other organic chemicals, metals and other inorganic chemicals, some recent FDA- and media-scrutinized compounds, and complex mixtures (e.g., cigarette smoke).

The CVS consists of the heart and the vasculature (arteries, arterioles, veins, venules, and capillaries). The focus will be on the heart and arteries as they display the bulk of characteristic toxic effects. The entry begins with a description of the normal anatomy and physiology of the heart. This is followed by a series of examples of agents that can act on ion movement, muscle function, and blood flow. The second part of the entry begins with a description of the anatomy and physiology of blood vessels followed by examples of vasculotoxic agents. A list of cardiotoxic agents and their mechanisms of toxicity is presented in **Table 1**. A listing of some vasculotoxic

Table 1 General mechanisms of cardiotoxicity; and cardiotoxicity of – key pharmaceutical agents, naturally occurring substances, and selected industrial agents

Mechanism	*Cellular perturbations*	*Organ manifestations*
General mechanisms of cardiotoxicity		
Interference with ion homeostasis		
Inhibition of Na^+/K^+ ATPase	↑ $[Ca^{2+}]_i$, ↓ Conduction velocity	Positive inotropic effect Proarrhythmic
Na^+ channel blockade	↓ Na^+ channel activity ↓ $^+$Conduction velocity	Proarrhythmic
K^+ channel blockade	↓ K^+ channel activity ↓ Repolarization ↑ Action potential duration	Proarrhythmic
Ca^{2+} channel blockade	↓ L-type Ca^{2+} channel activity ↓ Ca^{2+}-induced-Ca^{2+} release ↓ AV conduction	Negative inotropic effect Negative chronotropic effect Bradycardia
Altered coronary blood flow		
Coronary vasoconstriction or obstruction	Ischemia (ATP depletion, intracellular acidosis)	Myocardial infarction Cardiac myocyte death Cardiac remodeling
Ischemia/reperfusion injury	Oxidative stress, ↑ $[Ca^{2+}]_i$ Intracellular pH change	Cardiac myocyte death
Oxidative stress	Lipid peroxidation DNA damage Mitochondrial dysfunction, Altered $[Ca^{2+}]_i$ homeostasis	Cardiac myocyte death
Organellar dysfunction		
Sarcolemmal injury	Altered membrane integrity	Cardiac myocyte death
Sarcoplasmic reticulum dysfunction	Altered $[Ca^{2+}]_i$ homeostasis	Cardiac myocyte death
Mitochondrial injury	ATP depletion Cytochrome *c* release Altered mitochondrial $[Ca^{2+}]_i$ homeostasis	Cardiac myocyte death
Apoptosis	Cellular shrinkage Sarcolemmal blebbing Chromatin condensation Redistribution of membrane phospholipids DNA fragmentation	Cardiac myocyte death
Oncosis	Cellular swelling Sarcolemmal blebbing Chromatin clumping Mitochondrial swelling	Cardiac myocyte death

Table 1 Continued

Agents	*Cardiotoxic manifestations*	*Proposed mechanisms of cardiotoxicity*
Cardiotoxicity of key pharmaceutical agents		
Ethanol	↓ Conductivity (acute) Cardiomyopathy (chronic)	Acetaldehyde (metabolite) Altered $[Ca^{2+}]_i$ homeostasis Oxidative stress Mitochondrial injury
Antiarrhythmic drugs		
Class I (disopyramide, encainide, flecainide, lidocaine, mexiletine, moricizine, phenytoin, procainamide, propafenone, quinidine, tocainide)	↓ Conduction velocity Proarrhythmic	Na^+ channel blockade
Class II (acebutolol, esmolol, propranolol, sotalol)	Bradycardia, heart block	β-adrenergic receptor blockade
Class III (amiodarone, bretylium, dofetilide, ibutilide, quinidine, sotalol)	↑ Action potential duration QTc interval prolongation Proarrhythmic	K^+ channel blockade
Class IV (diltiazem, verapamil)	↓ AV conduction Negative inotropic effect Negative chronotropic effect Bradycardia	Ca^{2+} channel blockade
Inotropic drugs and related agents		
Cardiac glycosides (digoxin, digitoxin)	Action potential duration AV conduction Parasympathomimetic (low doses) Sympathomimetic (high doses)	Inhibition of Na^+, K^+-ATPase, ↑ $[Ca^{2+}]_i$
Ca^{2+} sensitizing agents (adibendan, levosimendan, pimobendan)	↓ Diastolic function? Proarrhythmic?	↑ Ca^{2+} sensitivity, Inhibition of phosphodiesterase
Other Ca^{2+} sensitizing agents (allopurinol, oxypurinol)	?	Inhibition of xanthine oxidase
Catecholamines (dobutamine, epinephrine, isoproterenol, norepinephrine)	Tachycardia Cardiac myocyte death	β_1-adrenergic receptor activation Coronary vasoconstriction Mitochondrial dysfunction ↑ $[Ca^{2+}]_i$ Oxidative stress Apoptosis
Bronchodilators (albuterol, bitolterol, fenoterol, formeterol, metaproterenol, pirbuterol, procaterol, salmeterol, terbutaline)	Tachycardia	Nonselective activation of β_1-adrenergic receptors
Nasal decongestants (ephedrine, ephedrine alkaloids, ma huang, phenylephrine, phenylpropanolamine, pseudoephedrine)	Tachycardia	Nonselective activation of β_1-adrenergic receptors
Appetite suppressants (amphetamines, fenfluramine, phentermine)	Tachycardia, pulmonary hypertension Valvular disease	↑ Serotonin? Na^+ channel blockade?
Antineoplastic drugs		
Anthracyclines (daunorubicin, doxorubicin, epirubicin)	Cardiomyopathy Heart failure	Altered $[Ca^{2+}]_i$ homeostasis Oxidative stress Mitochondrial injury Apoptosis
5-Fluorouracil	Proarrhythmic	Coronary vasospasm?
Cyclophosphamide	Cardiac myocyte death	4-Hydroxycyclophosphamid (metabolite) Altered ion homeostasis
Antibacterial drugs		
Aminoglycosides (amikacin, gentamicin, kanamycin, netilmicin, streptomycin, tobramycin)	Negative inotropic effect	↓ $[Ca^{2+}]_i$
Macrolides (azithromycin, clarithromycin, dirithromycin, erythromycin)	↑ Actin potential duration QTc interval prolongation Proarrhythmic	K^+ channel blockade

Table 1 Continued

Agents	*Cardiotoxic manifestations*	*Proposed mechanisms of cardiotoxicity*
Fluoroquinolones (grepafloxacin, moxifloxacin, sparfloxacin)	↑ Action potential duration QTc interval prolongation Proarrhythmic	K^+ channel blockade
Tetracycline	Negative inotropic effect	↓ $[Ca^{2+}]_i$
Chloramphenicol	Negative inotropic effect	↓ $[Ca^{2+}]_i$
Antifungal drugs		
Amphotericin B	Negative inotropic effect	Ca^{2+} channel blockage? Na^+ channel blockade? ↑ Membrane permeability?
Flucytosine	Proarrhythmic Cardiac arrest	5-fluorouracil metabolite Coronary vasospasm?
Antiviral drugs		
Nucleotide analog reverse transcriptase inhibitors (stavudine, zalcitabine, zidovudine)	Cardiomyopathy	Mitochondrial injury Inhibition of mitochondrial DNA polymerase Inhibition of mitochondrial DNA synthesis Inhibition of mitochondrial ATP synthesis
Centrally acting drugs		
Tricyclic antidepressants (amitriptyline, desipramine, doxepin, imipramine, protriptyline)	ST segment elevation QTc interval prolongation Proarrhythmic Cardiac arrest	Altered ion homeostasis Ca^{2+} channel blockade Na^+ channel blockade K^+ channel blockade
Selective serotonin reuptake inhibitors (fluoxetine)	Bradycardia, Atrial fibrillation	Ca^{2+} channel blockade Na^+ channel blockade
Phenothiazine antipsychotic drugs (chlorpromazine, thioridazine)	Anticholinergic effects Negative inotropic effect QTc interval prolongation PR interval prolongation	Ca^{2+} channel blockade?
Other antipsychotic drugs (clozapine)	Blunting of T waves ST segment depression	
General inhalational anesthetics (enflurane, desflurane, halothane, isoflurane, methoxyflurane, sevoflurane)	Negative inotropic effect Decreased cardiac output Proarrhythmic	Ca^{2+} channel blockade Altered Ca^{2+} homeostasis, β-adrenergic receptor sensitization
Other general anesthetics (propofol)	Negative inotropic effect	Ca^{2+} channel blockade Altered Ca^{2+} homeostasis, β-adrenergic receptor sensitization
Local anesthetics		
Cocaine	Sympathomimetic effects Ischemia/myocardial infarction Proarrhythmic Cardiac arrest Cardiac myocyte death	Na^+ channel blockade Coronary vasospasm, Altered Ca^{2+} homeostasis Mitochondrial injury Oxidative stress Apoptosis
Other local anesthetics (bupivacaine, etidocaine, lidocaine, procainamide)	Decreased excitability ↓ Conduction velocity Proarrhythmic	Na^+ channel blockade
Antihistamines		
(astemizole, terfenadine)	↑ Action potential duration QTc interval prolongation Proarrhythmic	K^+ channel blockade
Immunosuppressants		
(rapamycin, tacrolimus)	Cardiomyopathy Heart failure	Altered Ca^{2+} homeostasis
Miscellaneous drugs		
Cisapride	↑ Action potential duration QTc interval prolongation Proarrhythmic	K^+ channel blockade

Table 1 Continued

Agents	*Cardiotoxic manifestations*	*Proposed mechanisms of cardiotoxicity*
Methylxanthines (theophylline)	↑ Cardiac output Tachycardia Proarrhythmic	Altered Ca^{2+} homeostasis, Inhibition of phosphodiesterase
Sildenafil	?	Inhibition of phosphodiesterase
Radiocontrast agents (diatrizoatemeglumine, iohexol)	Proarrhythmic Cardiac arrest	Apoptosis?
Cardiotoxicity of naturally occurring substances		
Estrogens		
Natural estrogens (17β-estradiol, estrone, estriol)	QTc interval prolongation?	Gender differences in K^+ channel expression?
Synthetic estrogens (diethylstilbestrol, equilin, ethinyl estradiol, mestranol, quinestrol)	Cardioprotection?	Antiapoptotic effects? Antioxidant activity? ↑ Na^+, K^+-ATPase activity? Ca^{2+} channel blockade?
Nonsteroidal estrogens (bisphenol A, diethylstilbestrol, DDT, genistein)		Other mechanisms?
Progestins		
(desogestrel, hydroxyprogesterone, medroxyprogesterone, norethindrone, norethynodrel, norgestimate, norgestrel, progesterone)	Enhanced toxicity of cocaine?	Mechanisms?
Androgens		
Natural androgens (androstenedione, dehydroepiandrosterone, dihydrotestosterone, testosterone)	Myocardial infarction Cardiac hypertrophy	Mitochondrial injury? Altered Ca^{2+} homeostasis? Other mechanisms?
Synthetic androgens (boldenone, danazol, fluoxymesterone, methandrostenolone, methenolone, methyltestosterone, nandrolone, oxandrolone, oxymetholone, stanozolol)		
Glucocorticoids		
Natural glucocorticoids (corticosterone, cortisone, hydrocortisone)	Cardiac hypertrophy Cardiac fibrosis	Increased collagen expression Other mechanisms?
Synthetic glucocorticoids (e.g., dexamethasone, methylprednisolone, prednisolone, prednisone)		
Mineralocorticoids		
(aldosterone)	Cardiac fibrosis Heart failure	Increased collagen expression Other mechanisms?
Thyroid hormones		
(thyroxine, triodothyronine)	Tachycardia Positive inotropic effect Increased cardiac output Cardiac hypertrophy Proarrhythmic	Altered Ca^{2+} homeostasis
Cytokines		
Interleukin-1β	Negative inotropic effect Cardiac myocyte death	↑ Nitric oxide synthase expression Apoptosis
Interleukin-2	Negative inotropic effect	↑ Nitric oxide synthase expression
Interleukin-6	Negative inotropic effect	↑ Nitric oxide synthase expression
Interferon-γ	Cardomyopathy Proarrhythmic	↑ Nitric oxide synthase expression Altered ion homeostasis

Table 1 Continued

Agents	*Cardiotoxic manifestations*	*Proposed mechanisms of cardiotoxicity*
Tumor necrosis factor-α	Negative inotropic effect Cardiac myocyte death	↑ Nitric oxide synthase expression ↑ Sphingosine production ↓ Ca^{2-} transients Apoptosis
Cardiotoxicity of selected industrial agents		
Solvents		
Toluene (paint products)	Proarrhythmic	↓ Parasympathetic activity ↑ Adrenergic sensitivity Altered ion homeostasis
Halogenated hydrocarbons		
(carbon tetrachloride, chloroform, chloropentafluoroethane, 1,2-dibromotetra-fluoromethane, dichlorodifluoromethane, *cis*-dichloroethylene *trans*-dichloroethylene dichlortetrafluorethane, difluoroethane, ethyl bromide, ethyl chloride, fluorocarbon 502, heptafluoro-1-iodo-propane 1,2-hexafluoroethane, isopropyl chloride, methyl bromide, methyl chloride, methylene chloride, monochlorodifluoroethane monochlorodifluoromethane, octafluorcyclobutane, propyl chloride, 1,1,1-trichloroethane, trichloroethane, trichloroethylene, trichlorofluoromethane, trichloromonofluoroethylene trichlorotrifluoroethane, trifluoroiodomethane, trifluorobromomethane)	Proarrhythmic Negative inotropic effect Decreased cardiac output	↓ Parasympathetic activity ↑ Adrenergic sensitivity Altered ion homeostasis Altered coronary blood flow
Ketones		
(e.g., acetone, methyl ethyl ketone)	Proarrhythmic	↓ Parasympathetic activity ↑ Adrenergic sensitivity Altered ion homeostasis
Heavy metals		
(cadmium, cobalt, lead)	Negative inotropic effect Cardiac hypertrophy Proarrhythmic	Complex formation, Altered Ca^{2+} homeostasis
(barium, lanthanum, manganese, nickel)	Proarrhythmic	Ca^{2+} channel blockade

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therapeutic agents and related compounds is presented in **Table 2**. Neither the contents of this chapter nor the material in the tables is intended to be exhaustive or fully inclusive. The agents noted here serve as examples of cardio toxic and vasculotoxic agents. There are detailed chapters in specialty texts that discuss these and other agents more fully. A brief bibliography plus mention of some websites with excellent graphics of the CVS are presented at the end of this chapter.

The Heart

The Heart as a Pump

The mammalian heart (**Figure 1**) can generally be viewed as two side-by-side (left and right) pumps

Table 2 Vasculotoxic agents

Agents	*Sources*	*Prominent vascular effects*	*Associated diseases*
Industrial and environmental agents			
Allylamine	Synthetic precursor	Bioactivation of parent compound by amine oxidase to acrolein and hydrogen peroxide results in smooth muscle cell injury; intimal smooth muscle cell proliferation in large arteries	Atherosclerosis
β-Aminopropionitrile		Damage to vascular connective tissue; aortic lesions; atheroma formation, aneurysm	
Boron		Hemorrhage; edema; increase in microvascular permeability of the lung	Pulmonary edema
Butadiene	Synthetic precursor	Hemangiosarcomas in several organs	
Carbamylhydrazine		Tumors of pulmonary blood vessels	Cancer
Carbon disulfide	Fumigant/solvent	Microvascular effect on ocular fundus and retina; direct injury to endothelial cells; atheroma formation	Coronary vascular disease Atherosclerosis
Chlorophenoxy herbicides			Hypertension
Dimethylnitrosamine		Decreased hepatic flow; hemorrhage; necrosis	Occlusion of veins
Dinitrotoluenes	Synthetic precursor		
4-Fluoro-10-methyl-12-benzyanthracene		Pulmonary artery lesions; coronary vessel lesion	
Glycerol		Strong renal vasoconstriction	Acute renal failure
Hydrogen fluoride		Hemorrhage; edema in the lungs	Pulmonary edema
Hydrazinobenzoic acid	Constituent of *A. bisporus*		
Paraquat		Vascular damage in lungs and brain	Cerebral purpura
Polycyclic aromatic hydrocarbons	Environmental tobacco smoke		
Pyrrolidine alkaloids		Pulmonary vasculitis; damage to vascular smooth muscle cells; proliferation of endothelium and vascular connective tissue in the liver	Pulmonary hypertension; hepatic venoocclusive disease
Organophosphate pesticides			Cerebral arteriosclerosis
T-2 toxin	*Fusarium* mycotoxin		
Vinyl chloride		Portal hypertension; tumors of hepatic blood vessels	Cancer
Gases			
Auto exhaust		Hemorrhage and infarct in cerebral hemispheres; atheroma formation in aorta	Atherosclerosis
Carbon monoxide	Environmental	Damage to intimal layer; edema; atheroma formation	Atherosclerosis
Nitric oxide		Vacuolation of arteriolar endothelial cells; edema, thickening of alveolar-capillary membranes	Pulmonary edema
Oxygen		Vasoconstriction – retinal damage; increased retinal vascular permeability – edema; increased pulmonary vascular permeability – edema	Blindness in neonate; shrinking of visual field in adults; edema
Ozone		Arterial lesion in the lung	Pulmonary edema
Therapeutic agents and related compounds			
Antibiotics/antimitotics			
Cyclophosphamide		Lesions of pulmonary endothelial cells	
5-Fluorodeoxyuridine		GI tract hemorrhage; portal vein thrombosis	
Gentamicin		Long-lasting renal vasoconstriction	Renal failure
Vasoactive agents			
Amphetamine		Cerebrovascular lesions secondary to drug abuse	Disseminated arterial lesions similar to periarteritis nodosa
Dihydroergotamine		Spasm of retinal vessels	
Ergonovine		Coronary artery spasm	Angina
Ergotamine		Vasospastic phenomena with and without medial atrophy	Gangrene of the thrombosis; peripheral tissues

Table 2 Continued

Agents	*Sources*	*Prominent vascular effects*	*Associated diseases*
Epinephrine		Peripheral arterial thrombi in hyperlipemic rats	Participates in thrombogenesis
Histamine		Coronary spasm; damage to endothelial cells in hepatic portal vein	
Methysergide		Intimal proliferation; vascular occlusion of coronary arteries	Coronary artery disease
Nicotine	Tobacco	Alteration of cytoarchitecture of aortic endothelium; increase in microvilli	
Nitrites and nitrates		'Aging' of coronary arteries	Repeated vasodilation
Norepinephrine		Spasm of coronary artery; endothelial damage	
Metabolic affectors			
Alloxan		Microvascular retinopathy	Diabetes; blindness
Chloroquine		Retinopathy	
Fructose		Microvascular lesions in retina	Diabetes-like condition
Iodoacetates		Vascular changes in retina	
Anticoagulants			
Sodium warfarin: warfarin		Spinal hematoma; subdural hematoma; vasculitis	Uncontrolled bleeding; hemorrhage
Radiocontrast dyes			
Metrizamide; metrizoate		Coagulation; necrosis in celiac and renal vasculature	
Cyanoacrylate adhesives			
2-Cyano-acrylate-*n*-butyl		Granulation of arteries with fibrous masses	
Ethyl-2-cyanoacrylate		Degeneration of vascular wall with thrombosis	
Methyl-2-cyanoacrylate		Vascular necrosis	
Miscellaneous			
Aminorex fumarate		Intimal and medical thickening of pulmonary arteries	Pulmonary hypertension
Aspirin		Endothelial damage, gastric erosion obliteration of small vessels, ischemic infarcts	
Cholesterol; oxygenated		Atheroma formation; arterial damage	Atherosclerosis derivatives of cholesterol: noncholesterol steroids
Homocysteine		Increase of vascular fragility, loss of endothelium, proliferation of smooth muscle cells promotion of atheroma formation	Atherosclerosis; synthesis
Oral contraceptives		Thrombosis in cerebral and peripheral vasculature	Thromboembolic disorders
Penicillamine		Vascular lesion in connective tissue matrix of arterial wall, glomerular immune complex deposits, inhibits synthesis of vascular connective tissue	Glomerulonephritis
Talc and other silicates		Pulmonary arteriolar thrombosis, emboli	
Tetradecylsulfate Na		Sclerosis of veins	
Thromboxane A2		Extreme cerebral vasoconstriction	Cerebrovascular ischemia
Vitamin D	Dietary		

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that are joined together and pump in a simultaneous rhythm. Each pump, however, propels blood through a different pipeline circuit. The pumps each consist of two connecting chambers – an atrium and a ventricle – which contract in sequence to provide the pumping action. The ventricles, which are responsible for pumping the blood through the circuits, have thick muscular walls and are located beneath the thinner-walled atria, which function primarily as reservoirs for blood between the heart's contractions. To ensure one-way flow through the CVS, the heart is equipped with specialized valves. The atrioventricular valves prevent backflow of blood into the atria during ventricular contraction (systole), and the aortic/pulmonic (semilunar) valves prevent backflow of blood into the ventricles during

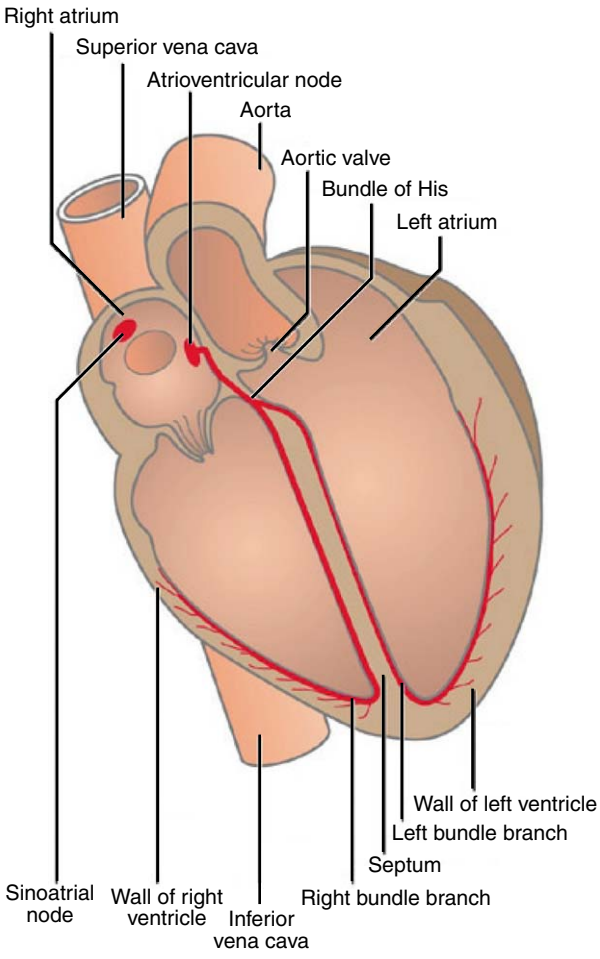

Figure 1 The conducting system of the heart. (Reprinted with permission from fleshandbones.com)

ventricular relaxation (diastole). During systole, the two ventricles develop pressure and eject blood into the pulmonary artery and aorta. At this time the atrioventricular valves are closed and the semilunar valves are open. The semilunar valves are closed and the atrioventriular valves are open during diastole. The right atrium receives blood flowing from the systemic venous system via the superior and inferior venae cavae. This blood initially passes passively through the right atrioventricular orifice into the right ventricle. An atrial contraction then propels a slight additional amount of blood into the right ventricle. A ventricular contraction closes the atrioventriular valve allowing blood now to be propelled past the pulmonic valve into the pulmonary circuit. As blood flows through the pulmonary vasculature, carbon dioxide in the venous return blood is exchanged for oxygen so that the blood pumped through the next (systemic) circuit to the rest of the body will be properly oxygenated. The left atrium receives freshly oxygenated blood from the pulmonary vasculature via the pulmonary vein. Again, blood passively traverses the atrioventricular orifice until an atrial contraction provides complete filling of the ventricle and closes the atrioventricular valve. The strong contraction of the thick-muscled left ventricle now opens the aortic valve, allowing blood to access the systemic circulation (under relatively high pressure) via the aorta. In the absence of injury and/or disease, the heart is a very efficient, durable, and reliable pump. In the 80 year life span of a person, and at a contraction rate of 72 beats per minute, a heart will beat $\sim 3\,000\,000\,000$ times. Two major features of the heart contribute to its unique characteristics: the nature of the heart muscle and the specialized electrical conduction system of the heart.

Cardiac Muscle

There are three distinct types of muscle tissue in vertebrates: striated, smooth, and cardiac. Striated, or skeletal, muscle is attached, at least at one end, to the skeleton via tendons. This muscle type is often referred to as the voluntary muscle, as it can be consciously controlled. Smooth muscle is usually arranged in sheets or layers in tubular systems, such as arteries and veins (see Blood Vessels), the gastrointestinal and respiratory tracts, and the genitourinary tracts. The activities of the smooth muscles are not under conscious control; rather they are coordinated by the autonomic (involuntary) nervous system. The cardiac muscle comprises the bulk of the heart wall proper; and small amounts are found in the superior vena cava and pulmonary vein. The cardiac muscle is not under conscious control; it has an automaticity center which responds to the autonomic nervous system when needed (see section Impulse Conduction). In the heart, cardiac muscle cells are joined in a network of fibers and are connected by gap junctions, which facilitate the conduction of electrical impulses through the cardiac muscle network. In addition to the typical cardiac myocytes, there are other cardiac muscle cells that are specialized to initiate, attenuate, or accelerate the electrical impulses for coordinated contraction of the cardiac network.

Impulse Conduction

The specialized electrical conduction system of the heart allows for the synchronous contraction of the left and right sides of the heart and the sequential contraction of the atria and ventricles (**Figure 1**). Electrical impulses most quickly arise in

the spontaneously-firing cells of the sinoatrial (SA) node commonly called the 'pacemaker'. The SA node is located at the junction of the superior vena cava and the right atrium; therefore, a wave of depolarization (see below) originating at the SA node is conducted first to the cells of the right atrium, then to the cells of both atria, finally converging on a second group of specialized cells – the cells of the atrioventricular (AV) bundle. AV bundle cells act as a conduit for the original impulse from the SA node to the AV node, which lies at the junction of the median wall of the right atrium and the septum separating the two ventricles. From the AV node, the impulse wave next passes into the ventricular conduction system – the bundle of His and Purkinje fibers – located within the ventricular septum, which allows for depolarization of ventricular muscle.

If a microelectrode is inserted into a resting muscle or nerve cell (termed 'excitable tissue'), an electrical potential difference will be recorded across the membrane of that cell. In the case of cardiac muscle cells, this resting potential is $-90\,\text{mV}$ (intracellular relative to extracellular). In other words, the cell membrane is electrically polarized with the inward facing surface of the membrane having a net negative charge with respect to the outer facing surface of the membrane. This polarity is maintained primarily by the presence of extracellular, positively charged ions and intracellular negatively charged proteins. The flux of ions through active (requiring cellular energy) and passive (concentration-driven) processes is responsible for changes in electrical potential. In the resting cardiac muscle cell, the concentration of potassium ions (K^+) is higher inside the cell than outside, while sodium ions (Na^+) are at a much higher concentration outside the cell than inside. Cellular energy is required to maintain the appropriate resting state distributions of the different ions across the cell membrane. In the case of potassium and sodium ions, there is a cell membrane pump, which requires energy derived from the hydrolysis of the terminal phosphate group from adenosine triphosphate (ATP). The associated enzyme responsible for this hydrolysis is the sodium–potassium ATPase. When an electrical stimulus is received by a cardiac muscle cell, voltage-gated channels in the cell membrane open allowing sodium to diffuse down its concentration and electrical gradient into the cell. This influx of positive charge causes the cell membrane to become 'depolarized' (i.e., to have less negative charge). As depolarization proceeds, the membrane may reach the threshold potential ($\sim -70\,\text{mV}$ for most cardiac muscle cells). Any further depolarization results in a phenomenon known as the action potential, which completely depolarizes the cell. At the peak of the action potential, the inside of the cell actually becomes positive relative to the outside ($+30\,\text{mV}$). The cell membrane then repolarizes relatively slowly and reaches the $-90\,\text{mV}$ resting potential before it can respond to another electrical impulse. The wave of depolarization moves very rapidly across the membrane of an individual cardiac muscle cell. In addition, the wave of action potentials is propagated to adjacent cells via the specialized gap junctions. This propagation allows for the complete depolarization of most cells in the network, thus initiating the contraction of the heart muscle as a group.

Cardiac muscle cells predominantly display a fast response action potential (**Figure 2**), and cells in the atria and ventricles exhibit a rapid conduction velocity due to the gap junctions. The depolarization–action potential–repolarization process is divided into five phases. Phase 0 begins when the threshold potential has been reached. At this time, many 'fast' sodium channels in the cell membrane open allowing an inrush of sodium ions to initiate the action potential. At the end of phase 0, the cell is completely depolarized. Toward the end of phase 1 and the start of phase 2, the sodium influx begins to decrease, as does the membrane potential. During the relatively long (200–300 ms) phase 2 plateau, calcium and sodium ions enter through 'slow' membrane channels. Movement of ions through these 'slow' channels only takes place after the membrane potential has dropped to $\sim -55\,\text{mV}$, that is, after the 'fast' sodium ion current has ceased. While these 'slow' inward currents occur, there is also a slow outward movement of potassium ions which keeps the plateau relatively steady. The calcium influx of phase 2 triggers the process known as excitation–contraction coupling, in which the myosin thick filaments slide past the thin actin filaments in the contractile unit of the muscle known as the sarcomere. This process requires energy and involves activation of a myosin ATPase that hydrolyzes ATP. The released energy is utilized to form cross-bridges between the actin and myosin molecules. Both the velocity and the force of contraction are dependent on the amount of calcium ions that reaches the site of contraction. Within the resting muscle cell, calcium is sequestered in a compartment called the sarcoplasmic reticulum. During the action potential, calcium and sodium ions that enter the cell cause depolarization of the sarcoplasmic reticulum membrane, resulting in the release of large amounts of calcium which are needed for effective contraction of the sarcomere. Between contractions, calcium is once again sequestered in the sarcoplasmic reticulum so that the actin–myosin interaction is not overly prolonged. During the long

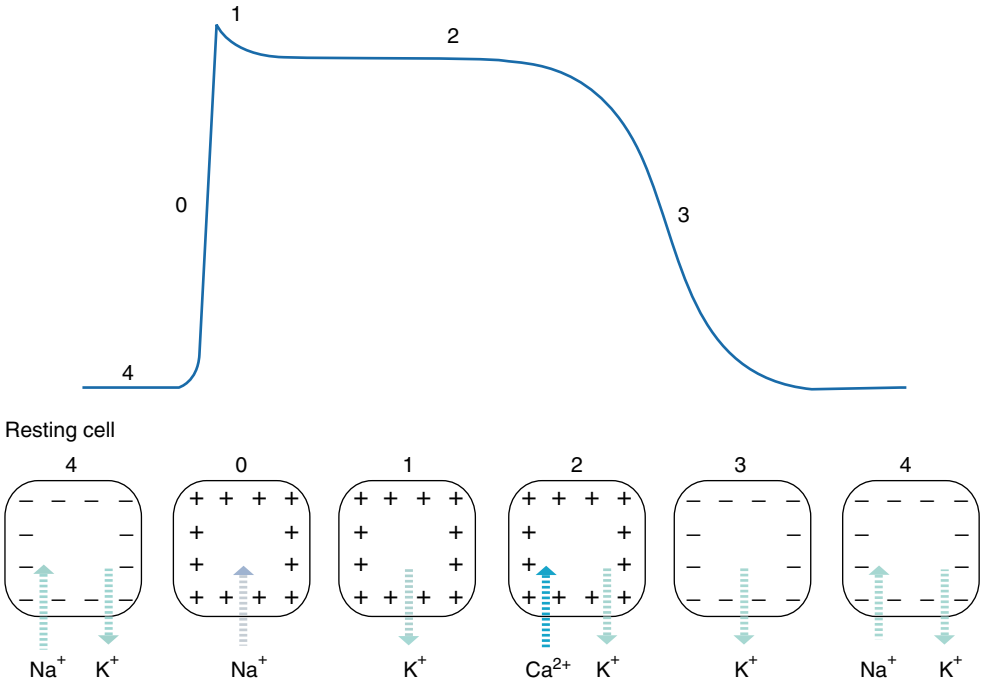

Figure 2 The principal ionic movements during the different phases of the action potential in a cardic muscle cell. (Reprinted with permission from fleshandbones.com)

duration of the plateau phase, a new action potential cannot be initiated because the 'fast' sodium channels are inactivated or refractory to further electrical stimulation. During phase 3, membrane permeability to potassium increases and the 'slow' calcium and sodium channels become inactive. The ensuing efflux of potassium ions allows for repolarization of the membrane until the normal resting potential is reached (phase 4).

In contrast, conduction velocity is slow in muscle fibers at the SA and AV nodes. Unlike the majority of cardiac muscle cells, these pacemaker cells have an unstable resting potential (~ -60 mV) due to a cell membrane alteration that allows sodium ions to leak into the cell without a concurrent potassium ion efflux. This sodium leakage reduces the membrane potential allowing even more sodium ions to move into the cell. In addition to the inward sodium movement, there is also an inward calcium flow which causes the pacemaker cells to have a more positive resting potential. Finally, the cell produces an action potential at ~ -40 mV. This phenomenon is called spontaneous diastolic depolarization. The overall effect is that pacemaker cells initiate waves of depolarization that move across the heart causing the muscle to contract. As noted previously, this phenomenon occurs ~ 72 times per minute (more or less depending on autonomic nervous system stimulation, periods of stress, or physical activity). The waves of electrical activity may be recorded in an electrocardiogram (ECG), which displays the net electrical changes relative to where the recording electrodes are placed on the surface of the body.

Intrinsic Modulators of Cardiac Activity

The heart responds constantly to hormonal and nervous system signals. Sympathetic nervous system terminals releasing norepinephrine are found in cardiac cells of the atria and ventricles. This allows for reflex regulation of heart muscle contractility. Sympathetic innervation is also present to the SA node and AV junction, where norepinephrine release acts to increase heart rate (enhanced phase 4 depolarization) and also to increase conduction velocity by reducing the AV junction impedance to conduction. Parasympathetic innervation is provided by cranial nerve 10, the vagus nerve, to the SA node and the AV junction. These fibers release acetylcholine, which slows SA node activity (decreasing the rate of phase 4 depolarization) and decreases conduction throughout the AV junction.

A practical example of normal nervous system regulation of cardiovascular activity is the processes of blood pressure regulation. Pressure receptors in the carotid sinus and aortic arch sense arterial wall

stretching. These receptors send impulses to the cardiovascular regulatory sensors in the medulla where reflex impulses are generated via the vagus nerve resulting in decreases of heart rate, peripheral vascular resistance, and thus decreased venous return. This results in a decreased blood pressure. Conversely, a decrease in blood pressure will decrease vagal stimulation in favor of sympathetic input. The sympathetic reflex is characterized by increases in heart rate, myocardial contractility, venous return, peripheral vascular resistance, and cardiac output. In addition, the sympathetic response can be produced by the release of naturally occurring catecholamines (epinephrine and norepinephrine) from the medulla of the adrenal gland.

Pathologic Changes in the Heart

The major pathologic changes that occur in the heart are associated with effects on heart rate, contractility of heart muscle, or electrical conduction. Regarding heart rate changes, an arrhythmia, as the name indicates, is a loss of rhythm and here refers to an irregularity of the heartbeat. Two of the more common forms are tachycardia, which is an abnormally rapid heart beat, and fibrillation, which is a rapid twitching of the muscle fibrils. Either of these can occur in the atria or the ventricles. Agents that alter ion levels and fluxes and thereby alter aspects of impulse transmission can produce arrhythmias. The most common site of arrhythmic impulse generation is the SA node. If depolarization after an action potential is accelerated or delayed anywhere within the heart, an aberrant action potential can be triggered and result in an arrhythmia.

Another set of pathologic changes is associated with effects on the force of contraction. The heart muscle exhibits a higher rate of oxygen consumption and a greater energy requirement than many other tissues. Thus, impaired contraction can result from interference with any of the major cycles critical for proper energy metabolism or from processes that interfere with delivery or utilization of the optimum levels of oxygen. For example, if blood flow through the coronary arteries is occluded, as occurs during atherosclerosis, there will be decreased delivery of oxygen to the heart muscle. When this occurs acutely a myocardial infarction, commonly referred to as an MI, may result leading to devitalization of a segment of the heart musculature. Even if death does not occur, there will likely be a decrease in the force or efficiency of contraction of the heart muscle. 'Recreational' use of psychoactive drugs (e.g., amphetamines, cocaine) can result in profound and sudden cardiovascular responses including increases in blood pressure and heart rate due to acute catecholamine release in response to the drugs. These effects can be life threatening in individuals with underlying, and possibly previously unknown, cardiovascular problems including coronary artery disease, high blood pressure, or cerebrovascular disease.

Cells with high energy requirements, such as heart cells, have large numbers of organelles called mitochondria, which produce and supply ATP. Enzymes are organic catalysts that interact with specific substrate molecules to help speed up chemical reactions. The ATPases are enzymes that catalyze the hydrolysis of ATP with its attendant release of energy, which is made available for cellular processes. The myosin ATPase involved in muscle contraction was mentioned above and ATPases involved in the energy-driven pumping of ions including sodium, potassium, and calcium were mentioned above and are noted again below. During oxidative metabolism of organic substrates, the process of electron transport to molecular oxygen in mitochondria is coupled to oxidative phosphorylation, which yields ATP. Some poisons and anticancer drugs, such as cyanide and doxorubicin, interfere with electron transport and/or uncouple phosphorylation. This causes a direct decrease in the amount of energy available to the heart muscle and results in reduced contractility.

As noted above, the inward calcium ion movement is vital for the contraction of the cardiac muscle. This inward movement is blocked by calcium antagonists, such as cobalt and barium, and is stimulated by catecholamines. Increased calcium influx leads to increases in the intracellular level of cyclic AMP, a compound that helps mediate numerous metabolic responses within cells. This, in turn, leads to increased availability of calcium ions for interaction with the contractile proteins. The same effect can be achieved by increased levels of free calcium ions outside of the cells or increased levels of cyclic AMP within cells, as is seen with the vasodilating drug papaverine. Another mechanism for increasing intracellular calcium levels in cardiac cells involves the cardiac glycoside drugs, for example, digitalis from the foxglove plant. This drug inhibits the ATPase that pumps sodium ions out of cells. This results in elevation of sodium ion levels inside the cell, which in turn leads to increases in intracellular calcium ion levels and therefore increased rate and strength of contraction. Toxins that increase the permeability of the cardiac muscle cell membrane to the sodium ion, for example, the marine compound, ciguatoxin or the Columbian frog poison active agent, batrachotoxin, have a similar effect. On the contrary, agents that decrease membrane permeability to sodium ions

will depress myocardial contractility. Included here are a diverse group of compounds including tetrodotoxin, from the Japanese pufferfish; the shellfish-derived poison, saxitoxin; and polyethylene glycol, the active ingredient in many antifreeze preparations. Local anesthetics such as lidocaine and procaine depress the fast inward sodium ion current, the slow inward current, and the potassium ion outward current. They tend to slow the heart rate and the force of contraction; thus, they are commonly used as anti-arrhythmic drugs.

Drugs prescribed to alleviate one set of medical problems can have striking and sometimes fatal effects on the cardiac system. Antipsychotics derived from phenothiazine, including chlorpromazine, depress myocardial contractility and cardiac output. Chlorpromazine can also impair cardiac reflex mechanisms and cause a focal myocardial necrosis. Cyclophosphamide, an anti-cancer agent, also causes myocardial necrosis as well as changes in ECG patterns. Another anticancer agent, adriamycin (doxorubicin), can produce cardiomyopathies with subsequent congestive heart failure. Severe dysrhythmias and some cases of sudden death have been reported. Overdoses of the tricyclic antidepressants, for example, amitryptaline, can result in severe cardiotoxicity, probably due to anticholinergic activity. At high doses, the antidepressant imipramine will depress contractility, lower heart rate, and depress cardiac output. Cardiac arrest may also occur. Some antibiotics, including gentamycin and neomycin, depress calcium ion uptake and therefore reduce contractility of the cardiac muscle. Although the sympathetic system transmitters, the catecholamines, are essential for maintenance of normal myocardial contractility, it has been long recognized that when administered at higher than normal levels for extended periods of time, they can lead to severe myocardial necrosis.

Profound cardiotoxic responses can result from inhalation of a number of halogenated alkanes. These are low molecular weight hydrocarbons with some or all of the hydrogen atoms being substituted by halogens, usually chlorine or fluorine. These agents depress heart rate, contractility, and electrical conduction. The effects are generally more pronounced as the number of halogen atoms increases. Some of these compounds have the additional and profound effect of sensitizing cardiac muscle cells to catecholamines. In humans without pre-existing cardiac disease, the effects of most of these compounds are reversible, although chronic exposure may cause some irreversible damage. As would be expected, the older halogenated hydrocarbon anesthetics such as halothane and enflurane had similar effects.

In contrast, low-pressure fluorocarbons, such as trichlorofluoromethane, can be particularly toxic. In most cases, the levels generally encountered in the environment are too low to have any major lasting effect and even at relatively high levels (up to 15%) fatalities are rarely recorded. However, at levels much above this, for example, over 20%, tragic results can ensue. Among people who inhale these agents from closed bags to 'get high', fatalities can result because the levels of these agents in the bags can reach 35–40%.

There are compounds that interfere with the regular activity of calcium ions in cardiac cells, either by replacing them (as is the case with a number of heavy metals) or by altering the flux of calcium ions across the cell membrane. Among metals, lanthanum, manganese, and nickel all block calcium channels in the cell membrane. Both barium and cobalt ions antagonize endogenous calcium ion levels and tend to shorten the action potential. Lead ions have multiple effects, including displacement of calcium and interference with calcium ion availability, energy metabolism, and ATP synthesis in heart muscle cells. Among organic chemicals, the opium derivative, papaverine, also blocks slow calcium ion channels. Cobra venom cardiotoxin and bacterial endotoxin both interfere with calcium ATPase activity and endotoxin also depresses calcium uptake by heart muscle cells.

Agents Causing Morphologic Changes

A number of cardiotoxic compounds have been listed to this point, including some that interfere with sodium/potassium ATPases; increase sodium or calcium influx; or depress myocardial function by replacing calcium, decreasing sodium permeability, or altering contractility. These agents produce toxic responses in the heart muscle often resulting in death, but do so without causing any major morphologic changes in the heart. Other cardiotoxic compounds produce characteristic morphologic lesions in the heart muscle. There are a few basic types of such pathologic alterations. The first is toxic myocarditis. Chemicals which produce this effect cause cell damage and, ultimately, cell death. Whether or not they produce damage acutely or chronically is generally a function of the dose of the toxic agent. The acute form is characterized by edema, that is, accumulation of excess fluid, as well as inflammatory cell responses and multiple regions of cardiac cell death. However, the inflammatory response will be attenuated or may even be absent if the toxic agent suppresses the immune system, for example, drugs given to prevent rejection of transplanted organs.

The second type of major morphologic alteration in the heart arises from a sudden insufficiency or local arrest of the blood supply to the heart that can result in necrosis of a region of the heart. This condition is called a myocardial infarction (MI). In advanced arteriosclerosis, occlusion of the major arteries supplying the heart muscle with blood can result in an MI. Even in the absence of arteriosclerosis, MIs can result, for example, from amphetamine abuse, which produces severe inflammations of critical arteries (i.e., an arteritis). Intravenous drug use can cause infective endocarditis (an inflammation of the internal lining of the heart), which can lead to vessel occlusion with an embolus, thus resulting in an infarction. Cocaine abuse can result in ventricular tachycardia (i.e., rapid heart beat) and fibrillation, MI, and sudden death. At higher doses, cocaine can increase the levels of catecholamines, ultimately resulting in increased calcium ion activity, accelerated heart beat, arrhythmias, etc. Chemicals that antagonize calcium ion movement through calcium-specific membrane channels prevent the ventricular arrhythmias induced by cocaine. Gross MI can result from toxic exposures to carbon monoxide, nitrates, ergot derivatives, and some potent anticancer drugs (see above).

Recent media attention and FDA guidelines have focused on the cardiovascular effects of appetite-suppressant drugs and nutraceuticals or herbal medicines. In 1997, the anti-obesity drugs fenfluramine and dexfenfluramine were withdrawn from the United States sales market due to convincing correlations made between drug usage and cardiac valvular abnormalities. Since then, the deleterious morphologic effects of these drugs have been described as valvular encasement and/or endocardial fibrosis. These lesions can have life-threatening consequences, including progressive aortic valvular regurgitation. The mechanism underlying the pathogenesis of these lesions remains unclear. There appears to be a correlation with increased serotonin levels in the blood and endocardial fibrosis; thus, there is speculation that these drugs may increase serotonin levels or may increase sensitivity of tissues to serotonin. In addition, the dietary supplement ephedra (ma huang), has been implicated in cases of myocardial infarction due to coronary artery vasoconstriction; thus, in 2004, the FDA issued a warning about ephedra with a proposal to ban its sale and use in the United States.

Another type of gross morphologic lesion in the heart muscle is hypersensitivity myocarditis. This is an inflammatory response that is the most common type of heart disease associated with drug use. There are five primary clinical criteria for diagnosis of this condition: (1) previous use of the drug(s) without deleterious incidents; (2) no apparent relationship between the size of the drug dose and the hypersensitivity response; (3) clinical symptoms consistent with responses to allergens or infectious disease agents; (4) independent confirmation of immunologic responses; and (5) persistence of the symptoms as long as drug use is continued. Histologically, there is infiltration of the heart muscle with numerous types of white blood cells and this is associated with local regions of lysis of the cardiac muscle cells. However, gross fibrosis and extensive regions of myocardial necrosis are usually absent. Among the drugs that have been reported to elicit this response are the antibiotics penicillin, streptomycin, ampicillin, tetracycline, and sulfadiazine.

The Blood Vessels

The second part of the CVS is composed of the blood vessels, which are an extensive series of tubular conduits of varying diameters. All but the narrowest of these vessels have a complex wall structure (see below). One major group of vessels, the arteries, distributes blood under various degrees of pressure to all parts of the body. A second major group of vessels, the veins, returns the blood to the heart. With the exception of the pulmonary artery, which brings blood from the heart to the lungs, the arteries carry blood that is more oxygenated than the blood in their venous counterparts. The large- and medium-sized arteries and veins share the same general structure, although the thicknesses of specific cell layers as well as the cell density within layers can vary considerably.

Blood flow

Despite the system's vital function of transporting blood throughout the body, it would be overly simplistic to view the vascular system as merely a series of pipes of varying diameter. When the left ventricle contracts to deliver blood to the aorta, the largest artery in the body, not only is the blood pressure generated at contraction relatively high, but it is also maintained at a moderately high pressure between contractions of the heart. If the arteries were a set of rigid pipes, the pressure in the artery system would fall to zero between contractions. The fact that this does not occur is due chiefly to the presence of numerous elastic layers (composed of the protein elastin) in the largest arteries. As the heart contracts, the blood pumped into these large arteries causes the elastin in the walls to stretch. Following contraction, the semilunar valves close (see description of valves

above) and the walls of the elastic arteries contract passively to maintain pressure within the system until the ventricles fill and contract once again. There are large, elastic arteries, which function primarily to maintain the pressure within the arterial system during diastole, the resting phase of heart contraction. There are also muscular arteries, which function primarily to distribute the blood throughout the body to organs and tissues, each of which may require different amounts of blood. To help ensure that appropriate volumes of blood are delivered on demand, the size of the lumen (the space through which the blood flows) of the muscular arteries must be regulated quickly and reliably. This is accomplished via innervation by sympathetic fibers of the autonomic nervous system.

Because capillary walls are thin (to permit diffusion) the blood that is delivered to them must be delivered under reduced pressure. This is accomplished by the arterioles, which combine relatively muscular walls with a narrow lumen. The arterial blood pressure is a function of cardiac output and the total peripheral vascular resistance, which is primarily a function of the degree of normal tension (tonus) of the smooth muscle cells in the walls of the arterioles. If this tonus increases above the normal range for extended periods of time, hypertension (high blood pressure) will result. This tonus is under the control of the autonomic nervous system and of adrenergic hormones (catecholamines).

From the capillaries, blood flows first into the narrowest members of the venous system, the collecting venules, and from there into the muscular venules, whose diameter is approximately twice larger than that of the former and whose walls contain one or two layers of smooth muscle cells. Blood then flows into progressively larger veins, first to the small and then to the medium-sized veins. Veins that are located deep within tissue tend to have thinner, less muscular walls than do superficial veins. The final sets of veins to receive blood before it is delivered back to the heart are the inferior and superior venae cavae. The outermost cellular layer in these veins is considerably thicker and the innermost layer is considerably thinner than those of the aorta, the first artery leaving the heart. Another difference between arteries and veins is that the latter have a more extensive vasa vasorum, an arterial blood supply to the vessel wall. Since venous blood is relatively poorly oxygenated, veins require supplemental oxygenation supplied by the vasa vasorum. Because venous blood is under low pressure, the vasa vasorum can penetrate closer to the innermost layer of the vein without being occluded by compressive pressures in the wall.

Pathological Changes

Approximately 90% of the pathologic alterations seen in veins are associated with one of three conditions: deep-vein thrombosis, which often appears following acute MI, thrombotic strokes and/or major surgery; varicose veins, which usually arise secondary to sustained increases of venous pressure; and superficial thrombophlebitis, which occurs in humans with varicose veins as well as in some females after pregnancy. A few venotoxic responses to exogenous (i.e., from outside the body) agents are noted below; however, the great majority of vasculotoxic agents have their effects on the arteries. Therefore, only a description of the artery wall structure is presented below along with listings and selected descriptions of agents that damage the arteries.

Artery Wall Structure

There are three principal cell coats (tunics) that have been identified in the wall of large- and medium-sized arteries (**Figure 3**). The outermost coat, the tunica adventitia, is composed of connective tissue cells plus extensive deposits of the proteins collagen and elastin. The adventitia in muscular arteries is approximately one-half the thickness of the middle coat, the media. In muscular arteries, the media is composed primarily of layers of smooth muscle cells. The principal extracellular protein component is elastin. In elastic arteries the tunica media also predominates, but in this case there are many layers of elastin with smooth muscle cells between the layers. The media is separated from the adventitia by a prominent elastic layer, the external elastic lamina. The adventitia of elastic arteries is thinner than that of muscular arteries. In large arteries a vasa vasorum will also be present. The innermost layer of the artery wall is the tunica intima, which is separated from the media by the internal elastic lamina. In photomicrographs, the inner elastic lamina appears fenestrated. This may serve as a relatively low-resistance pathway for migration of smooth muscle cells into the intima from the media, a process thought to be involved in the development of arteriosclerotic plaques. A single layer of endothelial cells (see below) borders the intima at the lumenal surface.

The media is the most heterogeneous in composition and the most variable in size of the three major coats of the artery. The predominant cell type in the media is the smooth muscle cell. Although some subtle differences both in appearance and behavior have been noted between smooth muscle cells in the intima versus those in the media, it is still not clear whether this is due to the presence of more than one

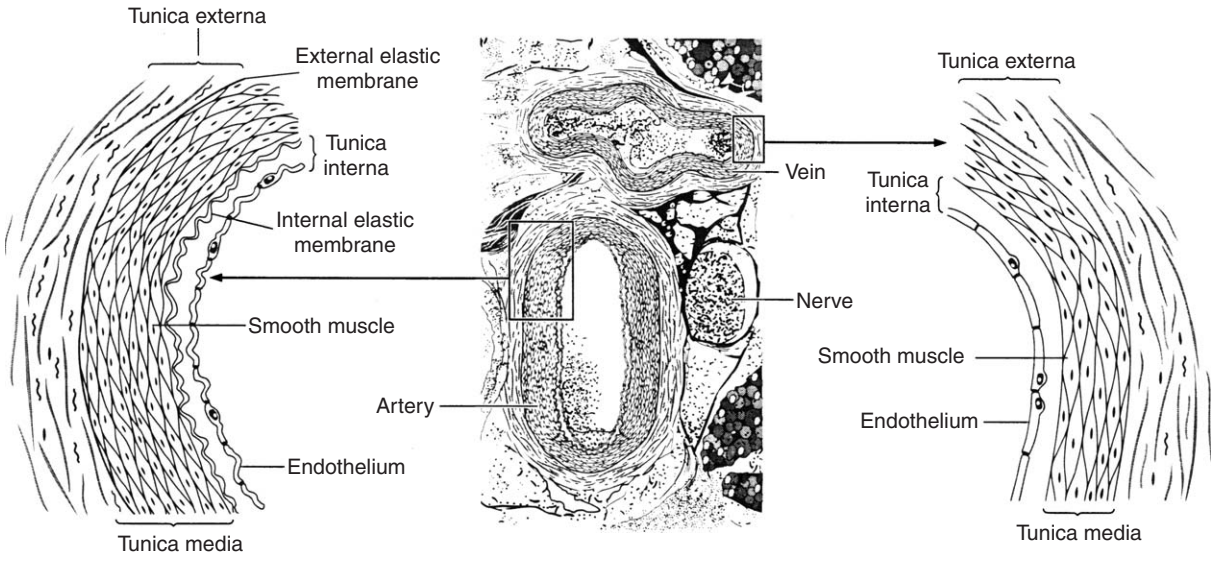

Figure 3 Comparision of typical artery and typical vein. (Reprinted with permission from Cotran R, Kumar V, and Collins T (eds.) (1999) *Robbins Pathologic Basis of Disease*, 6th edn. Philadelphia, PA: Saunders, with permission from WB Saunders.)

type of smooth muscle cell, or to differing microenvironments in these two adjacent regions of the artery wall.

Atherosclerosis is a major cause of death in most industrial societies. The characteristic lesion of this disease, the atherosclerotic plaque, is found in the intima of large- and medium-sized arteries. An additional problem with advanced plaques is that thrombus formation is likely to occur in regions of plaque rupture. The combination of the two events can lead to partial or even total occlusion of major arteries. If this occurs in one or more of the coronary arteries, a serious or even fatal MI may result. A discussion of arteriosclerosis and exogenous agents that can modulate this condition is presented below.

There exist a large variety of compounds, some of which are noted below, that evoke toxic responses within the arterial intima. These compounds are of interest not only because some humans suffer their cardiovascular effects each year but also because an understanding of the mechanism(s) whereby these agents act in living organisms may provide new insights into the complexities of the arterial intima. The existence of agents that can behave as vascular toxins should not be a primary public health concern, with the striking exception of cigarette smoke, discussed below. In fact, the extensive list of vasculotoxic agents listed in textbooks of toxicology notwithstanding, it is clear that if most deaths from heart disease and stroke were due only to those agents, then these two related conditions would quickly cease being the single greatest cause of death in the USA. As it is, there are nearly 900 000 deaths from these two diseases combined, every year. The major and largely avoidable vasculotoxic agent associated with these diseases is tobacco smoke, which is discussed in a subsequent section.

Endothelial Damage

Maintenance of the integrity of the single layer of endothelial cells that lines all of the vascular system is critical for normal vessel function. The intact endothelium is a dynamic system. It acts as a permeability barrier, preventing access of bloodborne contaminants to intimal cells. The intact endothelium also prevents adherence of white blood cells and thrombi; produces and secretes a wide range of growth regulatory molecules; and maintains vascular tone by releasing molecules that modulate dilation and constriction of blood vessels. The endothelium may even participate in its own injury on occasion. Endothelial cells are capable of oxidizing low-density lipoprotein (LDL), which is primarily responsible for transporting cholesterol through the blood to tissues. Oxidized LDL can injure the endothelium directly, produce molecules that allow specific types of white blood cells to adhere to the endothelial surface, and attract inflammatory cells to the inner surface of the artery. Presently, the prevailing view is that these events are critical to the early stages of arteriosclerotic plaque formation. Since the structural and metabolic integrity of endothelial cells is vital to normal arterial function, and since agents causing damage to endothelial cells might be present in

the blood at any time, there must be efficient processes available to repair the endothelium and maintain its integrity should it become damaged.

Blood vessels of similar anatomical structure have distinct responses to chemical stress depending on the organ system with which they are associated. This may be due to subtle differences at the cellular and subcellular levels between similar cells and/or to local responses to different stimuli, for example, due to specific hormone receptors or patterns of innervation. Consider the blood–brain barrier, which prevents many potentially toxic blood-borne agents from reaching the brain. If the metabolic status of the endothelial cells in vessels at the brain level is altered, one result can be a disruption of the tight junctions between the endothelial cells, with a resulting increase in permeability. As a result, the brain, which normally is shielded from a number of toxic agents, may now be exposed to them. Lack of oxygen or markedly reduced local blood flow (ischemia) will lead to swelling of the endothelial cells and a widening of the junctions. Chemicals, including alcohols and surfactants, that solubilize lipids, which are an important component of cell membranes, can also impair the barrier. Lead ions interact with sulfhydryl (–SH) groups that are critical to the functioning of many endothelial cell enzymes and structural proteins. Lead ions thus produce damage to the endothelial cells in blood vessels supplying the brain well before the typically recognized damage to nervous system cells is recognized. Chemicals that raise osmotic pressure, such as solutions of high salt or the alcohol, mannitol, can cause endothelial cells to shrink, thereby causing the tight junctions between the cells to separate.

The liver is the organ largely responsible for detoxification of xenobiotic (foreign biological) chemicals and partly, as a consequence, is also constantly at risk for damage by toxic chemicals. One such chemical, the carcinogen dimethylnitrosamine, first induces the proliferation of endothelial cells, followed by increased formation of vascular connective tissue, and, ultimately, total venous occlusion. Plant toxins of the pyrrolizidine alkaloid family, including monocrotaline, can produce identical effects. Monocrotaline, which enters the body in a non toxic form, is metabolized to its toxic form(s) by the liver. In addition to liver damage, this agent causes structural remodeling of blood vessels in the lung and a resultant increase in pulmonary arterial pressure. This effect is similar to the chronic pulmonary hypertension from which many people suffer.

Metals

A number of metals that cause kidney damage act on arteries supplying this organ. Elevated levels of cadmium are associated with hypertension, at least in animal studies. Cadmium has also been implicated in thickening of the wall of arterioles and deposition of fibrotic tissue in capillaries in the testes as well as the kidneys. Agents that chelate cadmium can reverse many of these effects, as can elevation of body levels of zinc. It appears that cadmium and zinc are antagonistic and that maintenance of a cadmium/zinc ratio within fairly well defined limits may be important in preventing cadmium-associated vessel wall changes.

Three other metal ions that have been implicated in damage to vessel walls are mercury, chromium, and arsenic. Mercury, which interferes with protein –SH groups, may cause vasoconstriction of preglomerular vessels in the kidney.

Arsenic, though an unlikely contributor to blood vessel damage on a worldwide level, represents a striking example of how local environmental alterations can have profound effects on a large portion of a population. On the southwest coast of Taiwan, the artesian well water consumed by the local population has high levels of arsenic and about one out of every 100 people suffer from blackfoot disease. In late stages of this disease, extremities can become gangrenous, leading to spontaneous or surgical amputation of extremities. People suffering from this disease exhibit much higher levels of both peripheral vascular disease and cardiovascular disease. The mechanism of the action of arsenic on the blood vessels remains unclear.

Primary Amines

Cardiotoxicity of primary amines (epinephrine, norepinephrine, isoproterenol) was noted earlier, and has been recognized for nearly 100 years. The vascular toxicity of these and related compounds has also recently been recognized. The effects seem to focus on medial cells of the artery wall, rather than on adventitial or endothelial cells. Early changes include loss of medial cells, mineralization, and loss of elastic fibers. Later there is a compensatory proliferation of intimal cells. The vascular toxicity of two related compounds is particularly striking. One of these compounds, allylamine, will be discussed near the end of this chapter. The second is β-aminoproprionitrile (β-APN), which is the active agent in the toxic sweet pea, *Lathyrus odoratus*. Consumption of flour derived from this plant results in lathyrism, a condition often seen in children and young

adults residing in Algeria, Ethiopia, and parts of India. Sudden death can result because of rupture of aortic aneurysms, which are ballooned and weakened segments of the artery wall. The toxicity of β-APN has been related to its inhibition of an enzyme which normally cross-links collagen and elastin in large elastic arteries, including the aorta, thereby strengthening them.

Atherosclerosis

Arteriosclerosis (literally 'artery hardening') is the general term used to describe thickening and stiffening that can occur for a variety of reasons in arteries of all sizes. From a clinical perspective, the lesion of greatest interest to cardiovascular disease is the atherosclerotic plaque (**Figure 4**). It is the principal lesion associated with human myocardial and cerebral infarction, which are the primary causes of death in the United States, Canada, Europe, and Japan. Plaque development is complex, involving processes as diverse as cell proliferation, cell death, synthesis, and deposition of a variety of extracellular macromolecules (e.g., collagens, elastin, proteoglycans), lipid accumulation and mineralization. The plaque typically appears in the arterial intima with a variety of associated cell types, including smooth muscle cells, macrophages, lymphocytes, platelets, and endothelial cells. Plaque formation has been classified both as a problem of proliferation and one of degeneration, as well as an inflammatory process, a response to injury, and a process related to benign tumor formation. In truth, there is evidence supporting each of these views.

Although in most cases atherosclerosis does not become manifest as a clinically serious condition until well into middle age or beyond, it is a disease that begins early in life. Autopsy studies on US soldiers killed during the Korean War revealed that many already had arterial deposits characteristic of the early stages of atherosclerosis. More recent studies on children through people in the third decade of life have confirmed and expanded these findings. The good news is that while there are genetic factors which may predispose an individual to develop atherosclerosis, there is considerable evidence that individual choices and lifestyle decisions can play a large role in preventing, or at least mitigating, the early onset of clinical symptoms of this disease. Further, results from a limited number of laboratory animal studies suggest that it may even be possible to reverse the clinical course of the disease.

There are three areas where lifestyle modification can have profound effects on moderating development of clinically significant atherosclerosis. In addition to exercise, the two areas most amenable to change are diet and smoking. There is strong epidemiological evidence associating elevated levels of serum cholesterol with increasing risk of atherosclerosis and subsequent heart attacks. As noted above, LDL is primarily responsible for transporting cholesterol and its esters through the bloodstream to the tissues. Oxidized LDL can damage vessel wall cells, including endothelial cells. Oxidized LDL can act as and also generate a chemoattractant, which attracts monocytes to the endothelial surface and possibly helps mediate passage of monocytes across the endothelium where they may differentiate into

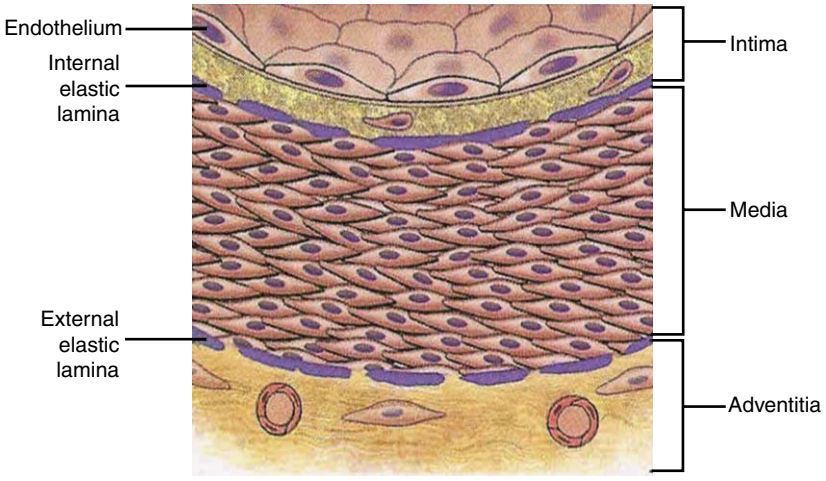

Figure 4 Diagrammatic representation of the main components of the vascular wall. (Reprinted with permission from Cotran R, Kumar V, and Collins T (eds.) (1999) *Robbins Pathologic Basis of Disease*, 6th edn. Philadelphia, PA: Saunders, with permission from WB Saunders.)

tissue macrophages. Monocyte-derived macrophages act as scavengers to help remove harmful molecules such as oxidized LDL. When normal control mechanisms are dysfunctional, macrophages filled with oxidized LDL can become foam cells, which are critical to the formation of early stage atherosclerotic plaques. Studies on experimental animals as well as humans have shown that reduction in levels of plasma cholesterol and LDL can lead to significant widening of the arterial lumen. There is evidence that probucol, a drug originally used for its plasma cholesterol-lowering capability, may function primarily as an antioxidant protecting the integrity of LDL.

Relaxation of blood vessels appears to be at least partially under the control of endothelial cells and their secreted products, especially endothelium-derived relaxation factor (EDRF). Oxidized LDL directly inhibits the endothelial cell-associated vessel relaxation. The generation of increased reactive oxygen species in association with elevated levels of blood cholesterol has also been reported. One of these reactive oxygen species, superoxide (O_2^-), may interact with vasoactive EDRF (nitric oxide) locally in the artery wall, preventing endothelial cell-dependent vasodilation. In addition, a product of the reaction of nitric oxide and superoxide, the reactive peroxynitrite, may act to stimulate lipoprotein oxidation, which, as noted above, is regarded as an early step in atherosclerotic plaque generation.

Oxidants arise from two sources. The first, which is internal, is related to various metabolic processes, including respiration, phagocytic activity to destroy bacteria- and/or virus-infected cells, and, paradoxically, attempts to detoxify foreign substances. In the process of carrying out the latter activity, toxic oxidant by-products can be produced. The second source is external. While the potential protective effects of dietary components and supplements, for example, vitamins, are still being debated, it is reasonable to conclude that decreasing exposure to oxidants from external sources would be beneficial not only in reducing chances of premature atherosclerosis, but also of other diseases, including cancer. By far the most common, avoidable, and dangerous source of external oxidants is cigarette smoke, which is considered a principal contributor to one-quarter of all heart disease cases, one-third of all cancers and $\sim$400 000 premature deaths in the United States every year. As economies of developing countries expand and as cigarette smoking becomes more popular throughout the world, health problems associated with cigarette smoking will increase rapidly.

Cigarette Smoke

Cigarette smoke is composed of active smoke, the smoke coming from the mouth end of the cigarette and breathed in by the smoker; and passive smoke (second-hand smoke; environmental tobacco smoke) which is composed mostly of the smoke coming off the burning end of the cigarette plus a small percentage of exhaled smoke. Active and passive smoke contain many constituents in common, but often in strikingly different concentrations. Among the more than 4000 different chemicals that have been identified in cigarette smoke, prominent candidates that have been considered as vasculotoxic agents include carbon monoxide and various carcinogens. In addition to interfering with transport of well-oxygenated blood, carbon monoxide may cause endothelial cell damage directly, although the mechanism is not clear. Another major class of potential vasculotoxins in cigarette smoke is the carcinogens. Most of these are found in the tar condensate fraction of cigarette smoke. Some, including benzo(*a*)pyrene, are well-known carcinogens that are found in other environmentally prominent substances including coal tar derivatives, charcoal-broiled meat and automobile exhaust. Other smoke carcinogens include the nitrosamines, some of which are tobacco-specific. Both benzo(*a*)pyrene and the parent nitrosamines require metabolic activation to become carcinogenic. The enzymes involved in these processes are members of the cytochrome P-450 system. During the course of detoxifying these agents so that they ultimately can be excreted readily, one or more toxic and possibly carcinogenic metabolites may be generated. Compounds such as benzo(*a*)pyrene induce the appearance of the cytochrome P-450 system enzymes, and smokers are constantly exposed to the P-450 inducers. Generation of endothelial cell-damaging agents during the metabolism of benzo(*a*)pyrene derived from cigarette smoke has been recently proposed , but not proved, as a mechanism to explain the initiation of atherosclerotic plaques. Oxidants derived from cigarette smoke can damage lipids, an important constituent of cell membranes, as well as cellular macromolecules, including DNA. These is no direct evidence that cigarette smoke causes damage to artery wall cell DNA in either living animals or humans; however, if such damage does occur it would provide independent support for the view that DNA alterations are characteristic of atherosclerotic plaques in animal models of the disease as well as in humans. In related experimental animal studies, the chemical allylamine caused both myocardial lesions and vascular fibrosis. Allylamine

toxicity is thought to be mediated via metabolism of this compound to the reactive aldehyde, acrolein, which is also a prominent component of cigarette smoke. Studies with cultured artery wall cells indicate that the primary arterial effect of allylamine is on the smooth muscle cells. Proliferation of intimal smooth muscle cells in response to allylamine exposure results in activation of a specific cellular DNA sequence, the H-ras oncogene, which is implicated in the development of certain forms of cancer. This lends further support to the contention that there may be molecular similarities between the development of the lesions of atherosclerosis and of cancer.

One of the problems researchers have faced in identifying specific health-threatening components of cigarette smoke is that while at moderate to high concentrations many of these agents can be toxic, in many cases the individual concentrations of these factors in cigarette smoke are likely too low to be able to account individually for the toxic and disease-promoting effects of cigarette smoke. The US Environmental Protection Agency sidestepped this problem in 1992 by declaring environmental tobacco smoke, with its thousands of components, to be a human class A carcinogen. The American Heart Association has classified environmental tobacco smoke as an environmental poison and as a major preventable cause of cardiovascular disease. Regarding environmental tobacco smoke, there have been estimates that as many as 60 000 excess heart disease deaths in the United States every year can be attributed directly to involuntary exposure to cigarette smoke. In support of this estimate, a number of laboratories have reported that inhalation of sidestream cigarette smoke accelerates arteriosclerosis in different experimental model systems of the disease. Since epidemiological and autopsy evidence strongly support the view that atherosclerosis begins as early as childhood, the experimental results with environmental tobacco smoke suggest that involuntary exposure of children to tobacco smoke may accelerate plaque development. The insidious nature of involuntary exposure to environmental tobacco smoke is further emphasized by recent findings in a mouse model of atherosclerosis. Male mice exposed only *in utero* to environmental tobacco smoke develop accelerated atherosclerosis as adults, even in the absence of a high fat diet. In the United States, where studies show that many children are less active physically and have poorer diets than children growing up a few of generations ago, involuntary exposure to second-hand smoke may well represent a major additional risk factor for the development of atherosclerosis. Fortunately, extensive epidemiologic evidence from both cancer and heart disease studies indicates that as the time since cessation of smoking increases, the chances of dying prematurely from either disease decrease. Thus, the vasculotoxic effects of cigarette smoke, both active and passive, may be largely reversible.

See also: Amphetamine; Arsenic; Batrachotoxin; Blood; Chemicals of Environmental Concern; Chromium; Cocaine; *hERG* (Human Ether-a-Go-Go Related Gene); Mercury; Tetrodotoxin; Tobacco Smoke.

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Relevant Website

http://www.fleshandbones.com – Pertains to the biomedical sciences including anatomy, physiology, pharmacology and general medicine. Among this website's features is an "Imagebank" containing color images that can be down-loaded.

Castor Bean

Brenda Swanson-Biearman

- Chemical Abstracts Service Registry Number: CAS 9009-86-3
- Synonyms: *Ricinus communis*; Castor-oil plant; Palma christi; Koll; Moy bean; Mole bean; Dog tick seeds
- Chemical/Pharmaceutical/Other Class: Toxalbumins

Uses

Ricin (castor bean) immunotoxin has been developed to attack the CD5 T-cell antigen (present in T-cell and some B-cell malignancies) as well as the interleukin-2 receptor of cancerous tumors. Neurobiological applications of ricin involve the study of brain function via lesioning. It is also used as a reagent for pepsin and trypsin, and as a commercial mole killer. Historically, it has been used as a biochemical warfare agent.

Exposure Routes and Pathways

Ingestion is the most common route, but ocular and dermatologic exposures to castor bean powder have been reported.

Toxicokinetics

The glycoproteins (ricin) are poorly absorbed from the gastrointestinal tract; however, once absorbed, they most likely follow a distribution pattern similar to that of albumin. Many cell surfaces contain receptors specific for the ricin molecules. This molecule consists of two subunits, A and B, bound by a disulfide link. When this link is broken, the B subunit binds to galactose-containing receptors in the cell wall and is transported intracellularly. The A subunit inhibits protein synthesis. The liver, spleen, adrenal cortex, and bone marrow are the primary sites of distribution. The biotransformation and elimination of toxalbumins are poorly understood. The elimination half-life in one patient was 2 days. The reported disappearance of ricin from the plasma is according to first-order kinetics when injected intravenously into mice and human cancer patients.

Mechanism of Toxicity

The principal toxicity of the toxalbumins is protein synthesis inhibition causing hemagglutination within the first hour, adrenal insufficiency, hepatic and renal failures, endothelial damage, and, in severe cases, profound capillary hemorrhage.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity of the castor bean in animals is similar to that in humans.

Human

The toxicity of the castor bean is variable due to erratic absorption patterns. Symptomatology can occur with the ingestion of one seed. The seeds are minimally toxic if the seed coat remains intact when ingested. Acutely, the toxalbumins cause an initial aggregation/sludge formation of red cells within the first hour, severe gastrointestinal lesions, retinal hemorrhages, rapid and weak pulse, and possible shock due to fluid and electrolyte loss from vomiting and diarrhea. Mild to moderate central nervous system depression may be seen. Seizures can occur, but are not common. Fever may be noted. Hepatic damage can occur in large overdoses with increases in alanine aminotransferase, total bilirubin, and aspartate aminotransferase. Elevated serum creatinine and hematuria are often seen. Unlike most toxalbumins, castor beans contain several allergens that can cause severe reactions in the hypersensitive individual. Late-phase complications (2 days postexposure) reflect the cytotoxic effects of the ricin. Patients may be asymptomatic prior to this phase, but damage to the hepatic, central nervous, renal, and adrenal systems may ensue. Laboratory radioimmunoassay is available for ricin (usually not on an emergent basis), but management must be based on symptomatology alone.

Clinical Management

No specific treatment is available for toxalbumin exposure. Aggressive gastric decontamination such as whole bowel irrigation is recommended. It is unlikely

that activated charcoal will be beneficial. Supportive care primarily consists of maintaining appropriate fluid volume and electrolyte balance.

See also: Ricin and Other Toxalbumins.

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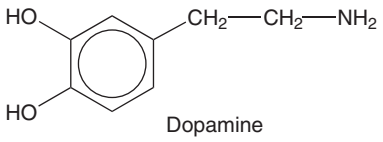
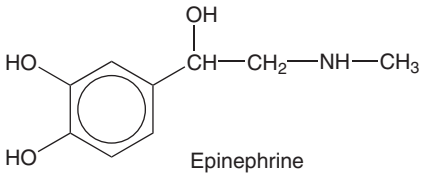
Catecholamines

Zhengwei Cai

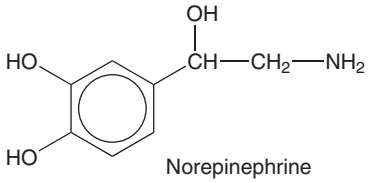
- Description: Catecholamine is the name of a group of compounds that contain a catechol nucleus (a benzene ring with two adjacent hydroxyl substituents) and an amine group. This group includes the mammalian neurotransmitters or hormones, such as dopamine, norepinephrine, and epinephrine, and nonmammalian compounds such as octopamine. Each compound has its own synonyms.
- Representative Chemical: Dopamine
- Chemical Abstracts Service Registry Number: CAS 51-61-6
- Synonyms: Pyrocatechol; 4-(2-aminoethyl)pyrocatechol; 3-hydroxytyramine; 3,4-dihydroxyphenethylamine; 4-(2-aminoethyl)-1,2-benzenediol; Dopastat; Intropin
- Chemical Formula: $C_8H_{11}NO_2$
- Representative Chemical: Epinephrine
- Chemical Abstracts Service Registry Number: CAS 51-43-4
- Synonyms: Benzyl alcohol; Adnephrine; Adrenal; Adrenalin; Adrine; Antiasthmatique; Asthma-Nefrin; Balmadren; Epifrin; Epirenamine; Glaucosan; Hemostatin; Methylaminoethanolcatechol; Renagladin; Renalina; Renostypticin; Soladren; Simplene; Supranefran; Suprarenin; Susphrine; Sympathin; Takamine; Vasoton; Vasotonin
- Chemical Formula: $C_9H_{13}NO_3$
- Representative Chemical: Norepinephrine
- Chemical Abstracts Service Registry Number: CAS 51-41-2
- Synonyms: 4-(2-Amino-1-hydroxyethyl)-1,2-benzenediol; α-(Aminomethyl)-3,4-dihydroxybenzyl alcohol; 2-Amino-1-(3,4-dihydroxyphenyl)ethanol; 1-(3,4-Dihydroxyphenyl-2-aminoethanol; Adrenor; Aktamine; Levarternol; Levonorepinephrine; Levophed; Noradrenaline; Nortrinal; Sympathin E
- Chemical Formula: $C_8H_{11}NO_3$
- Chemical/Pharmaceutical/Other Class: Catecholamines are endogenous neurotransmitters or hormones. Dopamine and norepinephrine are in the monoamine class
- Chemical Structures:



Dopamine



Epinephrine



Norepinephrine

Uses

Catecholamines are sympathomimetic drugs. Dopamine and norepinephrine are used as vasopressors (antihypotensives). Epinephrine is used as a vasoconstrictor, cardiac stimulant, or bronchodilator to counter allergic reaction, anesthesia, and cardiac arrest. It is also an antiglaucoma agent.

Background Information

Catecholamines are endogenous compounds and are synthesized in the brain, the adrenal medulla, and by some sympathetic nerve fibers. The biosynthesis of catecholamines begins with the hydroxylation of tyrosine by tyrosine hydroxylase to form L-dopa, which is decarboxylated by aromatic amino acid decarboxylase to form dopamine. Norepinephrine

is formed from dopamine by the enzyme dopamine β-hydroxylase, and epinephrine is formed from norepinephrine by enzyme phenylethanolamine *N*-methyltransferase. Dopamine is widely distributed throughout the CNS and is involved in the control of movement. Norepinephrine is an important neurotransmitter in both the CNS and the sympathetic part of the autonomic nervous system. The hormone epinephrine acts together with the sympathetic nervous system to initiate the body's quick response to stressful stimuli.

Exposure Routes and Pathways

When used therapeutically, intravenous injection or infusion is the most common route of administration. Epinephrine is available in nebulized racemic dosage form for inhalation.

Intoxication from catecholamine usually results from iatrogenic overdoses, accidental intravenous administration, and the injection of solution intended for nebulization.

Toxicokinetics

Epinephrine is well absorbed after oral administration but is rapidly inactivated in the gut mucosa. When intravenously injected or infused, the onset of drug effect is rapid (within 5 min for dopamine and 3–10 min for epinephrine) and the duration of drug effect is short (10 min for dopamine, 1 or 2 min for norepinephrine, and 15 min to hours for epinephrine depending on route of administration). Exogenous catecholamine in the circulation is rapidly and efficiently taken up by adrenergic neurons. Catecholamine is metabolized by monoamine oxidase, which is localized largely in the outer membrane of neuronal mitochondria, and by catechol-O-methyl transferase, which is found in the cytoplasm of most animal tissues, particularly the kidneys and the liver.

The primary metabolites of dopamine are homovanillic acid and dihydroxyphenylacetic acid (75%) and norepinephrine (25%). The primary metabolites of epinephrine and norepinephrine are vanilylmandelic acid and 3-methoxy-4-hydroxyphenethyleneglycol. Catecholamine metabolites and their conjugates are excreted in urine.

Mechanism of Toxicity

Catecholamines are sympathomimetic drugs. These drugs increase heart rate and cardiac output and may produce cardiac arrhythmias. Administration of norepinephrine also results in increased peripheral vascular resistance. Both effects may cause serious systemic hypertension, which may cause cerebral hemorrhage. Reduced hepatic and renal blood flow may cause tissue ischemia, increased glycolysis, and serum lactic acidosis. In very high doses, a paranoid state may be induced. Production of reactive oxygen species and formation of quinone during the metabolism of dopamine are involved in dopamine toxicity. Recent studies have demonstrated that norepinephrine may enhance or inhibit immune function under certain conditions.

Acute and Short-Term Toxicity (or Exposure)

Animal

Overdose of catecholamines may result in animal death. In test animals, there is evidence that death is the result of respiratory arrest caused by hypertension following overdose of epinephrine.

Human

At high infusion rates of dopamine, ventricular arrhythmias, and hypertension may occur. Nausea, vomiting, and angina pectoris are occasionally seen. Gangrene of the extremities may occur in patients with profound shock given large doses of dopamine for long periods of time. Norepinephrine may cause dose-related hypertension (sometimes indicated by headache), reflex bradycardia, increased peripheral vascular resistance, and decreased cardiac output. High doses of norepinephrine (in excess of 8–12 mg of base per min) cause intense vasoconstriction, which results in 'normal' blood pressure but decreased tissue perfusion. Local necrosis may result from perivascular infiltration and angina, mesenteric ischemia, and peripheral ischemia. Epinephrine may cause dose-related restlessness, anxiety, tremor, cardiac arrhythmias, palpitation, hypertension, weakness, dizziness, and headache. Anginal pain may occur when coronary insufficiency is present. A sharp rise in blood pressure from overdosage may cause cerebral hemorrhage and pulmonary edema.

Chronic Toxicity (or Exposure)

Human

Prolonged use and repeated injection of epinephrine may lead to tolerance and local necrosis. Prolonged use of norepinephrine may cause edema, hemorrhage, focal myocarditis, necrosis of the intestine, or hepatic and renal necrosis. It may also cause plasma volume depletion, which may result in

perpetuation of the shock state or recurrence of hypotension when the drug is discontinued.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Treatment is directed at ameliorating tachycardias, shock, cardiac arrhythmias, systemic hypertension, pulmonary edema, and lactic acidosis. In the case of severe toxicity, administration of a rapidly acting α-adrenergic blocking drug such as phentolamine may be considered.

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CCA-Treated Wood

C Charles Barton and Thomas T Newton

Chromated copper arsenate (CCA) is a chemical mixture registered by the US Environmental Protection Agency (EPA) for use as a wood preservative. It has been demonstrated to protect wood from dry rot, fungi, molds, termites, and other pests that can threaten the integrity of wood products. CCA-treated wood (also known as pressure-treated wood) is most commonly used in outdoor settings. Over 90% of all outdoor wooden structures are made with CCA-treated lumber. Around the home, CCA-treated wood is commonly used for decks, walkways, fences, gazebos, boat docks, and playground equipment. Other common uses of CCA-treated wood include highway noise barriers, signposts, utility poles, and retaining walls.

Untreated wood generally deteriorates within 3–5 years, depending on its exposure to soil and environmental conditions. CCA-treated wood, on the other hand, is relatively strong and long-lasting and maintains its integrity in conditions under which untreated wood would quickly degrade. CCA-treated wood products often retain their structural integrity 10–20 times longer than untreated woods.

In the pressure-treatment process, lumber is loaded into a horizontal cylinder. The cylinder door is sealed, and a liquid solution containing CCA is pumped in. The pressure in the cylinder is then raised, forcing the CCA into the wood. At the end of the process, the excess treatment solution is pumped back to a storage tank for reuse. The CCA solution is toxic. Therefore, it can be applied only by EPA-certified operators. However, wood that has been treated with CCA is not classified as hazardous because the CCA 'fixes' to the wood in a way that makes the chemical insoluble and somewhat leach resistant. Thus, CCA-treated wood is not considered to be a health risk unless burned in fireplaces or wood-stoves.

The arsenic penetrates deeply into the wood and remains there for a long time. However, some of the chemical may migrate from treated wood into surrounding soil over time and may also be dislodged from the wood surface upon contact with skin. The amount and rate at which arsenic leaches, however, varies considerably depending on numerous factors, such as local climate, acidity of rain and soil, age of the wood product, and how much CCA was applied. Interestingly, the leaching occurs more with newer structures and decreases with time.

Since excessive exposure to arsenic can be hazardous to health, precautions should be taken to decrease exposure. Applying a sealant on a regular basis (e.g., one reapplication every other year depending upon wear and weathering) should prevent the migration of arsenic from the wood. One should wash hands thoroughly after contact with treated wood, especially prior to eating and drinking; and ensure that food does not come into direct contact with any treated wood. Furthermore, workers should take certain precautions: wear gloves when handling wood, wear goggles and dust-mask when sawing and sanding, always wash hands before eating, and never burn CCA-treated wood.

During an 8 year investigation, the EPA examined the safety of using and handling CCA-treated wood. None of the EPA's investigations produced any findings showing increased risks of cancer or other toxic effects on humans handling CCA-treated wood.

In 1985, the EPA concluded that the benefits of CCA-treated wood far outweighed any risks. The EPA established modest use precautions, which the treating industry agreed to disseminate in a voluntary consumer-awareness program. The actual exposure levels to arsenic in CCA are considered to be minuscule. In 1990, the Consumer Product Safety Commission (CPSC) measured dislodgeable arsenic in eight samples of CCA-treated wood. In five of the samples, the amount was undetectable. Two other samples yielded small quantities of arsenic. The eighth sample, which yielded the greatest amount of arsenic, was rough-sawn lumber, a material classified by the wood-treatment industry as not acceptable for playground equipment. The CPSC concluded that the amounts of arsenic that people may be exposed to are below the level that may cause a health concern, and deemed it safe. Plants grown in soil touched by CCA-treated wood have the same minuscule exposure levels. For decades, CCA-treated wood has been used commercially near crops in the form of tomato stakes, vineyard supports, banana props, and mushroom trays. No problems have ever been recorded that indicate that the preservative migrates into plants and causes any health effects.

On February 12, 2002, the EPA announced a voluntary decision by industry to move away from using CCA to treat wood used in residential settings. This transition affects virtually all residential uses of wood treated with CCA, including wood used in play-structures, decks, picnic tables, landscaping timbers, residential fencing, patios, and walkways/boardwalks. Effective December 31, 2003, no woodtreater or manufacturer may treat wood with CCA for most residential uses. This decision will facilitate the transition in both the manufacturing and retail sectors to wood preservatives that do not contain arsenic, as well as other alternatives, such as naturally resistant woods and plastic alternatives. EPA does not believe there is any reason to remove or replace CCA-treated structures, including decks and playground equipment. Furthermore, the EPA is not recommending surrounding soils be removed or replaced. Also, CCA-treated wood can be disposed of with regular municipal trash (i.e., municipal solid waste, not yard waste).

See also: Arsenic; Wood Dust.

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Cell Cycle

Alice M Sheridan, Vishal S Vaidya, and Harihara M Mehendale

The cell cycle is the orderly progression of cells through specific stages during which DNA is replicated and distributed to two daughter cells resulting in cell proliferation. Precise regulation of the passage of cells through this cycle is necessary to assure the maintenance of DNA integrity through multiple generations. Cell cycle regulation also ensures that cell proliferation occurs only under defined conditions in response to growth factors and in the presence of a suitable environment. Loss of cell cycle regulation is a characteristic of cancer.

The cell cycle comprises four stages, which are called G1, S, G2, and M phases (**Figure 1**). S (for DNA synthesis) is the stage in which DNA is duplicated. G1 is the stage immediately prior to S during which the cell prepares for DNA synthesis. M (for mitosis) is the stage in which the cell divides and G2 is the stage preceding M during which the cell prepares for cell division. Two major points of regulation are at the transitions between G1 and S and between G2 and M phases. The progression of cells through late G1/S requires the presence of growth factors. A *restriction point* in late G1 marks the point at which cycle progression becomes growth factor independent. Cells that are actively proliferating progress from M phase back to G1 where preparations for DNA synthesis immediately start anew. Cells that are not actively proliferating are said to be quiescent and are in G0 phase. The entry of cells from G0 into the cell cycle is also a closely regulated step and requires an extracellular stimulus or growth factor. We describe below the critical proteins that have been identified to date that regulate G1/S and G2/M transitions. We emphasize that the cell cycle paradigm is rapidly evolving and expanding and that this description is likely incomplete.

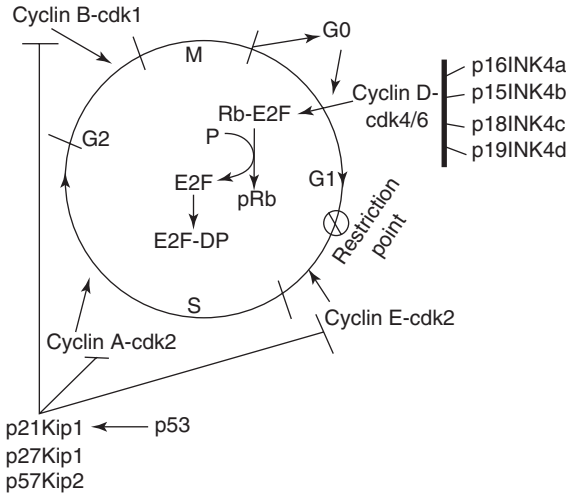

Figure 1 Overview of the different phases of the cell cycle. Quiescent cells are in G0 phase and reenter the cell cycle at G1 during which cells prepare for DNA synthesis. After passing the restriction point in late G1 cells are committed to enter S phase, during which DNA replication occurs. Cells in G2 phase prepare for mitosis (M phase). Cell cycle progression is controlled by various positive and negative cell cycle regulatory proteins including cyclins (A, B, D, E); cyclin dependent kinases (cdk 1, 2, 4, 6); cdk inhibitors (p15, p16, p18, p19, p21, p27, p57), retinoblastoma (Rb) and p53.

Cyclins and Cyclin-Dependent Kinases

Numerous proteins have been identified that stringently regulate the passage of cells at G1/S and G2/M phase transitions. Conserved serine/threonine kinases, called cyclin-dependent kinases (cdks), phosphorylate and activate specific regulatory proteins that drive cell cycle progression. The activity of cdk is controlled at three levels. First, cdks are activated by their interaction with proteins, called cyclins. Cyclins are proteins with very short half-lives of less than 30–60 min. Whereas the cdks are constitutively expressed throughout the cell cycle, the level of the cyclins varies throughout. Cyclin levels are controlled by both regulated synthesis and ubiquitin-mediated proteolysis. Specific cyclin–cdk complexes function at different cell cycle phases. Formation of the heterodimers cyclin D/cdk4, cyclin D/cdk6, and cyclin E/cdk2 are necessary for entry into and progression through G1. The induction of cyclin D family members is provoked by an extracellular signal or growth factor and initiates the entry of quiescent cells from G0 into G1. Cyclin D/cdk heterodimers phosphorylate and inactivate retinoblastoma protein (pRb) causing the release and activation of the E2F family of transcription factors. This family of transcription factors drives transcription of genes necessary for the G1/S transition, including cyclin E. Cyclin E/cdk2 also phosphorylates pRb but unlike cyclin D heterodimers, its activity is mitogen-independent. Both cyclin E/cdk2 and cyclin A/cdk2 drive entry and progression through S phase via the phosphorylation of non-Rb proteins that initiate DNA synthesis. Cyclins A and B form complexes with cdk1 (also called cdc2) and are called the mitotic cylins since these complexes regulate mitosis. Cyclin B/cdk1 controls the G2/M transition. Cyclin B is synthesized as the cell progresses through G2. Upon binding of

cyclin B to cdk1, the activated heterodimer phosphorylates proteins that are involved in mitosis. Activity of the cyclin/cdk complexes is also regulated by phosphorylation/dephosphorylation by cdk activating kinases (CAKs) and phosphatases. A third level of regulation is achieved by control of protein levels of cdk inhibitors. Cdk inhibitors are proteins that accumulate in response to multiple environmental stimuli including DNA damage, hypoxia, cell–cell contact and cytokines, and inhibit the activity of cyclin/cdk heterodimers. The cdk inhibitors include two classes of proteins. The INK 4 proteins, which include $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, and $p19^{INK4d}$, specifically inhibit the activity of cdk4 and cdk6 by competitive inhibition of cyclin D binding to the monomeric kinases. Mutations and deletions of the $p16^{INK4a}$ gene and inactivation by hypermethylation, have been shown to play a role in tumorigenesis in many different types of tumors. The Kip/Cip proteins include three structurally related proteins, p21, p27, and p57. In contrast to the INK4 proteins, the Kip/Cip proteins inhibit most cyclin/cdk heterodimers. Specific Kip/Cip proteins are induced by upstream events. p21 is induced in response to DNA damage and specifically inhibits cyclin E/cdk2. Protein levels of p27 are highest in quiescent cells and induce G1 arrest in response to conditions that typically result in cell quiescence such as growth factor deprivation or contact inhibition. Both the INK4 and the Kip/Cip proteins inhibit the phosphorylation and inactivation of pRb.

Retinoblastoma

The retinoblastoma gene (Rb) was the first tumor suppressor to be identified. Rb mutations were first shown to be causal in familial and sporadic retinoblastoma, a rare tumor of the eye, but have since been associated with many other tumors including osteosarcoma, small cell lung cancer, and prostate and breast cancer. In addition, mutations in the upstream Rb signaling pathway that result in the functional inactivation of the Rb gene product, pRb, are found in virtually all malignancies. Three Rb homologs have been described, including Rb, Rb 107, and Rb 130. All Rb homologs are characterized by a ‘pocket’ domain, which is highly conserved and necessary for pRb’s tumor suppressor function. All the Rb homologs bind viral oncoproteins as well as E2F family members. Binding of viral oncoproteins disrupts the pocket domain of pRb and impairs pRb’s tumor suppressor function. All pRb homologs cause G1 arrest. The primary role of pRb is the inhibition of transcription of genes that mediate passage across the G1/S transition. There are two mechanisms by which pRb inhibits transcription. First, pRb binds to and inhibits the E2F family of transcription factors. The binding characteristics of the homologs vary slightly as, whereas pRb binds preferentially to E2F1-4, p107 and p130 bind preferentially to E2F4 and E2F5. Phosphorylation of pRb regulates its interaction with E2F. The phosphorylation status of pRb fluctuates throughout the cell cycle. Hypophosphorylated pRb is active and binds to E2F family members thus sequestering E2F and inhibiting its transcriptional activity. Hyperphosphorylated pRb is inactive and releases E2F, which results in the transcription of genes that allow the cell to progress to S phase. Upon release from pRb, E2F binds to DP-1 or DP-2 and the resulting heterodimer activates genes necessary for DNA replication. The mechanism by which pRb inhibits E2F transcriptional activity is still debated but it may be via the recruitment of chromatin remodeling enzymes such as histone deacetylases (HDACs) which directly repress transcription by removing acetyl groups from chromatin which causes the chromatin to be less accessible to transcription factors. The role of the pRb-bound HDAC may be to counteract the activity of the E2F-bound acetyltransferase protein, p300/CBP, which transfers acetyl groups to chromatin and enhances transcriptional activity. In addition to its inactivation of E2F resulting in a decrease in transcription of E2F-responsive genes, the complex of pRb and E2F actively represses transcription, which may also be via the recruitment of HDACs to the promoter regions. The regulation of pRb activity is complex. There are 16 possible sites for cdk-mediated phosphorylation and data suggest that phosphorylation at each different site regulates a distinct pRb function. pRb is phosphorylated by multiple cyclin/cdk complexes. Cyclin D/cdk4/6 initiates phosphorylation in early G1 and cyclin E/cdk2 hyperphosphorylates pRb in late G1. Cyclin A/cdk2 maintains phosphorylation of pRb thoughout S phase. pRb may perform other roles in addition to regulation of G1/S including the regulation of apoptosis. A decrease in functional pRb results in the activation of p53-induced apoptosis, which appears to be mediated via the release of E2F1. Free E2F1 activates transcription of ARF (alternate reading frame of the p16INK4a locus), which inhibits a protein called mdm-2 ubiquitin ligase (mdm-2). mdm-2 targets proteins for ubituitin-mediated proteolysis. Since mdm-2 initiates the degradation of p53, its inhibition results in an increase in p53 and a corresponding increase in apoptosis. Thus, a decrease

in functional pRb, which could otherwise result in unchecked cell proliferation, triggers an apoptotic response. A decrease in functional pRb also creates a selection pressure for p53 mutations, since only cells that have mutated dysfunctional p53 survive. Not surprisingly, p53 mutations are often found to coexist with Rb mutations in malignant tumors.

Checkpoints

Checkpoints are surveillance mechanisms comprising numerous genes that detect DNA damage and induce either cell cycle arrest and DNA repair mechanisms, or, in the presence of extensive DNA damage, apoptosis. The data elucidating this surveillance network are very incomplete but have been advanced significantly since the isolation of the mutation that is associated with ataxia telangiectasia (AT). AT is a rare pediatric disease that is associated with immune deficiency and an increased susceptibility to cancer. Prior to the isolation of the AT mutation, it had long been observed that stimuli that induce DNA damage delay progression through the cell cycle. For years this phenomenon was assumed to be the passive response of the cell as a direct result of the DNA damage itself. By contrast, cells that harbor the AT mutation demonstrate a marked decrease in cell cycle arrest after DNA damaging radiation. These data suggested that an active system exists in normal cells that retards cycle progression in the presence of DNA damage. The checkpoint surveillance system comprises sensor proteins (proteins that detect DNA damage and initiate a signaling cascade); transducers (modifying enzymes such as kinases that relay the signal to effector proteins); and effectors (downstream target proteins that, upon activation by modifying enzymes, cause cycle arrest). Of these proteins, the least is known about sensor proteins, although several candidate genes have been suggested. The effector proteins include kinase inhibitors such as p21, or cyclin/cdk heterodimers that are either activated or inhibited to cause cycle arrest. Major transducer proteins include p53, ATM (AT mutated) and ATR (ATM and RAD-3 related).
p53 is a transcription factor that activates the transcription of genes that cause cell cycle arrest at either G1/S or G2. In addition, p53 activates genes that initiate DNA repair and cause apoptosis. Mutations of p53 are commonly described in association with human tumors. The result of p53 activation is cell type-specific and depends on the type and severity of injury. p53-induced G1 cell cycle arrest is mediated via the induction of p21 and p16. p53 has a very short half-life and is generally undetectable in healthy cells. In the presence of DNA damage induced by either ultraviolet or g-irradiation, p53 is activated by posttranscriptional modifications including phosphorylation and acetylation, that either enhance its stability or alter its affinity for binding proteins. ATM and its related protein, ATR, phosphorylate p53 which decreases its binding to mdm-2. A decrease in the interaction between p53 and mdm-2 causes a decrease in ubiquitin-mediated proteolysis of p53 and a resulting increase in p53 protein levels. As described previously, ARF also activates p53 via the inactivation of mdm-2. In addition to phosphorylation, the acetylation status of p53 also determines its stability. p53 is acetylated and stabilized by p300/ CBP which increases apoptosis. The recently described NAD-dependent deacetylase protein, SIRT1, removes acetyl groups from p53 and decreases apoptosis. ATM and ATR are closely related phosphoinositide 3-kinases that are activated by DNA damage. Upon activation, ATM phosphorylates and activates multiple proteins in addition to p53, including mdm-2 and a serine/threonine kinase called Chk-2. Activated Chk-2 phosphorylates p53. ATR phosphorylates many but not all of the same substrates as ATM. ATR phosphorylates and activates Chk-1, which also phosphorylates p53. p53, ATM, and ATR also contribute to G2 arrest. Upon activation, cdk1 initiates mitosis. cdk1 is activated via its interaction with cyclin B and via dephosphorylation by cdc25C phosphatase. Upon phosphorylation by ATM and ATR, Chk-2 and Chk-1 phosphorylate and inhibit cdc25C, which prevents the activation of cdk1. p53 activates transcription of two genes that inhibit cdk1 activity including GADD45 and 14-3-3 s. GADD45 disrupts the cyclin B/cdk1 heterodimer. The protein product of 14-3-3 s sequesters cdc25C, which prevents the dephosphorylation of cdk1. In the face of overwhelming DNA damage, checkpoints, in particular p53, induce cell death by apoptosis rather than cell cycle arrest. Apoptosis, or programmed cell death is an evolutionarily conserved, energy-requiring mechanism by which unwanted or irreparably damaged cells are removed from the organism. Apoptosis is a fundamental component of both normal embryogenesis and adult homeostasis. Apoptosis is also a physiologic response to diverse toxic stimuli including viral infection, DNA damage induced by irradiation or reactive oxygen species, hypoxia, growth factor deficiency or genetic aberration. Apoptosis is carried out by caspases, which are proteases that contain a cysteine nucleophile and cleave proteins whose sequence contains specific motifs that include an aspartic acid residue. Upstream or initiator caspases are activated by the binding of an extracellular ligand to a

death receptor. Death receptors are members of the tumor necrosis superfamily and are characterized by an intracellular death domain. An important example of a death receptor is CD95, or fas, which binds fas ligand. Upon binding of a ligand, the death receptor binds to intracellular adaptor proteins. Adapter proteins bind to initiator caspases 2, 8, 9, or 10, which provokes their autocleavage and activation. Initiator caspases activate downstream effector or executioner caspases, such as caspase 3 or 7, or proapoptotic BCL-2 proteins. The BCL-2 family includes proteins that contain BCL-2 homology domains. These domains allow for heterdimerization by which BCL-2 proteins activate other family members. BCL-2 proteins modulate the intrinsic apoptotic pathway and may have either pro or anti-apoptotic effects. Proapoptotic BCL-2 proteins increase mitochondrial membrane permeability which allows for the release of cytochrome c. Cytochrome c release from mitochondria results in dimerization of an adaptor protein called Apaf-1 (apoptotic protease activating factor) which binds procaspase 9 resulting in its cleavage and activation. Caspase 9 activates the downstream effector, caspase 3. Antiapoptotic proteins in the BCL-2 family inhibit the proapoptotic members and prevent the increase in mitochondrial membrane permeability. The downstream effector caspases target multiple proteins for degradation including enzymes, nuclear structural proteins such as lamins, cytoskeletal proteins such as actin, proteins critical for cell–cell interaction such as b-catenin, and DNA repair enzymes. p53 activates multiple genes that are involved in apoptosis, including genes that encode proteins that function via receptor-mediated signaling and those which encode proteins that modulate downstream effectors. p53-activated IGF-BP3 inhibits binding of IGF-1 to the IGF-1 receptor, which can induce apoptosis. p53 activates transcription of the death receptor ligands fas/Apo1/CD95 and the death receptor KILLER/DR5. p53 also induces the proapoptotic BCL-2 protein bax, as well as other proteins that enhance cytochrome c release from mitochondria, including p53, AIP1, PUMA, and Noxa. Apoptosis may also be induced via an increase in oxidative stress generated by multiple p53-induced genes that are homologous to NADPH-quinone oxidoreductase. Importantly, no singular p53-activated gene product has been conclusively shown to initiate apoptosis. It appears that many p53-induced proapoptotic genes need to be activated concurrently in order for apoptosis to occur. It is not clear which variables determine whether p53 induces cell cycle arrest or apoptosis. Certain cell types, such as T lymphocytes, are especially sensitive to apoptosis whereas fibroblasts are more likely to undergo cell cycle arrest. Whereas p53 induces arrest or senescence in normal cells, p53 activation usually causes apoptosis in transformed cells. The reason for the enhanced sensitivity to p53-induced apoptosis in transformed cells may be related to the deregulation of E2F due to the inactivation of pRb. Cycle arrest induced by p21 may protect the cell from apoptosis. Other factors that may predispose the cell toward p53-induced apoptosis include alterations in the bax/bcl-2 ratio, concurrent absence of growth factors, a greater intensity of stress and higher protein levels of p53. Posttranslational modifications may also determine p53 promoter specificity, which may play a major role in determining whether p53 expression results in cell cycle arrest or apoptosis.

Clinical Application

The normal regulation of the cell cycle plays an important role in tissue repair and inflammation. All tissues may be stratified by proliferative capability into three categories including labile, quiescent or permanently nondividing cells. Labile cells are continuously dividing and include surface epithelial cells such as stratified squamous epithelial cells of the skin and columnar epithelial cells of the gastrointestinal tract. Quiescent cells are nondividing under normal circumstances but can be induced to reenter the cell cycle by exposure to growth factors. Quiescent cells include parenchymal cells of the liver, kidney, and pancreas and mesenchymal cells such as fibroblasts. The cytokine-induced reentry of quiescent cells into G1 phase is an important component of the inflammatory response, which has been well characterized in the kidney. Glomerular mesangial cells proliferate in many models of glomerular disease, including lupus nephritis and diabetes. The proliferation of mesangial cells occurs in response to cytokines such as platelet-derived growth factor and basic fibroblast growth factor. Inhibition of mesangial cell proliferation may abrogate the glomerulosclerosis or the glomerular scarring that occurs as a result of inflammation. Permanently nondividing cells have lost all capacity for proliferation and include nerve cells and cardiac muscle cells. The deregulation of the cell cycle resulting in unchecked cell proliferation is a hallmark of cancer. All human cancers are characterized by defects of restriction point control, checkpoints, DNA repair, or apoptosis. Defects of restriction point control allow for uncontrolled proliferation and result in loss of terminal differentiation. While some cancers are characterized by loss of function mutations of Rb or by disruption of the Rb pocket domain by viral oncoproteins, many more are caused by functional

inactivation of pRb through cyclin D overexpression or INK4a mutations. Mutations of p53 are the most common mutations associated with cancer and occur in almost 50% of all human cancers.

Conclusion

The regulation of the cell cycle plays an important role in normal tissue repair and regeneration. Loss of cell cycle regulation is a chief characteristic of cancer. Cell cycle regulation involves numerous signaling pathways that determine whether cells will proliferate, remain quiescent, arrest or undergo apoptosis. While enormous progress has been made in the elucidation of these signaling pathways, our understanding of cell cycle regulation remains incomplete. Further studies may allow better understanding of diseases that result from deregulation of these pathways.

See also: Tissue Repair.

Further Reading

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Cell Proliferation

Sanjay Chanda and Harihara M Mehendale

Unicellular organisms, like yeasts, bacteria, or protozoa, have a strong selective pressure to grow and divide as rapidly as possible. The rate of cell division in these cases is limited only by the rate at which nutrients can be taken from the medium and converted into cellular materials. Multicellular organisms, on the other hand, are made up of different kinds of cells performing a variety of functions, and the survival of the organism as a whole is at stake rather than the survival or proliferation of one individual type of cell population. For the multicellular organism to survive, some cells must refrain from dividing even when nutrients are plentiful. Some cells do not divide after a certain stage of development as in the case of central nervous system. However, when need arises for new cells, as in the case of tissue injury, previously nondividing cells must be rapidly triggered to reenter the cell division cycle as part of the overall survival strategy.

Division Cycle of Cells

An adult multicellular animal must divide and supply millions of new cells just to replace the dead or dying cells. Cells go through a division cycle through a highly regulated process known as cell cycle progression (**Figure 1**). Cells divide by going through a cell cycle with the end-product being a duplication of the contents of the mother cell in two daughter cells. In an adult animal most of the cells are in resting or in G_0 (G = gap) phase of cell cycle. When needed to divide they enter the G_1 phase of cell cycle. In most cells the DNA in the nucleus is replicated during only a limited portion of the cell cycle called the S or synthesis phase of the cell cycle. After the S phase, the cells go into a second interval called G_2 phase. In mitosis or M phase, the contents of the nucleus condense to form visible chromosomes, which through an elaborately orchestrated series of movements, are pulled apart into two equal sets and then the cell itself splits into two daughter cells. Upon loss of tissues (e.g., a population of cells and a portion of the tissue) due to injury, the division cycle of cells is stimulated in tissue- or organ-specific fashion so that the lost tissues can be replaced promptly to restore tissue function. Each of the above phases of cell division is highly regulated and orchestrated by an intricate series of signaling mechanisms. When the lost tissue is replaced the entire repertoire is brought down to the normal resting level, thereby reestablishing the cellular, organ, and tissue homeostatic mechanisms.

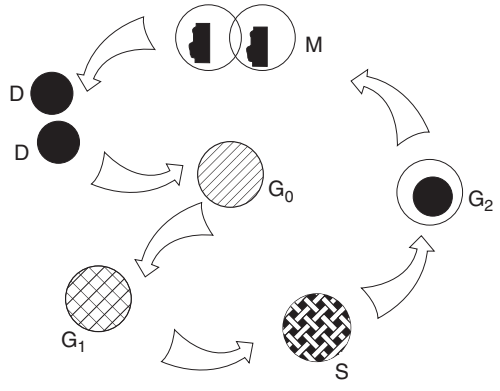

Figure 1 In adult organisms normally cells are in resting phase (G_0) of the cell division cycle. Upon appropriate stimulus the cells enter the division cycle which is characterized by G_1, S, G_2, and M phases. After division, the daughter cells (D) may either reenter the division cycle or enter the resting phase depending on the stimulus.

Genetic Control of Cell Structure and Function during and after Embryonic Development

Almost every multicellular animal is a clone of cells descended from a single original cell, the fertilized egg. Thus, the cells in the body, as a rule, are genetically alike. However, phenotypically they are different; some are specialized as muscles, others as neurons, others as hepatocytes, and so on. The different cell types are arranged in a precisely organized pattern, and the whole structure has a well-defined shape. All these features are ultimately determined by the DNA sequence of the genome, which is reproduced in every cell. Each cell must act according to the same genetic instructions, but it must interpret them with due regard to time and circumstance so as to play a proper part in multicellular organization. The development of vertebrates can be divided into three phases. In the first phase, the fertilized egg cleaves to form many smaller cells and these become organized into an epithelium and perform a complex series of gastrulation and neurulation movements, whose outcome is creation of a rudimentary gut cavity and a neural tube. In the second or organogenesis phase, the various organs, such as limbs, eyes, heart, and so on, are formed. In the third phase, the generated structures go on to grow to their adult size. These phases are not sharply distinct but overlap considerably in time.

Terminal Differentiation and Cell Division

After the embryonic development, cells in the normal adult human body divide at very different rates. Some, such as neurons and skeletal muscle cells, do not divide at all; others, such as liver cells, normally divide once every year or two; and certain epithelial cells in the gut divide more than twice a day so as to provide constant renewal of the gut lining. Most cells in the vertebrates fall somewhere between these extremes; they can divide but normally do so infrequently. Almost all the variation lies in the time cells spend between mitosis and the S phase, with slowly dividing cells remaining arrested after mitosis for weeks or even years. By contrast, the time taken for a cell to progress from the beginning of S phase through mitosis is brief (typically 12–24 h in mammals) and remarkably constant, irrespective of the interval from one division to the next. The time cells spend in a nonproliferative, so-called G_0 state varies not only according to the cell type but also according to the circumstances. Sex hormones stimulate the cells in the wall of the human uterus to divide rapidly for a few days in each menstrual cycle to replace the tissue lost by menstruation; blood loss stimulates proliferation of blood cell precursors; and acute liver damage provokes surviving liver cells to proliferate with a cycle time of only a day or two. Similarly, epithelial cells in the neighborhood of a wound are stimulated to divide so as to repair the injured epithelium. Delicately adjusted and highly specific controls exist to govern the proliferation of each class of cells in the body according to the need.

Role of Growth Factors and Cytokines in Cell Division

When put in an artificial culture medium completely devoid of serum, vertebrate cells will not normally pass the restriction point, even though all the requisite nutrients are present in the medium, and they will halt their growth as well as their progress through the chromosome cycle. Essential components of serum are some highly specific proteins (growth factors and cytokines), usually present in very small concentrations (in the order of 10^{-9}–10^{-11} mol l^{-1}). Different cells require different sets of these proteins. Some of these proteins are involved directly in stimulating cell division and are called complete mitogens. Some can directly inhibit cell cycle progression and thus can control cell division in the body. These are called growth inhibitors. Some of the proteins can cause cell cycle progression in an indirect way and are called growth triggers. **Table 1** provides examples of some of the growth factors and cytokines with their functions.

Cell Senescence and Reluctance to Divide

Most normal cells in the body of a mammal show a striking reluctance to continue proliferating forever. Fibroblasts taken from a normal human fetus, for example, will go through about 50 population doublings when cultured in a standard growth medium; toward the end of this time, proliferation slows down and finally stops, and the cells, after spending some time in quiescent state, die. Similar cells taken from a 40-year-old stop dividing after $\sim$40 doublings, while cells from an 80-year-old stop after $\sim$30 doublings. Fibroblasts from animals with shorter life span stop after a smaller number of division cycles in culture. Because of the correspondence with aging of the body as a whole, this phenomenon is called cell senescence. According to one theory, cell senescence is the result of a catastrophic accumulation of self-propagating errors in a cell's biosynthetic machinery that is unimportant under the conditions of life in the

Table 1 Example of growth factors and cytokines known to regulate cell proliferation

Factor	*Representative functions*
Platelet-derived growth factor (PDGF)	Stimulates proliferation of connective tissue cells and neuroglial cells
Epidermal growth factor (EGF)	Stimulates proliferation of many cell types
Insulinlike growth factors I and II (IGF-I and -II)	Work with PDGF and EGF to stimulate fat cell proliferation
Fibroblast growth factor (FGF)	Stimulates proliferation of many cell types including fibroblasts, endothelial cells, and myoblasts
Interleukin-2 (IL-2)	Stimulates proliferation of T lymphocytes
Transforming growth factor β (TGF-β)	Inhibits cell cycle progression of different cell types
Interleukin-1 (IL-1)	Inhibits proliferation of hepatocytes and other cell types
Hepatocyte proliferation inhibitor	Inhibits hepatocyte proliferation
Nerve growth factor (NGF)	Promotes axon growth and survival of sympathetic and some sensory and CNS neurons
Hematopoietic cell growth factors (IL-3, GM-CSF, M-CSF, G-CSF, and erythropoietin)	Promote division of different blood cells and various other types of cells

wild where most animals die from other causes long before a significant number of cells become senescent. An alternative theory is that the cell senescence is the result of a mechanism that has evolved to protect us from cancer by limiting the growth of tumors.

Cell Proliferation as a Compensatory Response to Toxic Tissue Injury

Human beings are exposed to numerous toxic insults every day. The body has several lines of defense mechanisms to combat the toxicants. Some of the toxicants are filtered out by virtue of their particle size, even before they can enter the body. Toxicants that enter the body can be metabolized and/or conjugated to be excreted out of the body. When these first lines of defense mechanisms are overcome, then the toxic substances cause cell death in the body. The site of cell death depends on the site of action of the toxicant. At this point the tissue can respond by stimulating its healthy cells to divide and to restore tissue structure and function. In response to cell death because of toxic insult, cells in the affected tissues (with the exception of neurons) start dividing in order to replace the dead or dying cells. One surviving cell can go through several cell cycles depending on the severity of the damage. The cell division stops at the precise point when all the dead cells have been replaced with new cells. At high doses of toxicants, the ability of the cells to go through the cell cycle is sometimes inhibited. This leads to two consequences. First, the dead cells are not replaced and failed cell division means loss of the organ and sometimes death. Second, in the absence of compensatory cell division, injury to the tissue can progress in an unrestrained manner. The ability of the cells to go through the cell cycle as needed decreases with age. This is why an 80-year-old can be more susceptible to the same dose of a toxicant as a 40-year-old.

Tissues vary in their compensatory responses to toxic chemicals. Skin, intestine, liver, and kidney are examples of tissues that can respond to toxic injury by stimulating cells to replace the lost tissue. Epithelial cells lining the criptae of the intestines are known to renew themselves every 72 h. Since these cells are subject to many foreign chemicals in the diet, many cells might be expected to be injured or affected in other ways. Therefore, these cells are completely renewed every 3 days. Although not as rapid, skin injury can result in replacement of injured skin rather promptly. The adult liver is normally a quiescent tissue with only an occasional cell dividing to replace dying cells to retain normal tissue homeostasis. Upon injury, however, the liver will respond promptly by stimulating its cells to divide and thereby restoring the lost tissue and function. Surgical removal of portions of the liver leads to restoration of the original liver mass through a very rapid cell proliferation and tissue repair response. Similarly, the kidney is also able to replace its cells upon toxic injury.

Stem Cells and Terminally Differentiated Cells

There are cell populations in the body that are renewed simply by duplication and then there are those that are renewed by means of stem cells. The defining properties of stem cells are (1) they themselves are not terminally differentiated cells – that is, they are not at the end of the pathway of differentiation; (2) they can divide without limit throughout the lifetime of the organism; and (3) when they divide, each daughter cell can either remain as a stem cell or it can take the path leading irreversibly to terminal differentiation.

Stem cells are required wherever there is a recurring need to replace differentiated cells that cannot themselves divide. There may be several reasons why a cell is terminally differentiated. The cell nucleus is digested, as in the outermost layers of the skin, or is extruded, as in the mammalian red blood cells. Alternatively, the cytoplasm may be heavily encumbered with structures, as in myofibrils of the striated muscle cells, which would hinder cell duplication. In other terminally differentiated cells the chemistry of differentiation may be incompatible with cell division. In any case, renewal must depend on stem cells.

The job of the stem cell is not to carry out the differentiated function but rather to produce the cells that will. Those stem cells that give rise to only one type of differentiated cells are called unipotent, those that give rise to a small number of cell types are called oligopotent, and those that give rise to many cell types are called pluripotent.

Cell Proliferation and Cancer

In multicellular organisms there are genes called social control genes, which are involved specifically in the social controls of cell division. A cell that undergoes a mutation or a set of mutations that disrupt the social restraint on cell division will divide without regard to the needs of the organism as a whole, and its progeny will become apparent as a tumor. Cancers, by definition, are malignant tumors; that is, the tumor cells not only divide in an ill-controlled way but also invade and colonize other tissues of the body to create widespread secondary tumors or

metastases. Approximately 10^{16} cell divisions take place in a human body in the course of a lifetime. Even in an environment that is free of mutagens, mutations occur spontaneously at an estimated rate of about 10^{-6} mutations per gene per cell division – a value set by fundamental limitations on the accuracy of DNA replication and repair. Thus, in a lifetime, every single gene is likely to have undergone mutations on about 10^{10} separate occasions in any individual human being. Among the resulting mutant cells, one might expect that there would be many that have disturbances in genes involved in the regulation of cell division and consequently disobey the normal restrictions on cell proliferation. To generate cancer, a cell must undergo a number of mutations occurring together to escape the multiple controls on cell division and then accumulate further changes to become endowed with the capacity for invasion and metastasis. From statistics it has been estimated that somewhere between three and seven independent random events, each of low probability, are typically required to turn a normal cell into a cancer cell; the smaller numbers apply to leukemia and the larger to carcinomas.

A protooncogene is a normal social control gene which can undergo mutation to become an oncogene. Oncogenes and protooncogenes contain DNA sequences that are closely similar but not identical. The mutations in the protooncogenes can result from spontaneous mutations or in response to chemical carcinogens or exposure to radiations. To date, more than 50 protooncogenes have been identified. It is likely, however, that many more social control genes remain to be discovered. Genes that stimulate cell division can be identified readily with current techniques, but there are several genes that have inhibitory effects on cell proliferation and recessive mutations in them are a common cause of cell transformation and cancer. The protooncogenes, code for various growth factors, growth factor receptors, and various intracellular mediators are involved in signaling cells to divide.

Importance of Understanding the Mechanisms in Control of Cell Division

Understanding of the mechanisms in control of cell division offers promising opportunities for developing new avenues for therapeutic intervention, with the aim of restoring and boosting tissue repair mechanisms in cases in which tissues have been damaged or lost as seen in burn wounds, trauma cases, in cases of drug overdoses, chemical poisoning, etc. The current armamentarium of clinical treatments for patients with drug overdoses or chemical poisoning aims to prevent additional injury either by blocking further formation of toxic metabolites or by increasing clearance out of the body. While this is important in preventing further damage to the affected tissue, or damage to unaffected tissue, survival depends heavily on the remaining cells in the tissue to proliferate to replace the dead or dying cells; this, in turn, depends on how soon after the injury the patient commences active treatment. In cases in which either there is a delay in treating the patient or the initial injury itself was massive, death or loss of the organ usually occurs because the damage compromises the regenerating ability of the cells, thereby paving the way for unrestrained progression of damage. If cellular regeneration could be 'actively' stimulated, even after the massive damage, by some therapeutically compatible mechanism, then it might be possible to prevent death or loss of organ. For example, animal experiments have shown that even after massive liver injury, liver failure and animal death can be obviated by stimulating tissue repair in the liver. The importance of cell division in tissue repair and recovery from injury is evident in experiments in which liver failure and animal death are observed in animals receiving an ordinarily nonlethal dose of a toxic chemical, if cell division is blocked by antimitotic agents. Perhaps carefully induced suppression of growth factors, cytokines, and protooncogenes involved in cell death or overexpression of those factors needed for cell division could stop the progression of injury and could restore the organ structure and functions. With the advent of gene therapy, specific genes could, one day, be delivered directly to the organ to induce expression/suppression of any of the factors implicated in fast recovery.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogenesis; Liver; Molecular Toxicology–Recombinant DNA Technology; Tissue Repair.

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Centipedes

Elizabeth J Scharman

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- SYNONYMS: Arthropoda (phylum); Chilopoda (class); Scutigeromorpha, Lithobiomorpha, Geophiulomorpha, and Scolopendromorpha (four orders)

Background Information

Over 2800 species of centipedes of various sizes and colors are found throughout the world in tropical and subtropical locations. The majority of species are 2.5–5.5 cm; tropical species may grow to 25 cm or longer. Centipedes are recognized by their long, multisegmented, flattened body. The body is composed of 15–181 segments, each of which (except the last one) has a pair of legs; the number of segments is always an odd number. Eyes may be simple, complex, or absent. The cranial segment bears multijointed antennae, three pairs of mouth parts, and a modified pair of legs, forcipules, which act as fangs.

Exposure Routes and Pathways

A bite is the usual route of envenomation from the centipede. The hand is a common bite site. There is one case reported in the literature of accidental ingestion.

Mechanism of Toxicity

The venom gland is found at the base of the forcipules. Venom passes through the ducts of the forcipules and is injected into the bite site. The components of centipede venom have not been completely identified. Known components include 5-hydroxytryptamine, histamine, lipids, polysaccharides, and enzymes including proteinases and esterases. Toxin S, which is a cardiotoxic protein, has been isolated from the species *Scolopendra subspinipes*. Cytolysin is found in the North American giant centipede, *Scolopendra heros*.

Acute and Short-Term Toxicity (or Exposure)

Human

Bites may result in localized burning pain, edema, erythema, and paresthesia. Superficial necrosis may occur in 1–2 h. The site may bleed. Swelling may persist for hours to days and may wax and wane during that time. Bullae near the bite site, and rashes at the bite site or other parts of the body, may also appear. Bites from tropical species may result in lymphangitis and lymphadenopathy; these effects are less common with other species. In the one reported case of ingestion, the 6-month-old child developed pallor, hypotonia, vomiting, and lethargy with full recovery.

Envenomation producing death has not been reported in the United States. In the Philippines, a 7-year-old girl is reported to have died following the bite from *Scolopendra subspinipes*, a tropical centipede.

Clinical Management

Treatment is symptomatic and supportive. The wound should be cleaned with soap and water; tetanus prophylaxis should be administered. Application of ice packs or the topical application of a corticosteroid, antihistamine, or local anesthetics may be useful in relieving symptoms. Severe pain has been treated with injection of a local anesthetic. Antibiotics are reserved for documented infections.

See also: Animals, Poisonous and Venomous.

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Cephalosporins

Shayne C Gad

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- Chemical/Pharmaceutical/Other Class: β-Lactam antibiotics (as are penicillins)
- Example Compounds: Cefaclor; Cefadroxil; Cefamandole; Cefazolin; Cefepidime; Cefoperazone; Cefotaxime; Cefoxitin; Ceftriaxone; Cephalexin; Cephalothin; Cephradine; Cephaprin; Cefmetazole; Cefonicid; Ceforanide; Cefotetan; Cefprozil; Loracarbef; Cefperazone; Cefpodoxime; Cefixime; Ceftazidime; Ceftizoxime; Moxalactarn

Uses

Cephalosporins induce their antimicrobial effect by inhibiting the integration of bacterial peptidoglycan. Individual peptidoglycan units are synthesized in the cytoplasm of the bacterial cell and are transported across the cytoplasmic membrane where they are inserted by peptidase enzymes into a crosslinked lattice that forms the structural support of the bacterial cell wall. The peptidase enzymes present in the outer cytoplasmic membrane are referred to as penicillin-binding proteins and represent the target sites for antibacterial action of cephalosporins and other β-lactam antibiotics. Cephalosporins are active *in vitro* against many gram-positive aerobic bacteria and some gram-negative aerobic bacteria. There are substantial differences among the cephalosporins in their spectra of activity as well as levels of activity against susceptible bacteria. Later-generation cephalosporins also are used in the later stages of livestock raising for adding weight.

Background Information

The cephalosporins have sustained their position as a very significant class of antibiotics worldwide for many years, comprising over one-half of the available β-lactam antibiotics. Initially, cephalosporin compounds produced by the fungal organism *Cephalosporium acremonium* were isolated in the early 1940s from fungus in sewage seawater in Calgiari, Sardinia, after it was observed that a natural pattern of periodic clearing of microbes was taking place from a local harbor area. Filtrates from *C. acremonium* cultures were found to have antimicrobial activity against infections in animals and humans, including the injection of filtrates into 'boils' and other cutaneous infections. The initial work later expanded to result in the discovery of cephalosporin C, the structural nucleus for cephalosporin compound development over the next four decades. Ongoing research and development have led to several additional cephalosporin antibiotics that have been released since the 1960s, with 24 unique, yet structurally similar compounds currently available for clinical use in the United States. The first three generations of cephalosporin antibiotics include both parenterally administered (i.e., intravenously or injection) and orally administered agents. The fourth-generation compound, cefepidime, is available for parenteral administration.

Exposure Routes and Pathways

The routes of exposure to cephalosporins are commonly oral, intravenous, or intramuscular. Accidental ingestion of oral dosage forms by children is the most common poisoning exposure.

Toxicokinetics

Cephalosporins are generally well absorbed following administration with bioavailability being greater than 75%. Many of these compounds are not stable in the acid environment of the stomach; therefore, only a limited number are useful for oral administration. The distribution is limited to the extracellular fluid space, with volumes of distribution for most ranging from 0.25 to 0.5 l kg^{-1}. Protein binding is primarily to albumin. Most cephalosporins are widely distributed to tissues and fluids, including pleural fluid, synovial fluid, and bone. Some of the third-generation compounds have good distribution to the cerebrospinal fluid. The metabolites possess antibacterial activity. The cephalosporins and their metabolites are rapidly excreted by the kidneys by glomerular filtration and/or tubular secretion. Serum half-lives of these compounds range from 0.4 to 10.9 h. Patients with immature renal systems or with renal compromise are at risk for toxicity due to decreased elimination. Cefamandole, cefmetazole, cefmenoxime, cefoperazone, and moxalactam have been associated with coagulopathies due to inhibition of platelet aggregation and prolongation of bleeding time.

Mechanism of Toxicity

Hematologic when given in higher doses, cephalosporins bind to cell membrane proteins and

act as haptens. High-dose treatments can induce a hemolytic anemia. They can also prolong bleeding times by reducing platelet adhesion and activation.

Many cephalosporins are substrates for the organic anion transport system in the proximal tubules and can accumulate in the kidney, competing for and inhibiting the transport system leading to renal necrosis. There is great variability in the potential for renal toxicity among different members of the family of compounds. Renal toxic members deplete glutathione levels in the renal cortex. There is evidence that this nephrotoxicity is due to action of the drugs on mitochondria.

Chronic Toxicity (or Exposure)

Animal

The extent of renal accumulation and effect is species dependent (rabbit > guinea pig > rat). There is also great variability in toxicity between different compounds.

Examples of the toxicity profiles of cephalosporins include:

- *Cefotan*: Has adverse effects on the testes of prepubertal rats. Subcutaneous administration of $500\,mg\,kg^{-1}\,day^{-1}$ ($\sim$8–16 times the usual adult human dose) on days 6–35 of life (thought to be developmentally analogous to late-childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence of severity of lesions was dose dependent; at $120\,mg\,kg^{-1}\,day^{-1}$ ($\sim$2–4 times the usual human dose) only one of 10 treated animals was affected, and the degree of degeneration was mild. Similar lesions were observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to $1000\,mg\,kg^{-1}\,day^{-1}$ subcutaneously for 5 weeks, or in infant dogs (3 weeks old) that received up to $300\,mg\,kg^{-1}\,day^{-1}$ intravenously for 5 weeks. *Pregnancy category B*: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan.
- *Cefixine*: Lifetime studies in animals to evaluate carcinogenic potential have not been conducted. SUPRAX did not cause point mutations in bacteria or mammalian cells, DNA damage, or chromosome damage *in vitro* and did not exhibit clastogenic potential *in vivo* in the mouse micronucleus test. In rats, fertility and reproductive performance were not affected by cefixine at doses up to 125 times the adult therapeutic dose. *Pregnancy category B*: Reproduction studies have been performed in mice and rats at doses up to 400 times the human dose and have revealed no evidence of harm to the fetus due to cefixine.
- *Cefoperazone*: Long-term studies in animals have not been performed to evaluate carcinogenic potential. The maximum duration of cefoperazone animal toxicity studies is 6 months. In none of the *in vivo* or *in vitro* genetic toxicology studies did cefoperazone show any mutagenic potential at either the chromosomal or subchromosomal level. Cefoperazone produced no impairment of fertility and had no effects on general reproductive performance or fetal development when administered subcutaneously at daily doses up to 500–$1000\,mg\,kg^{-1}$ prior to and during mating, and to pregnant female rats during gestation. These doses are 10–20 times the estimated usual single clinical dose. Cefoperazone had adverse effects on the testes of prepubertal rats at all doses tested. Subcutaneous administration of $1000\,mg\,kg^{-1}\,day^{-1}$ ($\sim$16 times the average adult human dose) resulted in reduced testicular weight, arrested spermatogenesis, reduced germinal cell population, and vacuolation of Sertoli cell cytoplasm. The severity of lesions was dose dependent in the 100–$1000\,mg\,kg^{-1}\,day^{-1}$ range; the low dose caused a minor decrease in spermatocytes. This effect has not been observed in adult rats. Historically, the lesions were reversible at all but the highest dosage levels. However, these studies did not evaluate subsequent development of reproductive function in the rats. *Pregnancy category B*: Reproduction studies have been performed in mice, rats, and monkeys at doses up to 10 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefoperazone.

Human

Like penicillins, cephalosporins are a relatively nontoxic group of antibiotics. The primary adverse effect reported is hypersensitivity, a rare event. Cross-allergenicity with penicillins may occur. Toxicity is unlikely in children less than 6 years of age who acutely ingest less than $250\,mg\,kg^{-1}$. Nephrotoxicity is a possible, but rare, occurrence with acute ingestion. Coagulopathies have been reported following chronic intravenous use of certain cephalosporins. At higher

concentrations, cephalosporins cause renal tubular injury, characterized by decreased glomerular filtration rate, glucosuria, enzymuria, and proteinuria.

Clinical Management

If a toxic or unknown amount of a cephalosporin has been ingested, gastric decontamination and the administration of activated charcoal is usually all that is needed. In the symptomatic patient, evaluation of renal function and electrolytes may be necessary. Chronic exposure usually requires discontinuation of the drug and supportive care. Anaphylaxis should be treated with epinephrine and/or diphenhydramine.

See also: Hemocompatibility; Kidney.

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Cerium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-45-1
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Ce^{3+}; Ce^{4+}

Uses

Although cerium is a rare earth element, it is relatively abundant in the earth's crust. Among the lanthanides, it is the most abundant. It is one of the 78 common elements in the earth's crust, and ranks 25th in occurrence at an average distribution of 20–60 ppm. Cerium is used in metallurgy as a stabilizer in alloys and in welding electrodes; in glass as a polishing agent, decolorizer, and to render glass opaque to near-ultraviolet radiation. It is also used in ceramics and as catalyst. Cerium is used as a component of some diesel fuel additives, and may be added to residual fuel oils to improve combustion. Cerium is found in portable rechargeable batteries.

Background Information

Cerium is a rare earth metal and the most abundant member of the lanthanide series discovered in 1803. It is the only material known to have a solid-state critical point.

Exposure Routes and Pathways

Inhalation, dermal, and oral are the possible exposure routes.

Toxicokinetics

Cerium is poorly absorbed by the intestine.

Mechanism of Toxicity

Cerium resembles aluminum in its biologic and chemical properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} values reported in rats ranged from 4 to 50 mg kg^{-1} for cerium nitrate with female rats being more sensitive than males. After peritoneal injection, the LD_{50} of cerium nitrate was 470 mg kg^{-1} in female mice and 290 mg kg^{-1} in female rats; the LD_{50} of cerium chloride was 353 mg kg^{-1} in mice and 103 mg kg^{-1} in guinea pigs. The oral toxicity of cerium nitrate was much lower (LD_{50} of 4200 mg kg^{-1} in female rats and 1178 mg kg^{-1} in female mice) than after intravenous or intraperitoneal administration. The LD_{50} of ingested cerium oxide could not be determined in rats when delivered at a dose of 1000 or 5000 mg kg^{-1}. An LD_{50} of 622 mg kg^{-1} has been reported for cerium oxide ingested by mice. The LC_{50} after inhalation of cerium oxide in rats was greater than 50 mg m^{-3}. The primary targets after

inhalation of cerium are the lung and the associated lymph nodes; other organs could be affected via clearance through the blood. Studies of cerium injected systemically have shown that, once in the circulation, cerium can cause liver toxicity with a no-observed-adverse-effect level of $1\,mg\,kg^{-1}$ after a single intravenous injection and a lowest-observed-adverse-effect level (LOAEL) of $2\,mg\,kg^{-1}$ for effects on liver detoxifying enzymes. Effects on other organs where cerium can accumulate (such as spleen, bones, and kidney) have not been studied. A single-dose study on the effects of *in utero* intravenous administration reported reduced weight in newborn mouse pups, with an LOAEL of $80\,mg\,kg^{-1}$. Cerium has been found to depress certain behaviors in mice administered this chemical, and cerium administered to pregnant mice on day 7 or 12 of gestation or 2 days postpartum caused significant decreases in open field activity of offspring. Fetal growth was impaired, as evidenced by weight decreases of 7–19%. The potential carcinogenicity of cerium-containing particles has not been studied in conventional rodent bioassays; *in vivo* mutagenicity studies have been negative.

Human

Cerium can increase blood coagulation rate and produce gastrointestinal effects. Inhalation can lead to polycythemia.

Chronic Toxicity (or Exposure)

Animal

An animal inhalation study involved exposure of rats to cerium oxide particles substantially larger than those in diesel emission. The exposure concentrations ranged between 5 and $500\,mg\,m^{-3}$ for 13 weeks. Effects observed included lung discoloration, enlargement of lymph nodes, and increased lung and spleen weight at all concentrations.

Human

Case reports of workers occupationally exposed to rare earth metals (including cerium) describe a condition termed rare earth pneumoconiosis with pathologic features including interstitial fibrosis, granulomatosis, and bilateral nodular chest X-ray infiltrates. Although the disease sometimes is associated with accumulation of cerium in particles, the role of cerium in this complex disease is unclear relative to other metals or gases to which workers may also have been exposed.

In Vitro Toxicity Data

In vitro mutagenicity studies have been negative.

See also: Aluminum; Metals.

Further Reading

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Relevant Website

http://www.healtheffects.org – Evaluation of Human Health Risk from Cerium Added to Diesel Fuel. Health Effects Institute Communication 9 (August 2001).

Cesium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-46-2
- Synonym: Caesium
- Chemical/Pharmaceutical/Other Class: Alkali metals
- Chemical Formula: Cs^+

Uses

Cesium is used in photovoltaic cells, vacuum tubes, scintillation counters, and atomic clocks.

Background Information

Cesium was discovered in 1860 by Robert Bunsen and Gustaff Kirchoff. It is used in the most accurate atomic clocks. Cesium melts at 28.4°C (just below body temperature) and occurs in Earth's crust at 2.6 ppm.

Exposure Routes and Pathways

Inhalation and ingestion are the routes of exposure.

Toxicokinetics

The metabolism and tissue distribution of cesium-137 were studied in rats injected intraperitoneally and sacrificed 1–300 days postinjection. In a chronic study, rats were administered cesium-137 in their drinking water daily. In the acute study, with the exception of the brain, muscle, and total animal, all tissues showed retention curves resolvable into three exponential components with half-lives of 1.5–2, 5–8, and 15–17 days. Retention in muscles was resolvable into a two-exponential function with half-lives of 8 and 16 days. In the chronic study, the highest equilibrium cesium-137 concentrations, 10% of the average daily intake per gram, occurred in the muscle. The authors concluded that the muscle should be considered the formal critical organ for cesium-137.

Mechanism of Toxicity

Cesium displaces potassium.

Acute and Short-Term Toxicity (or Exposure)

Animal

Primary skin and eye irritation, cutaneous sensitization, and oral acute toxicity studies were conducted on the hydroxide and iodide of cesium. Evaluation of cesium hydroxide has produced somewhat conflicting results. In one study, cesium hydroxide was markedly more toxic than the iodide, but was irritating only to abraded skin. Cesium iodide did not affect the eye or skin. These results indicate that cesium is only slightly toxic acutely and would pose an acute health hazard only when ingested in large quantities. In another study, cesium showed a low or very low acute toxicity orally or intraperitoneally to rats, except for cesium hydroxide, which had an intraperitoneal LD_{50} of $89\,mg\,kg^{-1}$. In a review of cesium hydroxide, it was concluded from the only animal studies available that cesium hydroxide is extremely irritating and corrosive to the eyes and skin after acute exposure.

Human

Cesium has been reported to cause hyperirritability and muscle spasms. Human data for cesium hydroxide are unavailable.

Chronic Toxicity (or Exposure)

Exposure to the radioactive form, cesium-137, can result in an increased risk of cancer. No data on long-term exposure, genotoxicity, mutagenicity, carcinogenicity, and reproduction toxicity have been found for cesium hydroxide.

Clinical Management

Prussian blue is administered by a duodenal tube to act as a chelating agent.

See also: Lithium; Metals; Potassium; Sodium.

Further Reading

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Johnson GT, Lewis TR, and Wagner WD (1975) Acute toxicity of cesium and rubidium compounds. *Toxicology and Applied Pharmacology* 32: 239–245.

Relevant Website

http://www.epa.gov – Cesium. US Environmental Protection Agency.

Channel Blockers *See* Calcium Channel Blockers.

Charcoal

William S Utley

- Chemical Abstracts Service Registry Number: CAS 16291-96-6
- Synonyms: Carbon; Activated charcoal; Wood charcoal

Uses

Charcoal is produced by the incomplete combustion of plant or animal products. The major use of charcoal is for outdoor cooking. The second largest use of charcoal is in industrial applications in the form of activated charcoal. The activation process involves heating the charcoal subjecting it to steam or treating with a chemical to both remove substances that have adhered to it as well as break it down into finer particles and thus increase the surface area. Activated carbon has been used for its adsorptive properties as a 'universal antidote' in cases of poisonings, as a filter aid agent, and in decolorization processes.

Exposure Routes and Pathways

The primary route of exposure is via inhalation of fine dust and ingestion.

Toxicokinetics

Charcoal is not absorbed through the skin or the gastrointestinal tract. When ingested it is excreted in the feces. This is readily apparent from the black color of the feces.

Mechanism of Toxicity

Charcoal is not generally considered to be toxic. It is possible to overwhelm pulmonary defense mechanisms if excessive dust is inhaled over a long period of time. The ability of charcoal to adsorb vitamins and enzymes can lead to nutritional deficiencies if ingested on a chronic basis.

Acute and Short-Term Toxicity (or Exposure)

Human

No reported human toxicity. The absorptive properties of charcoal may interfere with the enterohepatic circulation of certain drugs if it is taken orally.

Clinical Management

If toxicity should manifest, general life support should be maintained. Symptoms should be treated and decontamination undertaken if necessary.

Relevant Website

http://science.howstuffworks.com – Howstuffworks: What is activated charcoal and why does it work in filters?

Chemical Hazard Communication and Material Safety Data Sheets

Michele R Sullivan and Patricia M Nance

Introduction

The production and use of chemicals is fundamental to all economies. Chemicals directly or indirectly affect our lives and are essential to our food (e.g., as fertilizers, pesticides, food additives, and components of packaging), our health (e.g., in pharmaceuticals and in cleaning materials), and our life style (e.g., in appliances, fuels). The omnipresence of chemicals has resulted in the development of sector-specific regulations (e.g., transport, workplace, agriculture, trade, and consumer products). Having information on the hazardous properties and control measures of chemicals available throughout their life cycle allows their production, transport, use, and disposal to be managed safely, thus protecting human health and the environment.

What is Chemical Hazard Communication?

The sound management of chemicals includes systems through which chemical hazards are communicated to workers, consumers, and the public. It is important to know what chemicals are present

and/or used, their hazards to human health and the environment, and the means to control them. A number of classification and labeling systems, each addressing specific use patterns and groups of chemicals, exist at the national, regional, and international levels. The existing hazard classification and labeling systems address potential exposure to chemicals in all types of use settings, including production, storage, transport, workplace use, consumer use, and presence in the environment.

The primary purpose of all existing classification and labeling systems is to provide information to people who are potentially 'exposed' to chemicals, in order to minimize the possibility of adverse effects resulting from that exposure. While the audiences (workers, consumers, etc.) vary with the system, the purpose remains the same.

A goal of hazard communication is to ensure that employers, employees, and the public are provided with information on the hazards of chemicals so that they can take effective preventive and protective measures for their health and safety. This is sometimes referred to as the 'right-to-know' principle.

The first step in the safe handling of chemicals is knowing their identity, their hazards to health and the environment, and the means to control them. This complex information on the hazards and corresponding protective measures needs to be conveyed in a manner that is easily understood. The information can be conveyed in a variety of ways.

Information in the form of labels, placards, posters, or markings provided on or with the container of the hazardous material is common to all the systems currently in existence. This information generally includes some indication of the hazard(s), in text form and/or symbols. In addition to the hazard information, the container information may also include statements regarding safe use or handling, or other types of precautionary measures.

More detailed information may also be provided to those exposed to hazardous chemicals. In the workplace, for example, material safety data sheets (MSDSs) may be available. In the transport sector, a document such as the *North American Emergency Response Guidebook* may supplement the information on placards or markings. The details on these information documents vary from system to system for the same chemical.

In most workplace and transport chemical hazard communication systems, training is also a component. In consumer settings, however, the container label is the only communication mechanism available to provide information on safe handling and use.

Classification of Chemicals: A Starting Point for Hazard Communication

Classification is the identification of the hazard(s) of a chemical or mixture by assigning a category of hazard/danger using defined criteria. Hazard classification generally involves the following steps:

1. Identification of relevant data for a substance or mixture.
2. Comparison of the data to hazard classification criteria to determine whether the product is hazardous and the degree or level of hazard.

Health and physical hazards, and sometimes environmental hazards, are included in all systems. Typical hazards include:

- *Physical hazards*: Flammable liquids, solids, and gases, flammable aerosols, pyrophoric liquids and solids, self-heating substances, substances which in contact with water release flammable or toxic gases, oxidizing liquids, solids, and gases, organic peroxides, self-reactive substances, explosives, corrosive to metals, gases under pressure.
- *Health hazards:* Acute toxicity, skin irritation/corrosion, eye irritation/corrosion, respiratory or skin sensitization, mutagenicity, carcinogenicity, toxic to reproduction, target organ toxicity.
- *Environmental hazards:* Hazardous to the aquatic environment, hazardous to the terrestrial environment, hazardous to the ozone layer.

Chemical Hazard Communication Tools

Once a substance has been classified, the hazard(s) must be communicated to target audiences. The main tools of chemical hazard communication are 'labels' and MSDSs (sometimes called Safety Data Sheets (SDSs) and Material Data Sheets (MDSs)) that contain the hazard information. Their purpose is to identify the hazardous properties of chemicals that may constitute a health, property, or environmental risk during normal handling or use.

Labels

A label on a container of a product is designed to inform persons handling or using the chemical of its hazards. The label is the basic tool to keep the user informed of the hazards and the most important safety precautions. The label can be regarded as a snapshot of the chemical hazard(s) to be used as an alert for the worker who can get more detailed information from an MSDS or SDS, training, etc.

Many countries have developed hazard communication systems with their own standards for how

chemical information is to appear on a label. While the systems vary, the basic components of a label are similar. For example, labels commonly include the following information:

- product identifier/identities of the hazardous components,
- signal word,
- hazard statements,
- hazard symbols/pictograms,
- precautionary information, and
- supplier information.

Signal Words

Some systems require the use of signal words to give some indication of the severity of the hazards involved. Commonly used terms include 'danger', 'warning', and 'caution', depending on the type and severity of known or potential hazard.

Hazard Statements

Most existing systems require a statement of the hazards or effects of the chemicals. In some cases, all effects are required to be provided. In others, there is an established precedence of hazards and a limitation on what is provided.

Hazard Symbols

In addition to the written statements, several of the existing hazard communication systems use symbols to convey hazards. The United States (US) allows the use of symbols in the workplace, but does not require them. The placement and design of the symbols varies among systems. The European Union (EU) system places symbols in a square. The Canadian system requires the symbols to have a circle around them. And the United Nations (UN) transport system requires the symbols be placed in a diamond. These differences result in different labels even when the symbol itself is the same.

Precautionary Information

In addition to the signal words and statement of hazards, many labels are required to include other information that is precautionary in nature and provides more information on safe handling and use. The approach to these warnings and precautions varies among systems. In some systems, manufacturers have the discretion to determine what statements are necessary, and how they will be presented. In other systems, the regulatory authority has developed standard phrases and a decision logic to apply them to the label.

Colors

In addition to text and symbols, colors may be used to denote hazard or classification information. Under the EU system, an orange background is required for symbols. The transport system uses a variety of colors, such as a red background for the flammability symbol. The use of colors is intended to make the symbols stand out, or to more clearly delineate the hazard.

Label Format/Layout

There are a number of requirements in the existing systems that relate to size, appearance, and placement of the labels. For example, the Canadian Workplace Hazardous Materials Information System (WHMIS) system requires a border around the label that distinguishes it from labels of other systems.

The needs of the intended target audience influence what label components are used. In 'transport', for example, the label, placard, and transport documents are all used. In the 'workplace', the label is one element of a multicomponent system of chemical hazard communication, the other elements being the MSDS and training. In communicating the potential hazard of 'consumer products', the label plays the major role in providing the user with information about all the potential health, environmental, and physical hazards of the product and advice on using the product safely.

Material Safety Data Sheets (MSDS, or an SDS or MDS)

The supplier, manufacturer, or importer should be able to provide detailed information about the product on an MSDS (or an SDS or MDS). Chemicals that are used in the workplace are usually accompanied by an MSDS. Safety data sheet information can be found under several names, such as:

- chemical safety card,
- chemical info-sheet,
- SDS,
- MSDS,
- product safety data sheet, and
- health and safety data.

The MSDS provides comprehensive information about a chemical substance or mixture, including potential health, safety, and environmental hazards, and guidance on safe handling, use, and storage. Employers, workers, regulatory professionals, emergency personnel, and others use MSDSs as a source of information about hazards, advice on safety precautions, and regulatory information. The

information in an MSDS acts as a reference for the management of hazardous chemicals. The MSDS is product related and does not provide information for a specific workplace, although where products have specialized end-uses the MSDS information may be more specific.

An MSDS is usually composed of several sections, each containing a different kind of information. Each section is designed to provide useful information to users of the material and to individuals concerned with a variety of health, safety, and environmental issues. For example, the MSDS section on fire-fighting describes measures to be taken to extinguish fires involving the material. The toxicology section contains relevant toxicological information about the material.

In the United States, MSDSs originated in the shipbuilding industry using a format designated as the US Department of Labor, Occupational Safety & Health Administration (OSHA) Form 20. Chemical manufacturers expanded the original 2-page OSHA Form 20 in order to more adequately provide health and safety data on chemical products. Under the OSHA Hazard Communication Standard (29 Code of Federal Regulations (CFR) 1910.1200) issued in 1983, MSDS requirements are performance oriented and do not require a specific format. However, there is a nonmandatory OSHA Form 174.

Originally, MSDSs were intended to be used by health and safety professionals, workers, employers, and customers. In 1986, SARA or the Emergency Planning and Community Right-to-Know Act (EPCRA) expanded the use of MSDSs to fire departments, emergency responders, state and local emergency planning groups, and members of the community. In addition, a number of countries, regions, and international organizations developed guidance or requirements pertaining to MSDSs. Several of these are discussed below.

As result of these activities, MSDSs became diverse in overall format and content, making their use more complicated. In due course, the advantage of harmonizing the MSDS format to make information easier to find was recognized.

A 16-section MSDS format was developed in the 1990s and is common to many standards such as International Organization for Standardization (ISO) 11014-1, the European Union (EU) SDS, the International Labor Organization (ILO) standard under Chemicals Recommendation R177, and the American National Standards Institute (ANSI) Standard Z400.1. The initial 16-section MSDS sequence has been modified. According to the newly developed Globally Harmonized System (GHS) for the Classification and Labelling of Chemicals, information in the MSDS should be presented using the following 16 ordered headings:

1. Identification
2. Hazard(s) identification
3. Composition/information on ingredients
4. First-aid measures
5. Fire-fighting measures
6. Accidental release measures
7. Handling and storage
8. Exposure controls/personal protection
9. Physical and chemical properties
10. Stability and reactivity
11. Toxicological information
12. Ecological information
13. Disposal considerations
14. Transport information
15. Regulatory information
16. Other information

An example of a GHS 16-section MSDS for a fictional product is shown in the Appendix.

Chemical safety data sheets are prepared by various type organizations: chemical safety data sheets prepared by groups of experts and peer-reviewed; and chemical safety data sheets prepared by manufacturers or distributors. There are websites, such as the US National Institute for Occupational Health and Safety (NIOSH) that provide a listing of MSDS for various substances and mixtures. Peer-reviewed data sheets on chemical substances, the International Chemical Safety Cards (ICSCs) are available from the International Program on Chemical Safety (IPCS).

NJ RTK Hazardous Substance Fact Sheets are data sheets for over 1500 individual hazardous chemicals prepared under the New Jersey Right-To-Know Law. Information is available New Jersey State website.

Comprehensibility

The purpose of providing chemical hazard information is to effect a change in behavior causing the user to follow appropriate precautionary measures and avoid the occurrence of an adverse effect from handling or using the chemical. In order to bring about this behavior change, it is important that the information provided to the chemical user or handler is 'comprehensible'. Comprehensibility refers to the ability of the individual reading a label, warning, or safety data sheet to understand the information sufficiently to take the desired action.

Comprehensibility is different from readability because the latter is simply a measure of the educational level of the written material, while the former is a measure of how well the receiver of

the information understands it. A warning about incompatible chemicals may be written at the correct reading level for a specific target audience, for example, but may do such a poor job explaining the hazard that the warning is not understandable by most of the intended audience. Additionally, the same warning may be highly comprehensible to a population of chemical workers, but poorly understood by firefighters with the same educational level but different work experiences.

Training

In addition to labels and MSDS, appropriate 'training' for target audiences who are required to interpret label and/or MSDS information and take corresponding precautionary measures is a component of many hazard communication systems. Training is usually keyed to the nature of the work or exposure; and the target audiences that may include workers, emergency responders, those involved in label and MSDS preparation, and in the transport of hazardous chemicals.

Sectors/Audiences Involved in Chemical Hazard Communication

There are different sectors or target audiences that are users of chemical hazard information. The four primary sectors include industrial production, agriculture, consumers, and transport. Different target audiences receive and use the information conveyed about hazardous chemicals in different ways.

Industrial Production Sector

Workers at factories, storage facilities, construction sites, and at small- and medium-sized enterprises are potentially exposed to industrial chemical hazards. The elements common to workplace hazard communications systems include labels, MSDS/SDS, and training.

Agriculture Sector

Farmers and farm workers are potentially exposed to agricultural chemicals, such as pesticides and fertilizers. Labels are the primary source of information. Visual symbols and orally communicated information are particularly important in the agricultural setting. An MSDS may not be readily available or easily understood.

Consumer Sector

Consumers are exposed to a wide variety of chemicals in their daily lives, from bleaches and dyes, to flammable hair care products and pesticides used in gardens. Since consumers rely solely on label information, comprehensibility is of particular importance.

Transport Sector

The transport sector has long been a focus of international efforts on hazard communication. There is a wide range of target audiences, including transport workers and emergency responders, and also carriers and those who load and unload dangerous goods. Those involved in the transport sector need information concerning safe practices that are appropriate for all transport situations. Labels, placards, transport documents, and MSDSs are key tools.

Other Affected and Interested Sectors

There are others with an interest in chemical hazard communication. Emergency responders involved in responding to chemical emergencies such as spills, leaks, or explosions need hazard and safety information. Firefighters and those first at the scene of a transport accident also need information. Medical personnel responsible for treating victims require specialized information.

Chemical Classification and Hazard Communication Systems

As early as the 1950s, there was international work on the classification and labeling of chemicals. Initial efforts focused on the safe transport of dangerous goods. National and regional systems have more recently been created concerning chemical safety in the workplace. Several of these systems are discussed below. The principles for the various classification, labeling, and hazardous communication systems are related but there are differences among them.

Regional and National Examples

OSHA Hazard Communication Standard (29 CFR 1910.1200) The US OSHA's Hazard Communication Standard (HCS) ensures that information about chemical hazards and associated protective measures is provided to workers and employers. This is accomplished by requiring chemical manufacturers and importers to evaluate the hazards of the chemicals they produce or import, and to provide information through labels on shipped containers and MSDSs. Employers with hazardous chemicals in their workplaces must prepare and implement a written hazard communication program, and must ensure that containers are labeled, employees are provided access to MSDSs, and an effective training program is conducted for all potentially exposed employees. The HCS provides workers the 'right-to-know' the

hazards and identities of the chemicals they are exposed to in the workplace.

ANSI Z400.1: Hazardous Industrial Chemicals – Material Safety Data Sheets – Preparation The ANSI has adopted a voluntary consensus standard that gives guidance regarding preparation of MSDSs. The standard applies to the preparation of MSDS for chemicals and materials used under industrial occupational conditions. It presents basic information on how to develop and write a 16-section MSDS. It identifies information that must be included to comply with the performance oriented OSHA HCS.

Canada's Workplace Hazardous Materials Information System The WHMIS is Canada's hazard communication standard for the workplace. The key elements of the system are labeling of containers of WHMIS 'controlled products', MSDSs, and worker education programs. WHMIS is implemented through coordinated federal, provincial, and territorial legislations. Supplier labeling and MSDS requirements are set out under the Hazardous Products Act (HPA) and associated Controlled Products Regulations. The HPA and its regulations are administered by the Product Safety Bureau of the Government of Canada Department of Health, commonly referred to as Health Canada. Each of the 13 provincial, territorial, and federal agencies responsible for occupational safety and health has established employer WHMIS requirements within their respective jurisdiction. WHMIS includes a mechanism concerning disclosure of confidential business information.

Emergency Response Guidebook The *Emergency Response Guidebook* was developed jointly by Canada, the United States, and Mexico for use by fire fighters, police, and other emergency service personnel who may be the first to arrive at the scene of a transportation incident involving dangerous goods. It is primarily a guide to aid 'first responders' in quickly identifying the specific or generic hazards of the material(s) involved in the incident, and protecting themselves and the general public during the initial response phase.

EU Requirements for Classification and Labeling In keeping with Directive 67/548/EEC and its amendments, dangerous substances which are placed on the EU market have to be labeled according to their classification in the Annex I list, which contains ~2350 existing and 214 new substances. For dangerous substances not in the Annex I list, the manufacturer, distributor, and importer is obliged to apply a provisional classification and labeling following the criteria in Annex VI of the directive. Mixtures or preparations are also regulated by the EU (under 88/379/EEC, 99/45/EC, etc.), as well as SDSs (under 91/155/EC, 2001/58/EC, etc.).

EU Directives contain provisions on:

- Classification of dangerous substances and mixtures into categories characterizing the type and severity of the hazards.
- Packaging of dangerous substances and mixtures.
- Labeling of dangerous substances and mixtures with hazards and safety measures.
- SDSs for dangerous substances and mixtures.

International Agreements and Standards

'Earth Summit' – Agenda 21 At the 'Earth Summit' in 1992, governments agreed to language on the classification and labeling of chemicals in regards to the 'Sound Management of Chemicals':

- Adequate labeling of chemicals and the dissemination of safety data sheets such as ICSCs (International Chemical Safety Cards) and similarly written materials, based on assessed hazards to health and environment, are the simplest and most efficient way of indicating how to handle and use chemicals safely.
- For the safe transport of dangerous goods, including chemicals, a comprehensive scheme elaborated within the United Nations system is in current use. This scheme mainly takes into account the acute hazards of chemicals.

The mandate for the GHS originated at the 'Earth Summit':

- A globally harmonized hazard classification and compatible labeling system, including material safety data sheets and easily understandable symbols, should be available, if feasible, by the year 2000.

ILO Chemical Convention 170 and Recommendation 177 The purpose of the International Labor Organization (ILO) Convention 170 and Recommendation 177 concerning safety in the use of chemicals at work is to protect workers who use chemicals in their workplace. The Convention, which came into force in November 1993, covers hazard classification and communication of hazards, chemical identity, and precautions on labels. It also requires that chemical safety data sheets for hazardous chemicals be provided to employers. Chemical suppliers are responsible for ensuring that chemicals

have been classified, marked, and labeled and have chemical safety data sheets.

UN Recommendations on the Transport of Dangerous Goods The transport of dangerous goods is coordinated globally at the United Nations level by the 'Recommendations on the Transport of Dangerous Goods', also called the 'Orange Book'. The UN Recommendations on the transport of dangerous goods address the following main areas:

- Lists of commonly carried dangerous goods.
- Their classification.
- Labeling, marking, and transport documents.
- Packaging requirements including multimodal tank-containers.

The transport system is based mainly on physical and acute hazards. The elements of this system are widely adopted for the purpose of transporting dangerous goods by sea, air, road, rail, and inland waterways.

ISO 11014-1: International Standard for Safety Data Sheets In 1994, the International Organization for Standardization (ISO) developed a standard format for safety data sheets to create consistency in providing information on safety, health, and environmental matters for chemical products. In order to establish uniformity, certain requirements are provided as to how information on the chemical product shall be given (the titles and sequence of the headings and section content). The ISO SDS standard uses the 16-heading format.

Food and Agriculture Organization (FAO)

FAO International Code of Conduct on the Distribution and Use of Pesticides The 1985 International Code of Conduct, amended in 1989, was developed to address the use of pesticides in developing countries until the countries have established regulatory infrastructures for pesticides. The Code sets forth responsibilities and establishes voluntary standards of conduct for public and private entities engaged in or affecting the distribution and use of pesticides. The Code specifically addresses 'Labeling, packaging, storage and disposal' of pesticides.

FAO Guidelines on Good Labeling Practice for Pesticides The 1995 FAO *Guidelines on Good Labeling Practice for Pesticides* gives guidance on the preparation of labels and specific advice on content and layout. They are intended for use by industry and also by national pesticide regulatory authorities. The Guidelines contain: information that must appear on a label; comprehensibility considerations; pictograms for communicating safety information to users; and toxicity and hazard classifications for a product. The appendices contain examples of labels, hazard statements, agricultural practice statements, and other specific and generic label contents.

WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification

The World Health Organization (WHO) recommended classification of pesticides by hazard, first issued in 1978, has gained wide acceptance. The classification is based primarily on the acute oral and dermal toxicity to rats since these determinations are standard procedures in toxicology.

The Globally Harmonized System for the Classification and Labeling of Chemicals (GHS)

An important new tool to develop/harmonize chemical hazard communication systems is the UN Globally Harmonized System for the Classification and Labeling of Chemicals (GHS). While the existing chemical hazard communication systems are similar in intent, there are differences in their specific provisions. The GHS is an important tool to harmonize systems worldwide. As noted above, the need for a global system was endorsed internationally by the 1992 'Earth Summit'.

The harmonized elements of the GHS may be seen as a collection of 'building blocks' from which to form a regulatory approach for chemical hazard communication within the workplace, for the transportation system, for those involved in chemical work-related activities, and for consumers.

Overview

The GHS provides a comprehensive tool for chemical classification and hazard communication. It applies to all chemicals and mixtures of chemicals. The GHS includes the following elements:

- Harmonized criteria for classifying substances and mixtures according to their health, environmental, and physical hazards.
- Harmonized hazard communication elements, including requirements for labeling, symbols, and safety data sheets.

The GHS addresses the hazard communication elements common to the existing systems:

- Labeling: minimum data elements; hazard pictograms (symbols, colors, frames); comprehensibility;

signal words, hazard statements and precautionary measures.
- Chemical safety data sheets (MSDS/SDS): format; minimum data elements.
- Principles for hazard communication training.

The technical work on developing the GHS was completed in 2001. The technical GHS Document (the 'Purple Book') gives the classification criteria and the hazard communication elements, as well as examples of labels and classification of chemicals to illustrate how to apply the criteria. The UN Sub-Committee on the Globally Harmonized System (SCEGHS) will maintain update, and promote the technical GHS Document as well as manage implementation issues. More information about the GHS and the GHS Document is available at the Relevant Websites section.

Appendix

MSDS Example

1. Identification

Name of the product: Sticky Stuff
Recommended use: General adhesive

Producer:	GHS Ltd., UK London, SE, Southwarkbridge 1 Telephone no. +44-171717-555-555-5 Emergency no. +44-171717-333-333-3

2. Hazard(s) Identification

Classification:	Flammable liquid, Category 2 Eye irritation, Category 2A Hazardous to the aquatic environment; Acute Category 3
Labeling:	
Symbol:	Flame, Exclamation mark
Signal word:	Danger
Hazard statement:	Highly flammable liquid and vapor Causes severe eye irritation Harmful to aquatic life
Precautionary statement:	Keep away from heat, spark, or flame Do not smoke Wear safety glasses Wear impervious gloves Do not breathe fumes Use only with adequate ventilation

3. Composition/Information on Ingredients

Chemical identity: Component A 70–80%
Common name: Solvent A
Number of identity: CAS No.: 111111-11-1
Impurities: None
Chemical identity: Component C 20–25%
Common name: Not applicable
Number of Identity: CAS No.: 44444-44-4
Impurities: none

4. First-Aid Measures

Inhalation: Remove person to fresh air. If respiratory irritation, dizziness, nausea, or unconsciousness occurs, seek immediate medical assistance. If breathing has stopped, give artificial respiration.

Skin contact: Wash the contaminated area with soap and water. Remove contaminated clothing and wash before reuse. If irritation develops get medical attention.

Eye contact: Hold eyelids apart and flush eyes with plenty of water for at least 15 min. Get medical attention.

Ingestion: If swallowed, do NOT induce vomiting. Seek immediate medical attention.

Note to Physicians: Material if ingested may be aspirated into the lungs and can cause chemical pneumonitis. Treat appropriately.

5. Fire-Fighting Measures

Suitable extinguishing media: Foam, extinguishing powder, carbon dioxide, water fog. In case of fire, cool endangered containers with water fog.

Unsuitable extinguishing media: High-pressure water jet.

Specific hazards in case of fire: None are known.

Special protective equipment and precaution for fire fighters: For fires in enclosed areas, wear self-contained breathing apparatus. Do not inhale combustion gases.

6. Accidental Release Measures

Personal precautions: Depending on extent of release consider the need for fire-fighters/emergency responders with adequate personal protective equipment for cleaning-up.

Do not eat, drink, or smoke while cleaning up. Use a self-contained respirator, a mask with filter (type A class 3) or a filtering mask (e.g., EN 405). Wear protective clothing, safety glasses, and impervious gloves (e.g., neoprene gloves). Ensure adequate ventilation. Avoid all sources of ignition, hot surfaces, and open flames (see also Section 7).

Environmental precautions: Prevent spills from entering storm sewers or drains and contact with soil.

Methods and materials for containment and cleaning up: Eliminate all ignition sources. Runoff may create fire or explosion hazard in sewer system. Absorb on fire retardant liquid-absorbing material (treated sawdust, diatomaceous earth, sand). Shovel up and dispose of at an appropriate waste disposal facility in accordance with current applicable laws and regulations, and product characteristics at time of disposal (see also Section 13).

7. Handling and Storage

Precautions for safe handling: Avoid contact with eyes. Avoid prolonged repeated skin contact and breathing mists/vapours.

Use in well ventilated area away from all ignition sources. Switch off all electrical devices such as parabolic heaters, hot plates, storage heaters, etc., in good time for them to have cooled down before commencing work. Do not smoke, do not weld. Do not empty waste into sanitary drains. Take measures to prevent the build up of electrostatic charge.

Conditions for safe storage, including incompatibilities: Storage containers must be grounded and bonded. Store away from all ignition sources in a cool area equipped with an automatic sprinkling system. Ensure adequate ventilation. Store at temperatures between $+5^{\circ}C$ and $+50^{\circ}C$. Store only in the original container.

8. Exposure Controls/Personal Protection

Information on the system design: Draw off vapours directly at the point of generation and exhaust from the work area. In the case of regular work, provide bench-mounted extraction equipment.

Exposure Limits:

Component Name (CAS No.)	*Reference*	*TWA*		*STEL*	
		Ppm	*mg m^{-3}*	*ppm*	*mg m^{-3}*
Component A (111111-11-1)	ACGIH	500	1200	–	–
	UK OEL	500	1200	–	–
Component C (4444-44-4)	German MAK	200	950	–	–

Ventilation: Use in well ventilated area with local exhaust.

Respiratory protection: Approved respiratory equipment must be used when airborne concentrations are unknown or exceed the exposure limits. When processing large amounts use a light duty construction compressed air line breathing apparatus (e.g., in accordance with EN1835), a mask with filter (type A class 3, colour brown), or a filtering half mask (e.g., in accordance with EN 405) when there is inadequate ventilation.

Eye protection: Safety glasses with side shields or chemical goggles must be worn.

Skin protection: If prolonged or repeated skin contact is likely, neoprene gloves should be worn. Good personal hygiene practices should always be followed.

9. Physical and Chemical Properties

Physical state	Liquid
Color	Colourless, transparent
Odor	Solvent, ester-like
Odor threshold	Not available
PH value	Not applicable
Melting point	Not available
Freezing point	Not available
Initial boiling point	56°C
Flash point	− 22°C DIN 51755
Evaporation rate	Not available
Flammability (solid, gas)	Not applicable
Explosion limits	Lower limit = 1.4 vol.%; upper limit = 13.0 vol.% (literature)
Vapour pressure	240 mbar (highest partial vapour pressure) at 20°C
Vapour density	Not available
Relative density	0.89 g cm^{-3} at 20°C
Solubility	Partially soluble in water at 20°C
Partition coefficient	$\log K_{ow} = 3.3$
Autoignition temperature	Not available
Decomposition temperature	Not available
Viscosity	5 cSt at 40°C (ASTM D445)

10. Stability and Reactivity

Chemical stability: No decomposition, if used according to specifications

Possibility of hazardous reactions: None are known

Conditions to avoid: Heat, sparks, flame, and build-up of static electricity

Materials to avoid: Halogens, strong acids, alkalies, and ozidizers

Hazardous decomposition products: None are known

11. Toxicological Information

Acute Toxicity:

Test	*Results*	*Basis*
Oral toxicity (rats)	Not classified	Based on ingredients
Dermal toxicity (rats)	Not classified	Product test data
Inhalation toxicity, vapor (rats)	Not classified	Based on testing of similar materials
Eye irritation (rabbits)	Eye irritant category 2A	Based on testing of similar materials
Dermal irritation (rabbits)	Not classified	Product test data

Summary Comments: May cause severe eye irritation like ocular lesions, which are reversible.

Subchronic/Chronic Toxicity:

Test	*Results*	*Comments*
Dermal sensitization (guinea pig)	Not classified: negative response in Bueller, guinea pig test; 0% animals considered positive	Product test data

Summary Comments: Component A may have a drying effect on the skin, frequent or prolonged contact may cause flaking or cracking of the skin.

12. Ecological Information

Persistence and degradability: The total of the organic components contained in the product is not classified as 'readily biodegradable' (OECD-301 A-F). However, this product is expected to be inherently biodegradable.

Bio-accumulative potential: There is no evidence to suggest bioaccumulation will occur.

Mobility: Accidental spillage may lead to penetration in the soil and groundwater. However, there is no evidence that this would cause adverse ecological effects.

Aquatic Toxicity:

Test	*Results*	*Comments*
Acute toxicity	Acute Category 3: 96 h $LC_{50} = 65\ mg\ l^{-1}$	Product test data

13. Disposal Considerations

Waste Disposal: Product is suitable for burning in an enclosed, controlled burner for fuel value or disposal by supervised incineration. Such burning may be limited by local regulation. The product is suitable for processing at an appropriate government waste disposal facility. Use of these methods is subject to user compliance with applicable laws and regulations and consideration of product characteristics at time of disposal.

Recommended European waste code (EWC): 080406

14. Transport Information

UN number: 1993

UN proper shipping name: Flammable liquid, N.O.S. (Contains Component C)

Transport hazard class: 3

Packing group: II

Marine pollutant: No

15. Regulatory Information

Inventory Status: All components are on TSCA, EINECS/ELINCS, AICS, and DSL.

German: Regulations governing combustible liquids (German-VbF) class: AI

German water endangering class (WGK) = 1, slightly water-endangering product (manufacturer classification).

Australian Regulations:

AS 1940 Class: PGII

Poisons Schedule: S5

US Regulations:

US Superfund Amendments and Reauthorization Act (SARA) Title III:

SARA (311/312) Hazard Categories: Fire, Acute

SARA 313: This product contains the following SARA 313 Toxic Release Chemicals:

Chemical name	*CAS number*	*Concentration*
Component A	111111-11-1	70–80%
Component C	4444-44-4	20–25%

The following product components are cited on the lists below:

Chemical name	*CAS number*	*List citations*
Component A	111111-11-1	NJ RTK, TSCA 12b
Component C	4444-44-4	Prop. 65, NJ RTK

16. Other Information

The information contained herein is accurate to the best of our knowledge. My Company makes no warranty of any kind, express or implied, concerning the safe use of this material in your process or in combination with other substances.

See also: Hazard Identification; Hazard Ranking; Hazardous Waste; Risk Communication.

Further Reading

American National Standard for Hazardous Industrial Chemicals – MSDS Preparation (ANSI Z-400.1-2004).
American National Standard for Hazardous Industrial Chemicals – Precautionary Labeling (ANSI Z-129.1-2000).
Globally Harmonized System of Classification and Labelling of Chemicals (GHS), United Nations, 2003.
ISO 11014-1:2003 Draft Safety Data Sheet for Chemical Products.
OECD Series on Testing and Assessment Number 33, Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures (2001).
OECD Series on Testing and Assessment Number 27, Guidance Document ON THE Use of the Harmonized System for The Classification Of Chemicals that are Hazardous for the Aquatic Environment (2001).
UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria (4th revised edition.)
UN Recommendations on the Transport of Dangerous Goods, Model Regulations (13th revised edition, 2003).

Relevant Websites

http://www.ilo.org – International Chemical Safety Cards (ICSC) in many languages.
http://www.state.nj.us – NJ RTK Factsheets.
http://www.unece.org – GHS Document/'Purple book' in Arabic, Chinese, English, French, Spanish and Russian.
http://www.cdc.gov – A list of MSDS resource sites.
http://hpd.nlm.nih.gov – (US) National Library of Medicine, Household Products Database.
http://www.pp.okstate.edu – Oklahoma State University.
http://www.ilpi.com – Where to find MSDS on the Internet.

Chemical Interactions

Carey N Pope

In reality, people are never exposed to individual chemicals in isolation. For example, multitudes of chemicals comprise the food we eat (both in the food itself and as contaminants on/in food) and there are many contaminating chemicals in air and drinking water, around the home and in the workplace. Thus, there is a continual possibility of interactions among chemicals within our bodies. There are three basic types of interactions between chemicals that can modulate toxicity: antagonism, synergism, and potentiation. Antagonism refers to the ability of one chemical to impair or limit the toxicity of another, or the joint interference with the toxic action of two or more chemicals. To illustrate these examples, let us consider two chemicals referred to as A and B. In measuring functional toxicity, a graded scale has been devised for recording the severity of response from 0 (i.e., no signs of toxicity) to 6 (lethality). If chemical A alone elicits an average response of 4 on the toxicity scale while chemical B elicits no toxicity on its own (i.e., an average response of 0), but both chemicals given together yield an average toxicity score of only 2, then it can be concluded that chemical B antagonized the toxic action of chemical A.

Treatment	*Toxicity score*
Chemical A	4
Chemical B	0
Both A and B	2

If on the other hand, Chemical A and Chemical B both elicited some degree of toxicity using this same scale, for example, chemical A caused an average response of 3 and B also elicited an average response of 3, but when given together a response of only 1 was noted, it is concluded that both chemicals antagonized the toxic actions of each other.

Treatment	*Toxicity score*
Chemical A	3
Chemical B	3
Both A and B	1

A simple mathematical description of antagonism is

$$T_{\text{Both}} < T_{\text{A}} + T_{\text{B}}$$

where T_{Both} is the degree of toxic response when both chemicals are given together while T_{A} and T_{B} represent the degree of toxic response when either chemical A or chemical B is given alone.

Antagonism is a relatively common phenomenon, and antidotal strategies are often based on this type of interaction. Chemical antagonism is a simple interaction between two chemicals in which the formed complex is less toxic. Functional antagonism occurs when two chemicals have opposing actions on physiology, thus their combined effects counteract each other. Kinetic or dispositional antagonism occurs when the absorption, distribution, elimination, or biotransformation of a chemical is altered by

another such that less toxicant reaches its target site. Finally, receptor antagonism occurs when two chemicals bind to a specific receptor in the body, and competition between the two leads to lesser toxicity.

Synergism refers to a greater toxic response with exposure to two chemicals than would be expected based on the toxic response elicited by either of the chemicals alone. Potentiation is similar to synergism in that greater toxicity is seen with exposure to two chemicals than expected based on the responses elicited by those chemicals alone, but in this case one of the chemicals has no capacity to elicit the toxic response on its own. Using the toxicity grading scale above, examples of synergism and potentiation can be considered.

Treatment	*Toxicity score*
Synergism	
Chemical A	1
Chemical B	1
Both A and B	5
Potentiation	
Chemical A	2
Chemical B	0
Both A and B	6

A simple mathematical description of synergism or potentiation is

$$T_{\text{Both}} > T_{\text{A}} + T_{\text{B}}$$

where T_{Both} is the degree of toxic response when both chemicals are given together while T_{A} and T_{B} represent the degree of toxic response when either chemical A or chemical B is given alone.

As noted above, people are exposed to many chemicals at any given time through various environmental media. While interactions between two chemicals are relatively straightforward and easy to understand, with more than two chemicals the analysis becomes exceedingly complicated. However, study of the interactive effects of chemicals can often provide specific information on their mechanisms of action.

See also: Interactive Toxicity.

Further Reading

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Chemical Mixtures, Toxicology and Risk Assessment *See* Mixtures, Toxicology and Risk Assessment.

Chemical Warfare Agents *See* Anthrax; Arsenical Vomiting Agents; Bio Warfare and Terrorism:Toxins and Other Mid-Spectrum Agents; Blister Agents/Vesicants; Botulinum Toxin; BZ; Chlorine.

Chemical Warfare Delivery Systems*

Thomas Cain and George O Bizzigotti

From the initial inception of chemical warfare, various delivery systems have been developed for chemical warfare agents. Chemical weapons were generally designed with two objectives in mind:

- Compatibility with existing weapons systems, that is, chemical artillery shells could have been fired from the same guns as conventional artillery shells, chemical bombs could have been dropped

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from the same airplanes as conventional bombs, etc.
- Efficient dispersal of the chemical agent at the target.

This article surveys the various types of chemical weapons delivery systems that were developed during the twentieth century, concentrating on weapons produced by the United States. In general, chemical weapons delivery systems fit into one of several general types; weapons produced by other nations tend to have similar designs.

Cylinders

Figure 1 shows a diagram of a cylinder configured for a chemical warfare agent release. The German practice was to dig deep narrow trenches below the surface of the main trench. Gas cylinders were carried in at night and placed in deep trenches so that the tops were flush with the bottom of the main trench. The cylinders were covered with boards, on top of which were placed bags filled with peat moss and soaked with potash solution; these were intended to absorb any slow leaks. Three layers of sand bags were added to the top of the absorbent bags. The night before an attack, a protective cap was removed from the valve and a lead pipe attached, with the end directed over the parapet and weighted by a sand bag.

The first toxic chemical attacks of World War I employed gas cylinders. In March 1915, the Germans emplaced 1600 large and 4130 small cylinders of chlorine gas opposite Allied troops defending Ypres, Belgium. This attack illustrated the benefits and weakness of using cylinders: although the attack covered a much larger area than could be dispersed from artillery shells, it was at the mercy of the wind. The Germans waited for 43 days for the wind to shift to a westerly direction, releasing the gas on April 22, 1915. The Germans made more chlorine releases from cylinders, and in September 1915 the Allies launched their own chlorine attacks at Loos from cylinders. In this attack, the wind shifted shortly after the release, and some British troops were overcome when the gas blew back across their lines. There were ~200 chemical attacks during World

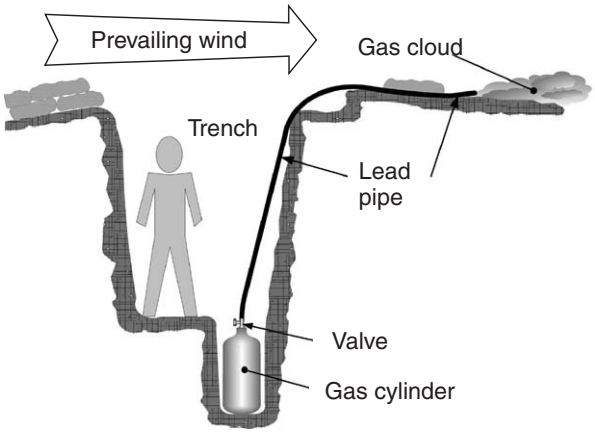

Figure 1 Setup of a gas cylinder for a chemical warfare agent release.

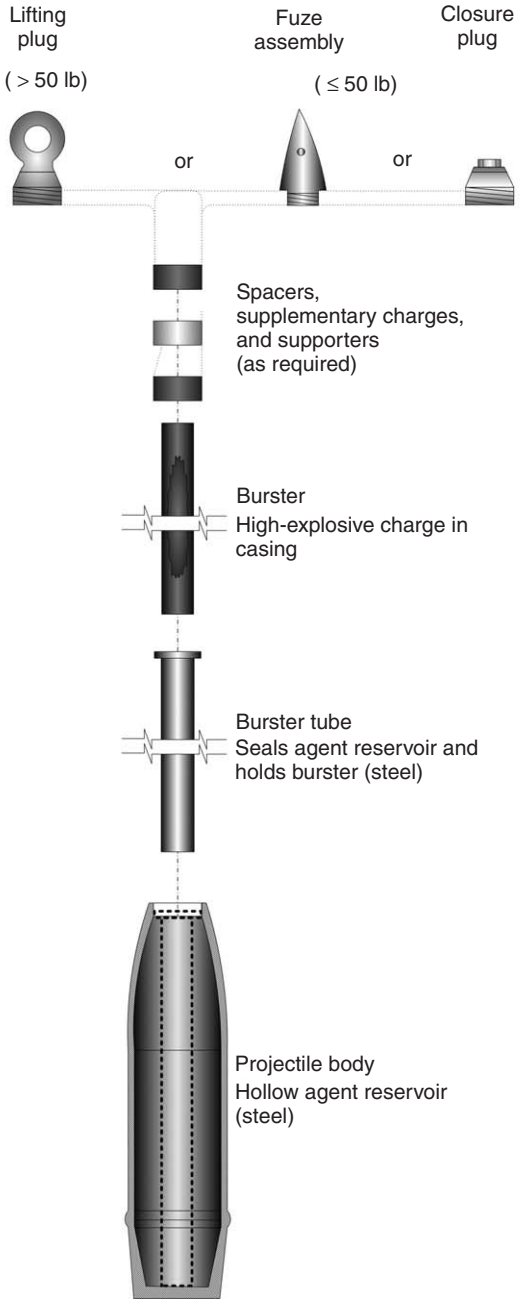

Figure 2 Configuration of a typical chemical artillery shell (projectile).

War I using chlorine and phosgene gas released from cylinders. The largest cylinder attack occurred in October 1915, when the Germans released 550 tons of chlorine from 25 000 cylinders at Rhiems.

Munitions

Chemical munitions consist of a chemical reservoir with a sequence of explosive devices, called the 'explosive train', to rupture the reservoir, disseminating the fill. Typically, an explosive train consists of a fuze with booster, a supplementary charge, and one or more bursters. Munition configurations are described by category in the following subsections.

Projectiles

Chemical projectiles consist of weapons that are fired through the use of an explosive charge and include artillery and mortar shells as well as other projectors. Artillery shells are fired from breech-loaded artillery (i.e., mechanically transported cannons, howitzers, etc., such as those on ships, tanks) while mortars are transported manually and their shells are fired by loading from the muzzle. Projectiles consist of a thick-wall reservoir filled with a chemical agent surrounding an axial explosive charge. When artillery shells are configured with the casing and propulsive charges or when mortar shells have the aft propulsive charge installed, they are referred to as cartridges. Examples of artillery shells are shown in **Figure 2** and a mortar cartridge (i.e., with propulsive charge) is shown in **Figure 3**.

Typical US projectiles range from 75 to 203 mm (3–8 in.) nominal diameters. In storage, small-caliber projectiles that can be lifted by one person (typically <50 lb) have fuzes or closing plugs in place, while larger caliber projectiles, too heavy to lift easily, have lifting lugs (unless recovered as 'duds'). If the projectile is unfuzed, the plug is removed and a fuze assembled to the projectile prior to adjusting the charge and loading the cartridge into the weapon. Fuzes may function on impact, instantaneous, or delay; they can function above ground either at a predetermined height based upon time of flight or function in proximity with the target area. Fuze function detonates the burster charge, resulting in projectile rupture and dispersal of the chemical agent as an aerosol. In the past, the United States has used several munitions that have the same configuration(s) and use identical shell bodies, bursters, and fuzes as chemical weapons, but contain different fills, including incendiary and obscurant chemicals.

Stokes Mortar The 4 in. Stokes mortar developed for chemical agent delivery was first fielded by the British in September 1915 at Loos, and was in wide use by the Somme battles of 1916; this represented the first use of projectiles filled with lethal chemicals in World War I. Chemical artillery shells (or 'projectiles') and mortars remained in chemical arsenals throughout the twentieth century. During World War I, the Germans produced chemical agent-filled projectiles for 77, 105, and 150 mm artillery pieces,

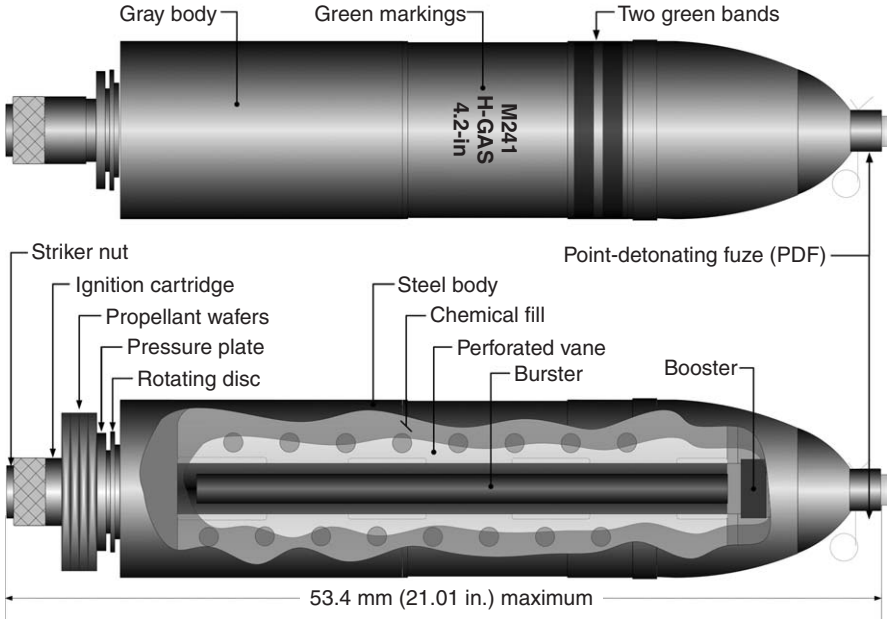

Figure 3 Configuration of a typical chemical mortar cartridge.

and the French produced agent projectiles for their 75 mm rapid firing gun. The United States used Stoke mortars, 75 mm, 4.7 in., 155 mm, 8 in., 9.2 in., and 240 mm projectiles during World War I; projectiles of the same sizes as well as 5 and 6 in., remained in the US arsenal through World War II. The 4.2 in., mortar was standardized in 1928; these remain in the current US stockpile. 105 mm., 5 in., naval, 155 mm, 175 mm and 8 in., naval projectiles were used by the US during the early Cold War years, with 105 mm, 155 mm, and 8 in., naval projectiles in the current stockpile. Virtually all chemical warfare agents have been used to fill artillery projectiles; US mortar shells were filled with mustard, phosgene, Lewisite, Tabun, and other older agents.

Livens Projector The Livens Projector was a large-scale mortar developed for delivering large amounts of chemical warfare agent. The Livens Projector, first used during the Somme offensive in 1916, consisted of a simple tube mortar closed at one end with charge box on which the projectile rested. The Livens Projectors were used in large numbers, often using electrical firing to deliver large numbers of projectiles simultaneously. The Livens Projectors could deliver quantities of agent similar to those in a cylinder attack, but originating a mile from the point of discharge. The Livens Projector was originally developed by the British, and remained in the US arsenal from World War I through the 1930s. **Figure 4** shows the configuration of Livens projectile.

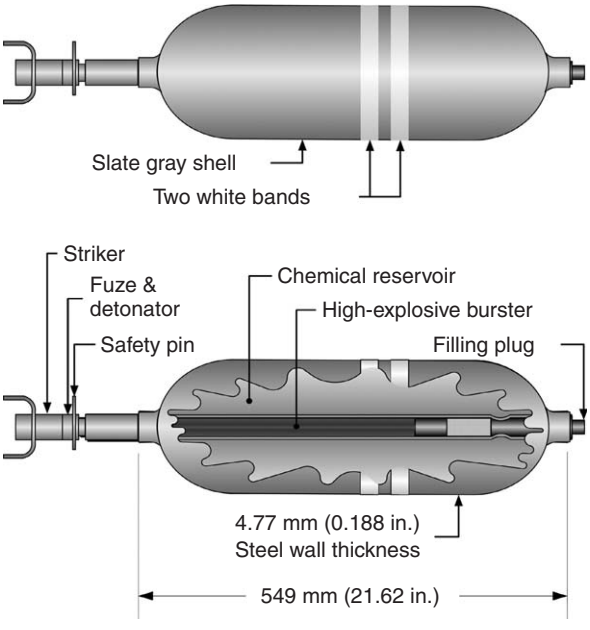

Figure 4 Configuration of a typical Livens projector.

Aerial Bombs, Submunitions, and Bomblets

Aerial chemical bombs were dropped from aircraft and range in weight from 14 to 454 kg (30–1000 lb). Bombs are typically transported and stored without explosive components (bursters, boosters, fuzes, or submunitions) or guidance systems (e.g., tail fins). Explosive components and guidance are generally installed on bombs just before loading onto an aircraft. Chemical bombs contain a reservoir filled with chemical agent surrounding an axial explosive charge. After the bomb is dropped, the fuze is armed. When the bomb arrives at its target, the fuze detonates the axial explosive train, shattering the bomb casing and dispersing the chemical fill as an aerosol. Illustrated examples of some aerial bombs are shown in **Figure 5**. An illustrated example of a bomb with submunitions is shown in **Figure 6**.

Aerial bombs containing chemical warfare agents were first developed around the end of World War I. The first US aerial bombs were 30 lb bombs that held roughly 10 lb of mustard agent. By World War II, 100 lb mustard bombs, 115 and 125 lb bombs filled with mustard, Lewisite, or GA, and 500 and 1000 lb bombs filled with hydrogen cyanide, phosgene, or

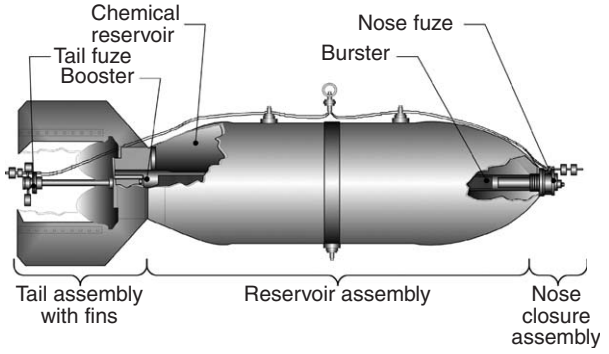

Figure 5 Configuration of typical aerial bombs.

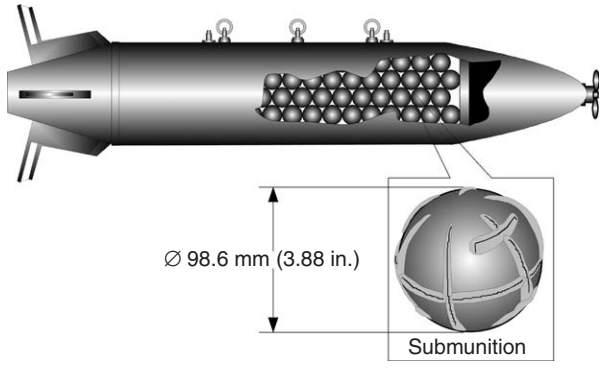

Figure 6 Configuration of bombs with submunitions.

cyanogen chloride were stockpiled. Moreover, 500 and 750 lb GB bombs were developed in the 1950s and remain in the US stockpile.

The payload of some larger munitions contain of a number of submunitions or 'bomblets', small munitions ranging in size from ~25–100 mm (1–4 in.) in diameter. Submunitions and bomblets frequently have explosive components. Bomblets are small bombs used to fill a larger munition. Bomblets are small cylindrical or spherical containers equipped with an axial explosive charge. After the cluster bomb is dropped, the bomblets are released. After release, aerodynamic forces cause the bomblets to spin, which arms the fuze. When the bomblets arrive on target, the fuze detonates the axial explosive charge, or burster. The burster shatters the bomblet casing and causes the chemical agent to disperse as an aerosol. **Figure 6** shows the configuration of a typical chemical cluster munition.

The first chemical cluster munitions were mustard-filled and were developed at the end of World War II. A 1000 lb GB-filled cluster bomb was developed during the Cold War; the weapon held 76 bomblets. Approximately 356 GB-filled bomblets filled the Honest John missile from the 1960s; a smaller warhead holding 52 bomblets was developed for the Little John missile. The Sergeant long-range missile had a warhead containing 330 bomblets; it had a range of 75 miles.

Rockets

Rockets or missiles are fully assembled, self-propelled munition that may have a single chemical reservoir or bomblets (bomblets are not normally present while in storage). Chemical rockets range in size from 60 mm (2.36 in.) diameter bazooka rounds to the Honest John Rocket with a 762 mm (30 in.) diameter and 364 bomblets. **Figure 7** illustrates the exterior configuration of some US chemical rockets.

The M55 chemical agent rocket was first developed in the early 1960s and remains in the US chemical stockpile. The rocket is in a shipping and firing container. The rocket includes (from the rear

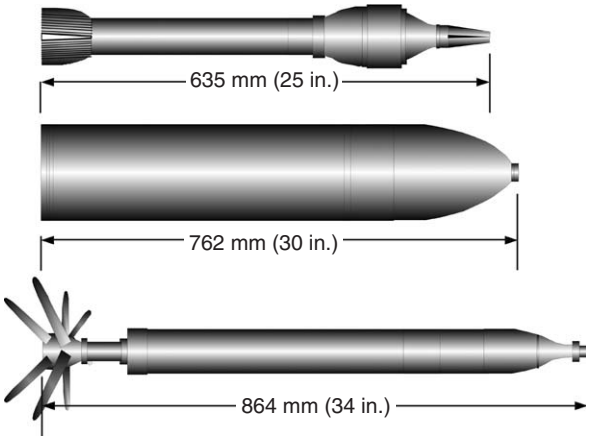

Figure 7 Configuration of typical US chemical rockets.

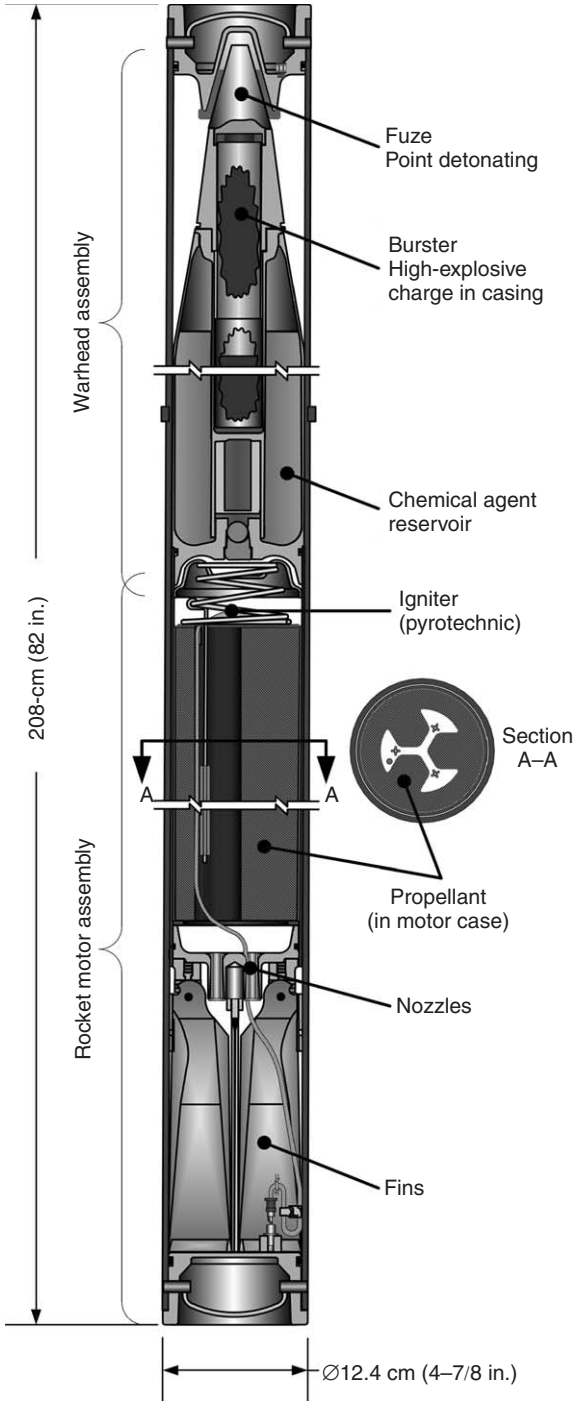

Figure 8 Configuration of the US M55 115 mm Chemical Rocket (82 in. long × 4–7/8 in. diameter in shipping and firing container).

forward) a set of spring-loaded guidance fins, an M28 double-base rocket propellant, a reservoir filled with chemical agent GB or VX surrounding an axial explosive charge, and a fuze. When the rocket propellant is ignited, it thrusts the rocket out of the shipping and firing container. Spring-loaded fins extend and the rocket follows a trajectory based on the angle on firing; the fuze is armed in flight. When the rocket arrives at its target, the fuze detonates the axial explosive charge, or burster. The burster shatters the rocket casing and causes the chemical agent to disperse as an aerosol. **Figure 8** shows an annotated cutaway of the M55 115 mm Chemical Rocket, which exists in the US stockpile.

Chemical rockets were first developed during World War II. The Germans produced 15 cm tractor rockets during the war, and the US standardized a 7.2 in rocket filled with cyanogen chloride or phosgene in 1945; these were stockpiled through the early Cold War. During the 1950s, a GB reservoir was developed for adaptation to a 3.5 in. heat rocket.

Spray Tanks

Spray tanks or spray apparatus range in size from portable units carried by one person up to tanks weighing ~900 kg (1 ton). These tanks were not designed to use explosives to disseminate chemicals but may have explosive components designed to either open valves or eject the spray tank from the aircraft. Spray tanks typically have an agent reservoir that feeds chemical warfare agent to a spray nozzle via a discharge tube. The pressure required to spray the agent can be generated in different ways. Some US spray tanks used a pressure tank filled with compressed gas; others used a scoop to generate pressure using ram air. **Figure 9** shows the configuration of the TMU-28 spray tank.

Spray tanks that allowed warplanes to deliver chemical warfare agents were initially developed during World War II and designed for use with mustard or lewisite. The current stockpile includes the TMU-28 spray tank filled with VX. Chemical spray tanks are containers designed for external use on aircraft for the dissemination of toxic chemical agents, smoke, and incapacitating chemical agents. The Aero 14B Spray Tank is pressure-controlled while the TMU-28/B uses a ram air system for airborne dispersion of chemical warfare agents.

Manually Delivered

Manually delivered munitions are small items that are placed by hand or delivered by an individual soldier.

Placed Placed munitions are delivered to a target by hand and include land mines and smoke pots. These weapons usually contained from 3.8 to 191 (1–5 gallons) of liquid so they could be transported by one person. Explosive components, when used, were either installed or stored in the same container used to store the weapon. As with other munitions, fuzes or activators were not typically installed until ready for delivery. An example of a chemical land mine is shown in **Figure 10**. Smoke canisters typically used a slow-burning material to cause chemical fills to form a smoke.

Chemical landmines were first developed during World War II; these mines were essentially a 1 gallon storage can filled with mustard and having an attached detonator. The M23 chemical agent landmine, first developed in 1960, contains a reservoir filled with chemical agent surrounding an explosive charge. The top of the land mine has a pressure

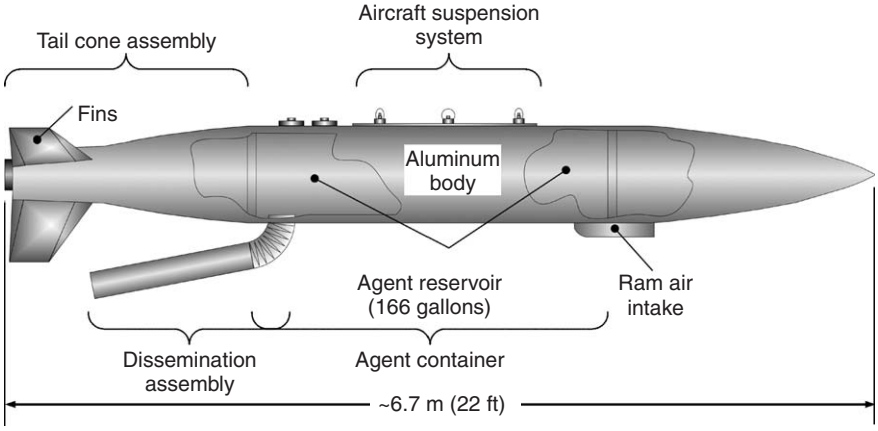

Figure 9 Configuration of the US TMU-28 chemical spray tank.

plate; when the pressure plate is depressed, the fuze detonates the explosive charge, or burster. The burster shatters the mine casing, blows away the soil covering the mine, and causes the chemical agent to disperse as an aerosol. The M23 could also be connected to a remote detonator via a wire. **Figure 10** shows the configuration of the M23 chemical landmine.

Thrown Thrown munitions are manually thrown or ejected by a small explosive charge and include grenades, ejection smoke canisters, and the like. These could be explosive, smoking, or frangible. Frangible, or breakable, munitions were designed to rely on the force of gravity and their impact with the ground to cause the munition case to rupture. The chemical in the munition would then be disseminated by splashing or evaporation.

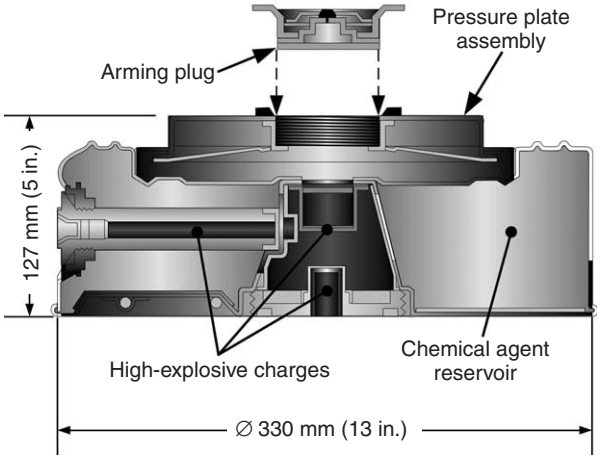

Figure 10 Configuration of the US M23 chemical land mine.

Binary Weapons

The United States began investigating binary weapons in the 1950s. Conventional 'unitary' chemical munitions contain the actual lethal agent, whereas binary weapons contain two components until the weapons are used. At the point that a binary projectile is fired or a binary bomb is dropped, the two components undergo a chemical reaction that forms the lethal agent. The US produced binary weapons during the 1980s; most of these weapons have already been destroyed.

In binary projectiles, the two components were contained in two separate sealed plastic containers. These containers could be stored separately and loaded into the rear of the shell immediately prior to firing. Upon firing, the setback and spin forces caused the containers to rupture, allowing the reactants to combine *en route* to the target. The burster for the binary projectile is container in the front of the projectile. **Figure 11** shows the configuration of a binary chemical projectile.

Storage Containers

Bulk chemical agent is stored in containers. Several different types of containers have been used to store or transport chemical agent. Types of containers that have been used include glass ampoules and bottles, drums, metal cylinders meeting US Department of Transportation specifications of various sizes, and up to a '1 ton container', which is a commercial metal

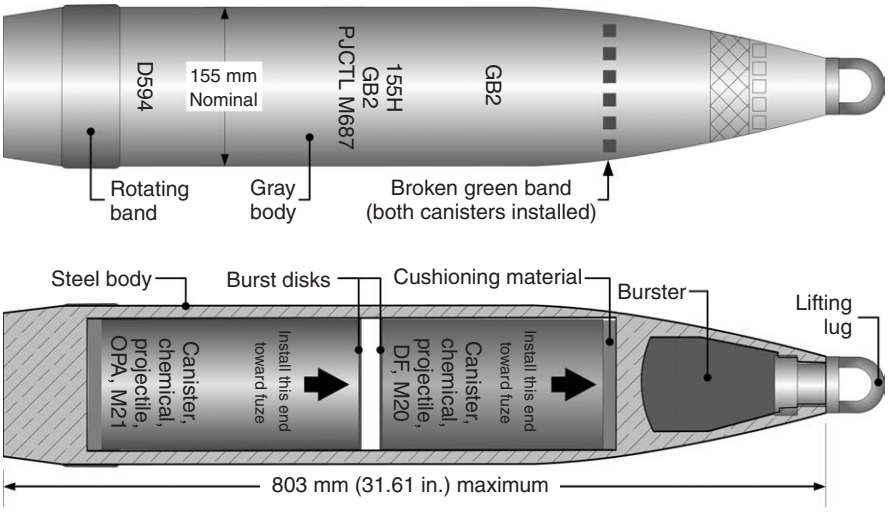

Figure 11 Configuration of a typical binary artillery shell.

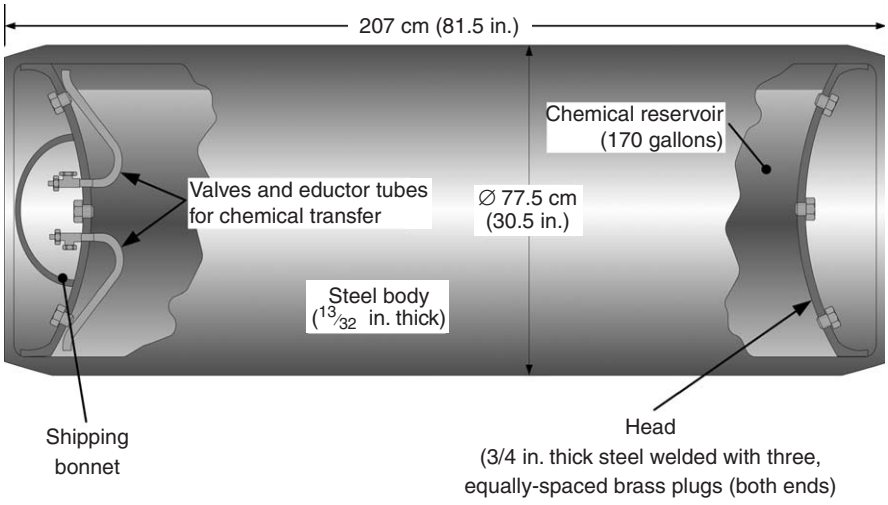

Figure 12 Configuration of a ton container.

cylinder used to transport chlorine gas and other chemical compounds.

The US uses containers similar to containers in general use in the chemical industry. They are probably the least hazardous way to store chemical agent because they do not contain any explosive or energetic components. **Figure 12** shows the configuration of a ton container used for bulk chemical agent storage.

See also: Ancient Warfare and Toxicology; Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents; Chemical Warfare During WW1.

Chemical Warfare During WW1*

George O Bizzigotti

Chlorine Gas

At 5 p.m. on April 22, 1915, German troops at Ypres discharged 180 000 kg of chlorine gas from 5730 cylinders on the line between Steenstraat on the Yser Canal, through Bixschoote and Langemark, to Poelcappelle. The gas cloud blew with the wind, and either killed or caused the French and Algerian troops in the opposing trenches to flee, opening an 8–9 km gap in the Allied line. On April 24, 1915, the Germans conducted a second chlorine gas attack at Ypres, this time against Canadian troops.

On May 31, 1915, chlorine was first employed on the eastern front, by the Germans at Bolimow, near Skierniewice, 50 km southeast of Warsaw. This attack employed 12 000 cylinders, releasing 264 tons of chlorine along a 12 km line. There were ~200 chemical attacks during World War I using gas released from cylinders; the largest of these occurred in October 1915 when the Germans released 550 tons of chlorine from 25 000 cylinders at Rhiems.

Prof. Fritz Haber was chief of the German chemical warfare service during World War I and personally directed the first chlorine gas attack. Haber, a Nobel laureate and known for his discovery of a process for synthesizing ammonia by the combination of nitrogen and hydrogen, is often referred to as the father of chemical warfare.

The Antecedents of Chemical Warfare

Many chronicles of the history of chemical warfare begin with "At 5 PM on 22 April 1915..." What is perhaps less appreciated is that the first chlorine attack represented merely an escalation of an existing use of irritant chemicals. The use of irritating smokes dates to antiquity; the Chinese used arsenical smokes as early as 1000 BC. The use of smoke from burning sulfur against enemy fortifications was a feature of classical Greek warfare described by Thucydides. The first international agreement limiting the use of chemical weapons, concluded in 1675 between

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France and Germany, prohibited the use of poison bullets.

Chemical warfare was debated at several points during the nineteenth century. Sir Lyon Playfair's proposed the use of cacodyl cyanide-filled shells to break the siege of Sebastopol during the Crimean War was rejected by the British Army. During the American Civil War, Mr. John Doughty suggested the use of chlorine-filled artillery shells, but this plan was never implemented. Several other proposals for chemical warfare appeared during the siege of Petersburg in 1864, but were not acted upon. During the late part of the century, there were several efforts to ban chemical warfare. The Brussels Convention of 1874 on the Law and Customs of War banned the use of poison gases. Signatories of Declaration (IV,2) at International Peace Conference at the Hague in 1899 agreed to abstain from the use of projectiles which had the sole objective of the diffusion of asphyxiating or deleterious gases. In addition, the vagaries of wind and weather and the lack of advanced chemical production technology had served as an effective limitation to the employment of chemicals in warfare prior to 1914.

In fact, several belligerents in World War I had been using munitions filled with irritants from almost the beginning of hostilities. The French first used shells filled with ethyl bromoacetate in August 1914, less than 1 month into the war, and chloroacetone was introduced into the French arsenal in November 1914. On October 27, 1914, the Germans at Neuve-Chappelle used the 'Ni-Schrapnell' 105 mm shell, which consisted of lead balls embedded in powdered *o*-dianisidine chlorosulfonate; the inclusion of shrapnel in the shell is generally considered to have been an effort to make the weapon not solely a chemical munition to comply with the Hague declaration. On January 31, 1915 at Boloimow, the Germans introduced 150 mm shells filled with 'T-Stoff', a mixture of brominated aromatics including xylyl bromide, xylylene bromide, and benzyl bromide. All these compounds are extreme irritants capable of severely limiting the effectiveness of unprotected troops. The use of chlorine in April 1915 was thus not entirely a novel type of warfare.

Development of Additional Agents

As the war continued, many toxic compounds in addition to chlorine were tested for utility as chemical warfare agents:

- Bromine was expensive and in limited supply, and was considered more valuable as a feedstock for the manufacture of brominated organic agents.
- Trichloromethylsulfuryl chloride.
- Phosgene (CG) was released from 4000 cylinders (88 tons) on December 19, 1915, at Nieltje in Flanders; phosgene remained a significant chemical warfare agent through World War II.
- Monochloromethyl chloroformate was first used by the Germans at the Somme in the summer of 1916.
- Trichloromethyl chloroformate (DP, diphosgene) was first used in the summer of 1915. It eventually replaced monochloromethyl chloroformate in German gas shells and was used extensively through the end of the war.
- Hydrogen cyanide (AC), an agent, was used extensively by the French. There was considerable debate about whether the French weapons systems were capable of creating lethal cyanide concentrations on the battlefield.
- Hydrogen sulfide.
- Trichloronitromethane (PS, chloropicrin), was mixed with diphosgene in the German 'Green Cross' shell.
- Cyanogen bromide.
- Cyanogen chloride (CK).
- Phenylcarbamine dichloride (phosgene anilide) was used in German gas shells.
- Dichloromethyl ether.
- Dibromomethyl ether.
- Methyl cyanoformate.
- Ethyl cyanoformate.
- Methanesulfonyl chloride.
- Ethanesulfonyl chloride.
- Ethyldichloroarsine, first used in March 1918.
- Methyldichloroarsine.
- Ethyldibromoarsine.
- Bischloroethyl sulfide (HS, mustard gas) was first used in an artillery attack on July 12, 1917 by the Germans. This agent caused the most casualties of any agent used during World War I and remained in chemical stockpiles for nearly a century; it is currently slated for destruction in the United States and Russia.

Of the many chemical warfare agents tried, chlorine, phosgene, diphosgene, chloropicrin, hydrogen cyanide, cyanogen chloride, and mustard were produced and used in significant quantities. Total production of the major agents is given in **Table 1**.

Development of Additional Means of Delivery

At the same time as they experimented with more lethal chemical agents, both sides worked to develop more effective methods of agent delivery. Gas cloud

Table 1 Production of chemical warfare agents during World War I (in tons)[a]

Country	*Chlorine*	*Phosgene*	*Diphosgene*	*Mustard*	*Chloropicrin*	*Cyanides*	*Total*
Germany	58 100	18 100	11 600	7600	4 100		99 500
France	12 500	15 700		2000	500	7700	38 400
Britain	20 800	1 400		500	8 000	400	31 100
United States	2 400	1 400		900	2 500		7 200
Austria	NA	NA	NA	0	NA	NA	5 245
Italy	NA	NA	NA	0	NA	NA	4 100
Russia	NA	NA	NA	0	NA	NA	3 650
Total	93 800	36 600	11 600	11 000	15 100	8100	189 195

[a]NA denotes figures not available. Note that a portion of the chlorine, phosgene, and cyanide were used for nonchemical warfare purposes. A total of 150 000 tons of chemicals were produced for chemical warfare purposes, and 125 000 tons of that were actually used on the battlefield.

attacks relied on the wind; in the absence of wind or if the wind blew from the wrong direction, gas cylinders were useless. If the wind shifted shortly after a release, the gas would blow back onto the attacking forces. Thus, a number of new means of delivering chemical warfare agents to the opposing forces were introduced:

- The 4 in. Stokes mortar developed for chemical agent delivery, first fielded in September 1915 at Loos; this represented the first use of projectiles filled with lethal chemicals in World War I.
- The Germans produced chemical agent-filled projectiles for 77, 105, and 150 mm artillery pieces, and the French produced agent-filled projectiles for their 75 mm rapid firing gun.
- The British Livens Projector was a large-scale mortar developed for delivering large amounts of chemical warfare agent.

The Toll of Chemical Warfare, 1914–18

It is difficult to find a definitive figure for the numbers of men injured and killed by chemical warfare agents during World War I. British casualties alone can be estimated at 185 000 injured and 8700 dead. Prentiss gives a figure of 1 296 853 casualties produced by ~125 000 tons of chemical warfare agents used by the combatants, but it is known that in many cases the official figures underestimate the number of casualties. Furthermore, it is unclear to what degree the official figures include individuals who survived gas attacks with little apparent effect but who developed serious complications only after the war. Given Prentiss' estimate of 10 000 000 battle deaths from the war, it is arguable as to whether chemical warfare was more or less horrific than the other methods of conducting the war.

See also: Chemical Warfare Delivery Systems; Chlorine; Phosgene.

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Chemicals of Environmental Concern

Steve J D'Surney and Mike D Smith

Introduction

Environmental contaminants are any physical, chemical, biological, or radiological substance or matter that has an adverse effect on air, water, soil, or living organisms. In some cases environmental contamination is a clear-cut phenomenon, whereas in others, the perception lies largely in the eyes of the beholder. Toxic organochlorine solvent residues leached into water supplies from a hazardous waste dump are pollutants in anybody's view. Frequently, time and place determine what may be called a pollutant. The phosphate that the sewage treatment plant operator has to remove from wastewater is chemically the same as the phosphate that a nearby farmer has to buy at high prices for fertilizer.

A reasonable definition of a pollutant is a substance present in greater than normal concentration as a result of human activity and having a detrimental effect upon its environment or upon something of value in that environment. Contaminants, which are not classified as pollutants unless they have some detrimental effect, cause deviations from the normal composition of an environment.

Pollutants can enter through direct dumping, piped outflow, and channeled waste streams as localized point sources, or as diffuse nonpoint sources they can enter rivers, lakes, streams, and groundwater through runoff and soil percolation. Nonpoint sources are considered to be major contributors to air, water, and soil pollution which include: runoff from paved streets and parking lots, agricultural lots, soil erosion from logging, atmospheric deposition of acidic or toxic air pollutants (**Table 1**). The source is particularly important, because it is the logical place to eliminate pollution. After a pollutant is released from a source, it may act upon a receptor. The receptor is anything, both biotic and abiotic, that is affected by the pollutant. Humans whose eyes water from atmospheric oxidants are receptors. Juvenile trout that die after exposure to pesticides in water are also receptors. Eventually, if the pollutant is long-lived, it may be deposited in a long-term sink, such as aquatic sediments and soils.

Mineral and Energy Exploration

The largest quantitative source of contamination derives from mining and energy extraction. Mining and mineral processing use a variety of chemicals for extraction, ore processing, water treatment, and many other supporting activities such as overburden removal. Mining and energy extraction generate large volumes of waste and have the potential to cause a number of environmental problems if improperly managed. Water and soil degradation can result from salinization, acidification, and chemical contamination. Streams and rivers can also experience severe siltation.

Coarse tailings and rock blasting produce large amounts of dust and mobilize heavy metal contaminants, such as lead, copper, aluminum, and zinc, which can leach into surface and subsurface waters. Cyanide and mercury are used to extract gold from soil and pulverized rock. Mineral processing generates a great deal of particulate matter released from bauxite and coal processing. Acid leaching into soil, ground water and riparian environments from mine wastes is common. Acid drainage from mine tailings, ore and waste dumps, contains sulfur and sulfides such as iron sulfide, which can be converted to acids through bacterial oxidation in the presence of moisture and oxygen.

Table 1 Common sources of contaminants to the environment

Sources	*Contaminants*
Mining and mineral processing	Heavy metals, chemicals via cyanide and acids, hydrocarbon products resulting from spills and coal mining, and metallic salts
Fossil fuel combustion	Sulfur dioxide, carbon dioxide, nitric oxide, heat, acids, acid rain, ozone, soot, polycyclic aromatic hydrocarbons, and volatile organic compounds
Agriculture and forestry	Pesticides, nitrates, phosphates, greenhouse gases, and mineral salts
Industrial production	Numerous synthetic organic and inorganic compounds, organochlorines, dioxins, heavy metals, hydrocarbons, chlorinated phenols, sulfates, sulfides, surfactants, solvents, acids, bases, salts, pharmaceuticals, plastics, resins, explosives, and natural organics
Consumerism	Residential and commercial chemicals, pesticides, fertilizers, hydrocarbons, solvents, surfactants, paints, sealants, medicines, volatile organic compounds, resins, plastics, metals, salts, acids, and bases

Metal contaminants may become mobilized to cause potential health and environmental problems resulting from leaching into soil, water, and sediment. Acid mine drainage and slag leachate can contain high concentrations of heavy metals and acids. Sulfuric acid can be formed via oxidation of sulfides. As a great deal of attention is paid to the containment and remediation of acid mine drainage, the neutralization of acid pH usually results in the precipitation of many contaminants usually as metallic salts. These salts would then become soluble and may enter surface and ground water.

Oil spills and coal mining command considerable attention from the media because they are often large scale and visually very dramatic. Nothing seems worse than a mass of toxic crude oil and tarry hydrocarbons smeared over the natural habitats of some foreshore or the sight of strip mining operations. As a result, there is a massive public response and a frenzy of activity by agencies, community groups, and politicians.

There are demands for 'the environment to be saved'. Often, as in the case of large spills such as 'Torrey Canyon' or 'Exxon Valdez', very large sums of money change hands in order to mobilize whatever resources can be found to clean up the mess. For many spills, however, the ecological issues are different from those being touted in public discussion. There is, in fact, plenty of evidence that for many marine organisms large, dramatic sudden impacts are not really the most serious threat. Long-term, chronic, low-level contamination of habitats by complex exogenous agents may be more compromising in terms of environmental outcomes. In addition, coupled with the destruction, deterioration, and fragmentation of natural habitats, there exists considerably greater threats to long-term sustainability of coastal biodiversity. Attempts to disperse oil spills with surfactants may be potentially hazardous.

Fossil Fuel Combustion

Humanity's major sources of energy are derived from fossil fuels, principally oil, gas, coal, and wood. The major combustion by-products of fossil fuel burning include sulfur dioxide (SO_2), carbon dioxide (CO_2), and nitric oxide (NO_2), and partially oxidized hydrocarbons. The process of burning fossil fuels in thermal power plants, factories, homes, and motor vehicles emits enormous amounts of the aforementioned pollutants. The most important environmental concerns resulting from fossil fuel use are global climate change, acid rain, surface ozone, and particulate-/aerosol-bound toxins.

Many scientists now believe that global warming is taking place, though the magnitude of the change and the contribution from anthropogenic sources are controversial. A component of the warming observed since the 1880s may be attributed to increases in the concentration of the so-called 'greenhouse gases' such as CO_2 and methane (CH_4) in the atmosphere.

Another side effect of fossil fuel burning is acid rain. In the process of burning organic fuels, some gases, in particular SO_2 and NO_2, combine with atmospheric water vapor to form sulfuric and nitric acids. Acidified rainwater can attain pH values below 3. Acid rain can cause damage to plant life, in some cases seriously affecting the growth of forests and lakes due to acid-stimulated metal leaching from soils and rock.

Besides gaseous fossil fuel emissions that contribute to global warming and acid rain, emissions of particulate matter from incomplete burning also contribute to poor air quality. Coal burning and diesel engines are a major source of particulate organic particles. Additionally, fuel combustion and evaporative emissions from motor vehicles are also major sources of anthropogenic volatile organic compounds (VOCs). Motor vehicles account for a considerable fraction of the total emissions of nitrogen oxides, particulate hydrocarbons, and VOCs in developed countries. Of particular concern is the production of polycyclic aromatic hydrocarbons (PAHs) resulting from incomplete combustion of fossil fuels. These compounds, especially diesel soot emissions, contain some of the most potent mutagens/carcinogens known to mankind.

Compared with solid fossil fuels, natural gas and oil are less polluting. Natural gas is the least polluting fossil fuel. The main environmental problems resulting from the production and transportation of primary energy are related to mining of solid fuels (mainly coal) and oil transportation. Coal mining operations produce large amounts of slag wastes and results in acid water drainage. The continuous acid discharges from mines seriously affects aquatic ecosystems, since acid waters containing heavy concentrations of dissolved heavy metals will support only limited water flora, and will not sustain fish and many invertebrates. The major impacts from oil are associated with accidental spillages during transportation both at sea and on land. The resultant damage to coastal areas and marine life can be dramatic in the short term and may also have long-term consequences. Solid wastes and ash disposal (spoil tips) from coal mines lead to the contamination of water percolating through slag heaps that cause groundwater and soil pollution. The combustion of liquefied petroleum gas (LPG) causes the problems of liquid residual disposal.

Agriculture and Forestry

The global concentration of greenhouse gases has increased measurably over the past 250 years, partly due to land use activities such as agriculture and forestry. Carbon dioxide, methane, and nitrous oxide emissions have increased by ~31%, 131%, and 17%, respectively, since 1750. Agriculture and forestry practices have contributed to trends in emissions of these greenhouse gases through fuel consumption, land use conversions, cultivation and fertilization of soil, production of ruminant livestock, and management of livestock manure. Additionally, the irrigation of formerly arid lands leaches minerals from soils at accelerated rates resulting in toxic concentrations of agricultural pollutants which include nutrients (nitrogen and phosphorus), pesticides, pathogens, selenium, and salts. While farmers do not intend for these materials to move from the field or enterprise, they often do, carried by rainfall, snowmelt, or irrigation water. After passage of the Reclamation Act of 1902, the United States Government began building and subsidizing irrigation projects to foster settlement and development of the arid and semiarid areas of the western USA.

A wide variety of pesticides are applied to agricultural crops to control insect pests (insecticides), weeds (herbicides), fungi (fungicides), and rodents (rodenticide). Annually, 500 million pounds of pesticides are applied to farmland, and certain chemicals can travel far from the point of application. Pesticide residues reaching surface water systems may harm freshwater and marine organisms, damaging recreational and commercial fisheries. Pesticides in drinking water supplies may also pose risks to human health. Long-lived pesticides such as dichloro-diphenyl-trichloro-ethane (DDT), Aldrin, Dieldrin, mercuric and arsenic compounds still persist in the environment. Shorter lived pesticides such as chlorpyrifos, methyl parathion, 2,4 D herbicides, and numerous new compounds are a global concern.

Industrial Production

The global expansion of industrial and consumer-oriented societies is linked to large-scale industrial production and consumerism that utilize a vast array of numerous chemical compounds. The listings of such chemicals are too vast to present in this paper but some examples will be discussed here. Environmental contaminants in nature typically involve complex mixtures, partitioning factors, chemical transformations, and abiotic and biotic interactions. The biological and environmental effects are complex and may be additive, synergistic and even antagonistic in nature.

Pulp and paper mill sludge is a complex and changeable mixture of dozens or even hundreds of compounds. Some are well known, like natural wood extractives, organochlorines, organosulfides, and dioxins. Priority pollutants and chemicals of concern that must be analyzed in pulp mill residues include heavy metals, chlorinated hydrocarbons, chlorobenzenes, PAHs, chlorinated phenols, chlorinated catechols, chlorinated guaiacols, phthalates, resin acids, alkylphenols and alkylphenol ethoxylates, and plant sterols.

In 1775, PAHs were the first group of compounds known to cause cancer in humans. Nowadays, many of these compounds are well-known carcinogens in humans and animals. PAHs are produced in the environment as the result of heating organic matter to high temperatures like tobacco smoke, soot, coal tar, creosote production, wood burning, smoked foods, roasted coffee, charbroiled meat, and fossil fuel combustion exhaust. However, the major environmental source comes from asphalt, tar, used motor oil, diesel exhaust, and coal burning.

Dioxins, a by-product of herbicide and pulp and paper production, are highly toxic members of a class of organochlorine chemicals including chlorinated dibenzo-*p*-dioxins (CDDs), dibinzofurans (CDFs), polychlorinated biphenals (PCBs), brominated dibenzo-*p*-dioxins (BDDs), brominated dibenzofurans (BDFs), and polychlorinated pesticides. Dioxins and its related compounds are cytotoxic and genotoxic, and have hormonal effects that may disrupt the endocrine system and cellular signaling pathways in wildlife and humans. Dioxins have both estrogenic and antiestrogenic effects depending on the organ or tissue affected. Exposure to relatively low levels of these chemicals has had catastrophic effects on populations of Beluga whales, alligators, turtles, mink, otters, bald eagles, osprey, cormorants, terns, herring gulls, migratory birds, chickens, lake trout, chinook and coho salmon, etc., throughout the United States and Canada.

Polychlorinated dibenzofurans (PCDFs) are formed as inadvertent by-products in the production and use of polychlorinated biphenyls (PCBs), formerly used as an insulator in electrical transformers and, in combination with polychlorinated dibenzo-*p*-dioxins (PCDDs), in the production of chlorophenols and have been detected as contaminants in these products. PCDFs and PCDDs also may be produced in thermal processes such as incineration and metal processing and in the bleaching of paper pulp with free chlorine. PCDFs are also found in residual waste from the production of vinyl chloride and the chlor-alkali process for chlorine production. The relative amounts of PCDF and PCDD congeners produced

depend on the production or incineration process and vary widely.

Like PCDDs, PCDFs are ubiquitous in soil, sediments, and air. Excluding occupational or accidental exposures, most background human exposure to PCDFs occurs as a result of eating meat, milk, eggs, fish, and related products, as PCDFs are persistent in the environment and accumulate in animal fat. High exposures have occurred in relation to incidents in Japan (*yusho*) and Taiwan (*yucheng*) involving contamination of rice oil and in accidents involving electrical equipment containing PCBs. Occupational exposures also may occur in metal production and recycling, and in the production and use of chlorophenols and PCBs.

Chemical wood preservatives account for the single largest pesticide use in the United States and one of the greatest pesticide threats to public health and the environment. Wood preservatives protect wood products from fungus and insect decay. The three principle wood preservatives include chromated copper arsenate (CCA), pentachlorophenol (penta), and creosote. The Environmental Protection Agency (EPA) has classified many chemicals and even certain heavy metal contaminants, as known or probable carcinogens, teratogens, cellular toxins, endocrine disrupters, and reproductive toxins. The arsenic in CCA, certain PAHs, and dioxins are known human carcinogens and are linked to disorders and birth defects.

Consumerism

Everything we put down the drain or flush (down the commode) ends up in our watersheds which can affect the health of terrestrial and aquatic wildlife, plants, the atmosphere, and the water quality in our area. Residential and commercial use of chemicals constitutes a very large, nonpoint source of environmental contamination. A typical source of environmental contaminants are products used for household use such as cleaning agents, surfactants, pesticides, fertilizers, lawn and garden treatments, paints, sealants, and even discarded or flushed medicines. It is imperative that those seeking a healthy lifestyle and reduction in pollutant exposure choose with care the products they use to clean and maintain their homes, yards, and pets.

The three primary ways household hazardous products impact our health and the environment are through their manufacture, usage, and disposal. When one purchases a hazardous product for the home, it creates a market for these toxic chemicals. Once we open the container to use the substance, the vapors released and the water contaminated can have an unhealthy effect on humans, marine life, and water and air quality. Long after the need to use that cleansing agent, these products still have lasting effects. Once disposed of, they release chemicals into the ground and wastewater stream which may contaminate our groundwater and present a problem to wastewater treatment facilities. Most often, hazardous products are flushed into the wastewater system or disposed of in landfills which may leach toxins for many years.

The invention of chlorofluorocarbons (CFCs) in the late 1920s and early 1930s stemmed from the call for safer alternatives to the sulfur dioxide and ammonia refrigerants used at the time. Chlorofluorocarbons were chosen for their safety and for their advantageous chemical properties. These compounds are low in toxicity, nonflammable, noncorrosive, and nonreactive with other chemical species, and have desirable thermal conductivity and boiling point characteristics. These features led to increased demand as more applications arose for CFC use. However, CFCs are human-made substances that release chlorine atoms which destroy ozone in the atmosphere, which causes an increase in UV radiation at the ground level. The amount of CFCs produced (and therefore likely released into the atmosphere) steadily increased over several decades until an international agreement called the Montreal Protocol was signed in September 1987. In this agreement the world's nations agreed to phase out CFC production.

In 1962, Rachel Carson's novel, *Silent Spring*, implicated pesticides such as DDT for the decline of some wildlife populations. Continued research in the late 1960s and 1970s discovered elevated use of manufactured organic chemicals in industrial and domestic applications. Chemicals of concern included PCBs, 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD), and related dioxin and furan congeners, PAHs, and various organic solvents. Regulatory efforts by the Environmental Protection Agency resulted in a decreased production of these xenobiotics. In addition to concerns involving genotoxins, cytotoxins and teratogens are reproductive and developmental effects associated with endocrine disruptors. An endocrine disruptor has been broadly defined as an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones responsible for the maintenance of homeostasis and the regulation of developmental processes.

Suspected endocrine-disrupting chemicals are found in insecticides, herbicides, fumigants, and fungicides that are used in agriculture as well as in the home. Other endocrine disruptors are found in industrial chemicals such as detergents, resins, plasticizers, organometals, halogenated aromatic hydrocarbons, and monomers in many plastics. Exposure to these chemicals occurs through direct contact in

the workplace or at home, or through ingestion of contaminated water, food, or air. Studies have found that some of these chemicals do leach out of plastics, such as the PVC plastics used to make IV bags. When these plastics, or other materials, are burned (as well as in their production) many unwanted by-products that are endocrine disruptors or suspected endocrine disruptors are released into the air or water.

Cancer is the uncontrolled proliferation of cells of the body caused by chemical (carcinogens), physical (radiation, X-rays, UV light), and biological agents (viruses). A carcinogen is an agent that causes or induces neoplasia. Most carcinogens react with the growth control genes of the DNA of cells, causing mutations that lead to cancer. Other carcinogens cause cancer through other mechanisms that do not involve DNA. Some of these cause hormonal imbalances that cause rapid cell proliferation. One example is DDT which mimics the actions of the hormone estrogen. Estrogen stimulates cell division in the breasts and uteri of women. DDT is thought to cause certain forms of breast and uterine cancer.

Conclusions

The twentieth century has brought with it tremendous gains in science and technology as well as gains in the quality of human life and longevity. However, these gains have been accompanied by certain hazards, many associated with the 100 000 chemicals which are now commonly in use.

As stated earlier, environmental contaminants are materials that can pollute our surroundings and adversely impact living organisms. Often these pollutants are chemical compounds produced by human endeavors, although environmental contamination can also come from nonhuman sources such as naturally occurring metals, animal waste, oil seeps, and algal blooms. Environmental contaminants may pollute soil, surface water, or aquatic sediments. Many compounds also leach through soils into groundwater, potentially impacting drinking water supplies. Numerous pollutants are discharged directly into the atmosphere by human industry, where winds may transport them to Earth's most remote corners. It is important, however, to note that industry is not the sole source of contaminants; individuals also contribute to this problem through the use of household pesticides and fertilizers, improper disposal of hazardous materials (e.g., used motor oil, paints, cleaning products), and even by driving the family car. Consequently, sites with one predominant contaminant are a rarity; complex mixtures and subsequent exposures define the real world.

See also: Coke Oven Emissions; Dioxins; Organochlorine Insecticides; Polychlorinated Biphenyls (PCBs); Polycyclic Aromatic Hydrocarbons (PAHs); Volatile Organic Compounds (VOC).

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Chemical-Specific Adjustment Factor (CSAF)

Bette Meek

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Introduction

In the development of reference or tolerable concentrations or doses, where kinetic and/or dynamic data are adequate, commonly adopted default values for interspecies differences and human variability can be replaced by more certain chemical-specific adjustment factors (CSAFs). These CSAFs represent part of a broader continuum of approaches to incorporate increasing amounts of data to reduce uncertainty, ranging from default (presumed protective) to more 'biologically based predictive' approaches. Guidance

for the adequacy of data to serve as the basis for development of CSAFs is available.

Framework for Development of CSAFs

A framework was proposed to address kinetics and dynamics separately in considering uncertainty related to interspecies differences and interindividual variability in the development of reference or tolerable concentrations/doses. Quantitation of this subdivision is supported by data on kinetic parameters and pharmacokinetic–pharmacodynamic (PKPD) modeling for a range of pharmacological and therapeutic responses to pharmaceutical agents (Renwick, 1993; Renwick and Lazarus, 1998). This framework allows the incorporation of quantitative chemical-specific data, relating to either toxicokinetics or toxicodynamics, to replace part of the usual 100-fold default uncertainty factor for interspecies differences or interindividual variability but collapses back to the usual 100-fold default in the absence of appropriate information (**Figure 1**). Owing to the nature of the data on which the subdivision is based, in the context of the framework, 'toxicokinetics' relates to the movement of the chemical around the body (i.e., the absorption, distribution, metabolism, and excretion). 'Toxicodynamics' relates specifically to the processes occurring in the target tissue(s), including metabolism.

Chemical-Specific Toxicokinetic Adjustment Factors [AK_{AF}, HK_{AF}]

The CSAFs for the toxicokinetic components of interspecies differences and interindividual variability are ratios of measurable metrics for internal exposure to the active compound such as area under the plasma or tissue-concentration time curve (*AUC*), the maximum measured concentration in blood (C_{max}) or clearance (*Cl*). For interspecies differences, this is generally determined on the basis of comparison of the results of *in vivo* kinetic studies with the active compound in animals and a representative sample of the healthy human population. For humans, relevant data on *AUC*, C_{max}, or *Cl* are generally derived from *in vivo* experimentation in volunteers given very low doses of the relevant chemical. Alternatively, relevant information on such parameters may be derived from *in vitro* enzyme studies combined with suitable scaling to determine *in vivo* activity.

For interindividual variability, most often, factors responsible for clearance mechanisms are identified (e.g., renal clearance, CYP-specific metabolism, etc.) and a CSAF derived based on measured or physiologically based pharmacokinetically (PBPK) modeled human variability in the relevant physiological and biochemical parameters. The population distribution for the relevant metric (e.g., *AUC*, C_{max}, *Cl*) for the active entity is analyzed and the CSAF (HK_{AF}) calculated as the difference between the central values for the main group and given percentiles (such as 95th, 97.5th, and 99th) for the whole population (**Figure 2**). These differences are analyzed separately for any potentially susceptible subgroup (**Figure 2**).

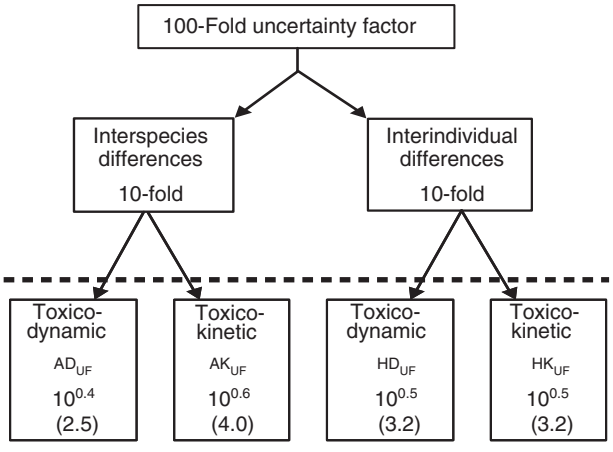

Figure 1 Subdivision of the 100-fold uncertainty factor to allow chemical-specific data to replace part of the default factor. AD_{UF} – Animal to human dynamic uncertainty factor; AK_{UF} – Animal to human kinetic uncertainty factor; HD_{UF} – Human variability dynamic uncertainty factor; and HK_{UF} – Human variability kinetic uncertainty factor. Chemical-specific data can be used to replace a default uncertainty factor (UF) by an adjustment factor (AF).

Chemical-Specific Toxicodynamic Adjustment Factors [AD_{AF}, HD_{AF}]

The CSAFs for toxicodynamic components are most simply, ratios of the doses which induce the critical toxic effect or a measurable related response *in vitro* in relevant tissues of animals and a representative sample of the healthy human population (interspecies differences) or in average versus sensitive humans (interindividual variability). At its simplest, then, replacement of the dynamic component of the default factor for interspecies differences is the ratio of the effective concentrations in critical tissues of animals versus humans (e.g., $EC_{10\ animal}/EC_{10\ human}$) for interspecies differences and in healthy human and susceptible subpopulations for interindividual variability (e.g., the $EC_{10\ average}/EC_{10\ sensitive}$).

Guidance for Development of CSAF

The International Programme on Chemical Safety provides guidance on several aspects of the

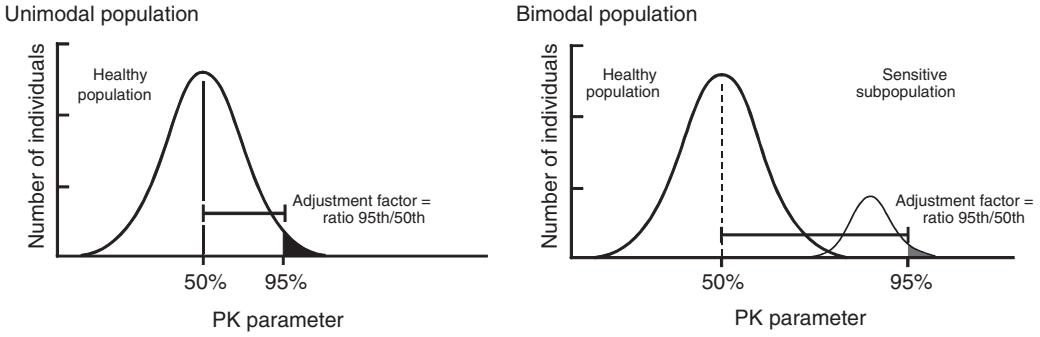

Figure 2 Development of CSAFs for interindividual variability.

development of CSAF, which are only briefly outlined here. For example, data for application in the four components of the framework must relate to the active form of the chemical. For the components of the framework addressing toxicokinetics [AK_{AF}], [HK_{AF}], choice of the appropriate metric is also an essential first step.

Choice of the appropriate end point is critical for the components addressing toxicodynamics [AD_{AF}], [HD_{AF}]. The selected measured end point must either be the critical effect itself or intimately linked thereto (with similar concentration-response and temporal relationships) based on an understanding of mode of action.

In addition, the metric for toxicokinetics or the measure of effects for toxicodynamics as a basis for CSAF needs careful consideration in relation to the delivery of the chemical to the target organ. Measures of various endpoints *in vivo* may represent purely toxicokinetics, or toxicokinetics and part or all of the toxicodynamic processes, as defined based on the subdivision of defaults. This necessitates consideration of the impact of specific data to replace the toxicokinetic and potentially a proportion or all of the toxicodynamic components of the default uncertainty factors.

For data that serve as the basis for all components, relevance of the population, the route of exposure, dose/concentration and adequacy of numbers of subjects/samples must also be considered and the potential impact on the validity of the calculated ratio addressed. For example, for *in vitro* studies which inform primarily dynamic components [AD_{AF}] and [HD_{AF}], the quality of the samples should be considered, and evidence provided that they are representative of the target population, e.g., viability, specific content, or activity of marker enzymes.

Conclusions

Consideration of relevant data in the context of a framework that addresses kinetic and dynamic aspects, explicitly, should result in greater understanding of contributing components and transparency in risk assessment. It is also anticipated that consideration in this context will lead to clearer delineation and better common understanding of the nature of specific data required which would permit development of more informative measures of dose response.

See also: Risk Assessment, Human Health; Uncertainty Factors.

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Chemotherapeutic Agents *See* Cancer Chemotherapeutic Agents.

Chernobyl

Amy Bickham Baird and Ronald K Chesser

Introduction

The meltdown of Reactor IV at the Chernobyl nuclear power plant on April 26, 1986 was the worst nuclear disaster in history. Radioactive fallout from the accident impacted not only Ukraine and its residents, but countries along the path of the radiation plume all the way to Scandinavia. A total of 31 people died as a result of the accident but the complete extent of the health effects are unknown. Despite obvious negative effects, some good came of the accident in terms of helping shape safety measures in nuclear power plants, as well as furthering our understanding of the consequences of exposure to radiation. But what about the long-term effects of exposure to radiation? Now, over 15 years after the accident, we are able to obtain a clearer understanding of these long-term effects. Scientists have been interested in the health effects of those exposed to high amounts of radiation following the meltdown, as well as effects on the nearby environment, where there still remains a high level of contamination.

Dose Estimates

Dose rates in the regions surrounding Reactor IV were highly variable due to distinct plumes of radioactive fallout released subsequent to the explosion. The first plume, designated the Western Trace, yielded doses in excess of $6\,\mathrm{Gy\,h^{-1}}$ in some areas, resulting in the death of over 400 ha of pine (*Pinus sylvestris*) forest. Radiation doses to firemen and reactor personnel exposed shortly after the explosion reached as high as 15 Gy, leading to the deaths of 29 persons (two died as a direct result of the explosion) within 4 months. Exposure of adolescents to ^{131}I in Chernobyl fallout has contributed to elevated cases of thyroid cancers in northern Ukraine and southern Belarus. Thyroid cancers are usually treatable and have not lead to substantial increases in deaths attributable to Chernobyl.

Dose rates rapidly declined subsequent to the Chernobyl accident and ensuing fire. Most of the isotopes released had short half-lives, so their energy rapidly generated absorbed radiation doses. Ninety-eight percent of the isotopes released at Chernobyl has now dissipated. The predominant radionuclides remaining are ^{137}Cs and ^{90}Sr, each having half-lives of $\sim$30 years. These isotopes, however, have high biological affinities and are readily incorporated into living tissues. Because of this affinity, the animals living in the regions near the reactor (Red Forest) are the most radioactive organisms living in otherwise natural environments. Some rodents, for example, are receiving up to $0.1\,\mathrm{Gy\,day^{-1}}$ from cesium and strontium in their muscle and bone. Documenting the doses received at Chernobyl is an important step in performing empirical studies for observing and noting the responses to exposure to the radiation.

Environmental and Genetic Impacts – Empirical Evidence

Living in a radioactive environment such as the areas at Chernobyl could have many potential impacts on the surrounding ecosystem, such as a reduction in lifespan of resident species, increased cancer risks, and a reduction in diversity (both genetic and species diversity) to name a few.

Several recent studies have examined the genetic effects of chronic exposure to radiation using naturally occurring species in the Chernobyl area. One examined population structure of the bank vole, *Clethrionomys glareolus*, from both contaminated and noncontaminated sites. This species was chosen as a model system because it contained the highest levels of internal radiation (^{137}Cs and ^{90}Sr) among rodents in the area. The researchers used DNA sequence data from the mitochondrial control region to define haplotypes and compared haplotype frequencies of chronically exposed individuals to those in control sites. This study, along with a subsequent study, monitored the structure of these populations over several years. They found that genetic diversity was consistently higher in contaminated sites as compared to control sites. They concluded that these observations could be caused by several factors, including increased mutation rate as a result of exposure to radiation, or increased genetic variation due to immigration into the exposed areas. Another study done with the same model system looked at the genetic effects above the DNA level showed that frequencies in micronuclei (chromosome breaks) are not increased in bank voles living in Chernobyl when compared to those outside the exposed area.

Additional experiments were designed to show whether or not an increase in mutation rate was occurring in animals chronically exposed to radiation at Chernobyl. One particular study exposed a transgenic mouse, Big Blue, to radiation in the most contaminated area at Chernobyl (along with individuals at a control site) for 90 days. The mice received a cumulative dose of 3 Gy of gamma-irradiation. Following exposure, mutation rates were estimated from control and experimental groups. They found that mutation rates in exposed individuals were not significantly different from those not exposed to radiation. This study was important not only for understanding the genetic consequences to animals living at Chernobyl, but it showed that chronic exposure to low doses of radiation does not have the same effect as acute doses of the cumulative amount.

Experiments like these performed at Chernobyl have immensely increased the understanding of genetic effects of exposure to radiation in a natural setting. Although there were undoubtedly extreme negative effects on populations immediately following the meltdown, populations have rebounded and are flourishing. The studies examining the effects on rodents can be used to estimate the risks to humans exposed to the same toxicant. Future studies are being planned to examine the possibility of hormesis occurring, such that exposure to small amounts of radiation could increase the production of repair mechanisms in order to decrease the amount of DNA mutations. With all of the advancements in our understanding of the effects radiation on genetics and the environment, there is still much work to be done. The disaster at Chernobyl, though detrimental in many ways, has been a valuable learning experience for many disciplines.

See also: Ecotoxicology; Radiation Toxicology, Ionizing and Nonionizing; Three Mile Island.

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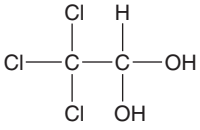
Chloral Hydrate

Michael Wahl

This article is a revision of the previous print edition article by Gregory P Wedin, volume 1, pp. 298–299, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 302-17-0
- Synonyms: Noctec; 'Knockout drops'; 'Mickey finn'; Choral; Hydrated choral; Chloralex; Chloralvan; Novochlorhydrate; Chloraldural; Chloraldurat; Tricholralacetaldehyde, hydrated; Trichloroethylidene glycol; 2,2,2-Trichlorethane-1,1-diol
- Chemical/Pharmaceutical/Other Class: Chloral derivative
- Chemical Formula: $CCl_3CH(OH)_2$
- Chemical Structure:



Uses

Chloral hydrate is used as a sedative–hypnotic agent. It is also a drug of abuse.

Exposure Routes and Pathways

Ingestion of oral dosage forms is the most common route of both accidental and intentional exposures to chloral hydrate. It is available as capsules, tablets, an oral solution, and rectal suppositories.

Toxicokinetics

Chloral hydrate is rapidly absorbed from the gastrointestinal tract following oral or rectal administration. It produces its pharmacologic action within ~30 min. Chloral hydrate is rapidly metabolized by alcohol dehydrogenase to trichloroethanol, which is pharmacologically active. A small amount is metabolized to an inactive metabolite, trichloroacetic acid. Trichloroethanol, in turn, is either conjugated with glucuronic acid to form urochloralic acid or oxidized to trichloroacetic acid. Chloral hydrate has a half-life of only a few minutes, whereas the half-life of trichloroethanol ranges from 4 to 14 h. The half-life of trichloroethanol may be prolonged following overdose.

Both chloral hydrate and trichloroethanol are highly lipid soluble. The apparent volume of distribution of chloral hydrate and trichloroethanol is 0.6–0.75 and 0.6–1.6 l kg^{-1}, respectively. Trichloroethanol is ~40% protein bound. The active and inactive metabolites of chloral hydrate are excreted primarily in the urine. The principal metabolite excreted in the urine is trichloroacetic acid and its glucuronide conjugate.

Mechanism of Toxicity

Chloral hydrate is a central nervous system (CNS) depressant. It is probably responsible for early depressant effects, but prolonged CNS depression is largely due to trichloroethanol. The mechanism by which chloral hydrate and trichloroethanol depress the CNS is not completely known.

Acute and Short-Term Toxicity (or Exposure)

Animal

As in humans, chloral hydrate is used in veterinary medicine as a sedative and hypnotic. Similar therapeutic and toxic effects are seen in humans and in animals.

Human

Chloral hydrate is an irritant to the gastrointestinal tract. Ingestion of chloral hydrate may cause nausea, vomiting, and diarrhea. Gastric perforation and esophageal stricture have been reported in cases of chloral hydrate overdose. Acute ingestion of 2 g is likely to lead to toxic symptoms. The lethal dose in adults is ~5–19 g. Lethargy progressing to deep coma, respiratory depression, hypotension, and hypothermia are characteristic toxic manifestations of chloral hydrate overdose. Unlike most other sedative–hypnotic agents, overdose with chloral hydrate may result in serious atrial and ventricular arrhythmias. Hepatic and renal dysfunction may develop.

Chronic Toxicity (or Exposure)

Animal

Two carcinogenicity studies of chloral hydrate in drinking water in rats showed no increase in tumors at any site. In a separate study of chronic chloral hydrate exposure in female mice, a slight increase in hyperplasia and slight increase in the incidence of adenoma in

the pituitary gland pars distalis were noted at the highest dose. Some studies have shown that chloral hydrate causes hepatocellular tumors in male mice.

Human

Prolonged administration of chloral hydrate may lead to the development of gastritis, skin eruptions, and renal damage. Chronic use of high doses may produce psychologic and physical dependence. Abrupt discontinuation may lead to delirium and seizures.

In Vitro Toxicity Data

Studies of chloral hydrate on porcine brain tubulin assembly assay demonstrated reduced assembly. Chinese hamster embryonic diploid cell studies of carcinogenicity demonstrated chromosomal damage only at the higher concentrations tested.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Gastrointestinal decontamination procedures should be used as appropriate based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used to adsorb chloral hydrate. The patient's level of consciousness and vital signs should be monitored closely. Obtunded patients with reduced gag reflex should be intubated. Respiratory support including oxygen and ventilation should be provided as needed. There is no antidote for chloral hydrate. Hypotension should be treated with standard measures including intravenous fluids, Trendelenburg positioning, and dopamine hydrochloride by intravenous infusion. Cardiac arrhythmias have been succesfully managed with beta blockers such as propenolol or esinolol. Class IA antiarrhythmics should be avoided, as these may worsen conduction disturbances especially with Torsades. Forced diuresis is of no value as a means to enhance the elimination of chloral hydrate. Hemodialysis and hemoperfusion may be useful in severe cases in which standard supportive measures are inadequate. Withdrawal reactions should be managed with barbiturates or other sedative–hypnotic agents.

See also: Charcoal; Gastrointestinal System.

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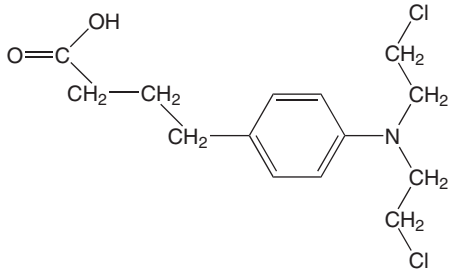
Chlorambucil

Larry J Dziuk

- Chemical Abstracts Service Registry Number: CAS 305-03-3
- Synonyms: CB 1348; 4-[Bis(2-chloroethyl)amino]benzenebutanoic acid; 4-[*p*-Bis(2-chloroethyl)aminophenyl]-butyric acid; Amboclorin; Leukeran; Chloraminophene
- Chemical Formula: $C_{14}H_{19}Cl_2NO_2$
- Chemical Structure:



Uses

Chlorambucil is an alkylating agent that retards or stops growth of cancer cells. It is used to treat chronic lymphocytic leukemia, malignant lymphomas such as lymphosarcoma, giant follicular lymphoma, ovarian cancer, and Hodgkin's disease.

Chlorambucil is also used as an immunosuppressive agent for treatment of lupus erythematosus, Waldenstrom's macroglobulinemia, glomerular nephritis, nephritic syndrome, psoriasis, Wegener's granulomatosis, chronic hepatitis, vasculitis associated with rheumatoid arthritis, and autoimmune hemolytic anemia with cold agglutins. The Food and Drug Administration (FDA) approved chlorambucil for use as a prescription drug in 1969.

The chemical also has been investigated for use as an insect chemosterilant.

Exposure Routes and Pathways

Ingestion is the primary route of exposure. Chlorambucil is administered orally in tablets containing

2 mg active ingredient. Continuous and intermittent oral schedules are used as part of the treatment regimen. Continuous treatment consists of initial daily doses of 0.1–0.2 mg kg^{-1} of body weight for 3–6 weeks. Intermittent treatment consists of 2 week treatments of 10–20 mg daily followed by rest periods of 2–4 weeks.

Occupational exposure may occur during production, formulation, packaging, and administration of the pharmaceutical. Possible exposures consist of inhalation, incidental ingestion, and dermal contact.

Toxicokinetics

Orally administered, chlorambucil is 70–80% bioavailable. It is extensively metabolized in the liver. From 15% to 60% of metabolites of the drug appear in urine after 24 h. Less than 1% of urinary excretion is the intact drug.

Mechanism of Toxicity

Chlorambucil is a cytotoxic agent. As a bifunctional alkylating agent, it causes breaks in DNA thus interfering with DNA replication and transcription.

Acute and Short-Term Toxicity (or Exposure)

Animal

When administered intraperitoneally, the LD_{50} values were 30 and 14 mg kg^{-1} in mice and rats, respectively.

Human

Possible effects of overexposure in the workplace include bone marrow toxicity and symptoms of hypersensitivity (skin rash, hives, itching, and difficulty breathing). Seizures rarely occur after administration of high doses. Children with nephritic syndrome and patients receiving high pulse doses of chlorambucil or with a prior history of seizures are at greatest risk of developing seizures. Myelosuppression, hyperuricemia, and pulmonary toxicity characterized by dry cough, fever, rales, and tachypnea may develop.

Nausea, vomiting and loss of appetite may occur during treatment. Chlorambucil chemotherapy may cause a temporary reduction in the production of blood cells by the bone marrow. Reduction in blood cells can result in anemia, risk of bruising or bleeding, and infection. This effect can begin from 10 to 14 days after the treatment has been given and may last for a few days. The extent to which the blood cell count is reduced depends on the dose of chemotherapy received and which other chemotherapy drugs, if any, were given in combination.

Chronic Toxicity (or Exposure)

A known or probable human mutagen, chlorambucil has been shown to be teratogenic, mutagenic, and carcinogenic in experimental models.

Animal

Chlorambucil is carcinogenic in experimental animals. When administered intraperitoneally to rats, lymphosarcomas, myelogenous leukemia, and reticulum cell sarcomas were noted. Mice receiving the material intraperitoneally developed lymphosarcomas, lung adenomas, and adenocarcinomas. Female mice developed ovarian neoplasms.

Human

Chlorambucil is known to be a human carcinogen based on sufficient evidence of carcinogenicity in humans. Leukemia was reported in a number of epidemiological studies in which chlorambucil was used as a chemotherapeutic agent either alone or in combination with other agents. The risk of developing cancer increases with increasing dose and time of treatment.

In Vitro Toxicity Data

Mutagenic in bacterial mutation assay (Ames), *in vitro* cytogenetics assay, and mouse micronucleus test.

Clinical Management

Medical treatment in cases of overexposure should be treated as an overdose of a cytotoxic agent. No specific antidotes are recommended. Symptoms of adverse reaction include mouth blistering, fatigue, rash, seizures, dizziness, unusual bruising or bleeding, cough, sore throat, congestion, difficulty in breathing, black tarry stools, and red urine. Because chlorambucil is cytotoxic, bone marrow toxicity can occur. Dosages are reduced if the results of medical monitoring indicate that blood cell counts are lowered.

Environmental Fate

Chlorambucil has limited water solubility, will not readily enter air, will not partition into fats, and is not likely to partition into and persist in soil or sediments. It is chemically unstable in water; hydrolysis

may be a significant depletion mechanism. Photolysis is likely a significant depletion mechanism as well.

Ecotoxicology

Chlorambucil is harmful to aquatic organisms such as daphnids. Solutions as low as 0.13 mmol caused mortality in sea urchin embryos.

The material is not toxic to activated sludge microorganisms.

Other Hazards

Chlorambucil is noncombustible, but toxic, corrosive, or flammable thermal decomposition products such as carbon monoxide, hydrogen chloride, and oxides of nitrogen may form if involved in a fire. Because of possible aquatic toxicity concerns, measures should be taken to keep firefighting water from entering surface waters.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration regulates chlorambucil under the Hazard Communication Standard and as a chemical hazard in laboratories, although there is no specific occupational exposure standard for the chemical. The Food and Drug Administration regulates clinical use of the drug and labeling requirements under the Food, Drug, and Cosmetic Act.

Relevant Website

http://ehp.niehs.nih.gov – The National Toxicology Program's Tenth Annual Report on Carcinogens.

Chloramphenicol

Greene Shepherd

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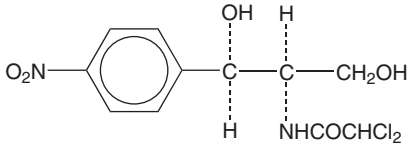
- Chemical Abstracts Service Registry Number: CAS 56-75-7
- Synonyms: AK-Chlor; Alcon opulets chloramphenicol; Amphicol; Aquamycetin; Antibiopto; Arcomicetina; Biomicin; Bioticaps; Cafenolo; Cèbenicol; Chemyzin; Chloramol; Chloromycetin; Chloroptic; Chemicetina; Chlomin; Chloratets; Chloramex; Chlorofair; Chlorsig; Chlorcol; Chloromycetin; Cloromicetin; Cloramffen; Cloramplast; Clorbiotina; Clorfenicol wolner; Clorofenicina; Cloromisol; Cloromoin; Cloroptic; Cloromicetin; Cutispray No. 4; Detreomycin
- Chemical/Pharmaceutical/Other Class: Antibiotic with both bacteriocidal and bacteriostatic properties
- Chemical Formula: $C_{11}H_{12}Cl_2N_2O_5$
- Chemical Structure:



Uses

Chloramphenicol is used as an antibiotic to treat infections of gram negative and anaerobic microorganisms.

Exposure Routes and Pathways

Oral and parenteral are the most common routes of exposure with chloramphenicol.

Toxicokinetics

Chloramphenicol is well absorbed from the gastrointestinal tract; peak serum concentrations are reached 1 or 2 h after an oral dose. Peak serum concentrations after ingestion equal those achieved after intravenous administration. Absorption after intramuscular injection is highly variable with peak concentrations achieved being 5–65% of those reached after intravenous or oral administration. The apparent volume of distribution is 0.6–1.6 l kg^{-1}. Approximately 50% of the drug is bound to plasma proteins (primarily albumin). Chloramphenicol diffuses into breast milk and readily crosses the placenta; fetal blood levels are 30–80% of maternal serum concentrations. Inactivation occurs primarily by hepatic glucuronidation. Hepatic insufficiency is known to decrease metabolism but rarely requires dose modification. Chloramphenicol has an elimination half-life of 1–4 h. Urinary excretion of unchanged

chloramphenicol is ~12% in adults and 20% in children; the remainder is eliminated as drug metabolite. Dosage modifications may be necessary for patients with renal insufficiency.

Mechanism of Toxicity

Chloramphenicol has a narrow therapeutic index with serum concentrations of 10–20 $\mu g\,ml^{-1}$ considered therapeutic and $>25\,\mu g\,ml^{-1}$ is considered toxic. Toxicity occurs through suppression of DNA and RNA synthesis in human as well as bacterial cells.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chloramphenicol is used as a veterinary antibiotic. It has a low level of animal toxicity.

Human

Acute, single overdoses of chloramphenicol in children and adults produce no significant toxicity. However, in neonates, a syndrome ('gray baby syndrome') of vomiting, irregular respiration, abdominal distension, diarrhea, cyanosis, flaccidity, hypothermia, and death may occur. The gray baby syndrome typically begins 2–9 days after the initiation of chloramphenicol. It has been associated with the administration of doses >200 mg daily. Its etiology may be the combination of immature hepatic glucuronidation in conjunction with diminished urinary excretion of the parent drug, secondary to an immature renal system.

Chronic Toxicity (or Exposure)

Animal

Three-day-old Swiss mice given IP injections of 20, 40, or 100 $mg\,kg^{-1}$ body weight for 3 months developed splenomegaly, hepatomegaly, and adenopathy. Mice chronically fed chloramphenicol in drinking water for 2 years had higher rates of lymphomas than controls.

Human

Bone marrow suppression occurs in all individuals who take chloramphenicol regularly. This is primarily manifested by a reversible fall in reticulocyte count. Depressions in platelet count may also occur. Dose-related bone marrow suppression is associated with serum concentrations $\geqslant 25\,\mu g\,ml^{-1}$. Chloramphenicol can also produce severe, idiosyncratic bone marrow toxicity resulting in aplastic anemia. This may occur more commonly in those who undergo prolonged therapy. The reported incidence of severe bone marrow toxicity ranges from 1:30 000 to 1:100 000. Greater myelotoxicity occurs in uremic patients. Other adverse effects include hypersensitivity reactions (including rash and fever), paresthesias, and optic neuritis. Chloramphenicol may also inhibit hepatic microsomal activity, specifically impairing the clearance of drugs including warfarin, phenytoin, and many other drugs.

In Vitro Toxicity Data

Chloramphenicol has been shown to decrease DNA synthesis on rat bone marrow and liver as well as in rabbit bone marrow cells *in vitro*.

Clinical Management

No specific treatment is available for chloramphenicol exposure. Hemodialysis is ineffective. Charcoal hemoperfusion or whole blood exchange transfusion has been recommended in infants with serum concentrations of $>50\,\mu g\,ml^{-1}$. Management is primarily supportive.

See also: Toxicity, Chronic; Warfarin.

Further Reading

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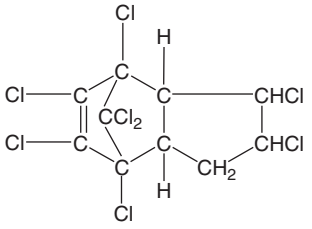
Scott JL, Finegold SM, and Belkins GA (1965) A controlled double-blind study of the hematologic toxicity of chloramphenicol. *New England Journal of Medicine* 272: 1137–1142.

Chlordane

Benny L Blaylock

- Chemical Abstracts Service Registry Number: CAS 57-74-9
- Synonyms: 1,2,4,5,6,7,8,8-Octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan; Chlordane; Chlordane; Belt; Chlor Kil; Chlortox; Corodane; Gold Crest C-100; Kilex Lindane; Kypchlor; Niran; Octachlor; Synklor; Termex; Topiclor 20; Toxichlor; Velsicol 1068
- Chemical/Pharmaceutical/Other Class: Synthetic organochlorine cyclodiene insecticide
- Chemical Formula: $C_{10}H_6Cl_8$
- Chemical Structure:



Uses

Chlordane is used as an insecticide.

Exposure Routes and Pathways

Oral, dermal, and inhalation are all primary exposure pathways. In addition, since chlordane readily crosses the placenta, *in utero* exposure may also occur.

Toxicokinetics

Absorption of chlordane occurs through the skin, the gastrointestinal tract, and the lungs. This is enhanced when the agent is in an organic solvent. Rats retained 77% of the dose absorbed through the respiratory tract. Gastrointestinal absorption is dependent on the amount of lipid-containing material in the gut.

As with other organochlorine cyclodiene compounds, chlordane is metabolized mainly by the liver microsomal cytochrome P-450 system. Several metabolites are produced including chlordene chlorohydrin, monohydroxylated dihydrochlordene, oxychlordane, and relatively smaller but similar amounts of 1,2-dichlorochlordene, 1-hydroxy-2-chlorochlordene, 1-hydroxy-2-chloro-2,3-epoxychlordene, 1,2-hydroxychlordene, trihydroxydihydrochlordene, and β-glucuronide-1-hydroxydihydrochlordene. Oxychlordane is considered the main toxic metabolite and is 20- to 25-fold more toxic than the parent compound.

Due to its high degree of lipophilicity, chlordane is readily sequestered in adipose tissue and in the kidneys, muscles, liver, and brain. The α isomer is primarily stored in adipose tissue while the γ isomer is stored to a greater extent in kidney than in fat.

Due to slow absorption and metabolism, $\sim$80% of an oral chlordane dose is excreted in urine and feces. Chlordane has also been found in human breast milk. Excretion of orally administered chlordane is slow and can take days or weeks. In two accidental poisoning reports, the half-life of chlordane in blood serum of the patients was 88 and 21 days.

Mechanism of Toxicity

Chlordane blocks the neuronal uptake of chloride ions by blocking the activity of γ-amino butyric acid. This results in only a partial depolarization of activated neurons leading to an uncontrolled excited condition. Additionally, chlordane inhibits Ca^{2+},Mg^{2+}-adenosine triphosphate (ATPase) and Na^{+},K^{+}-ATPase functions, leading to increased concentrations of intracellular free calcium in neurons and the release of neurotransmitters. This neurotransmitter release potentiates depolarization of adjacent neurons in a chain reaction manner, propagating stimuli through the central nervous system (CNS).

Acute and Short-Term Toxicity (or Exposure)

Animal

Rats exposed to 413 mg m^{-3} by inhalation showed neurological signs of toxicity including death, abnormal respiratory movements, salivation, and convulsions. Epithelial degeneration and debris in the alveoli were also observed. Hepatotoxicity included centrilobular hepatocytes enlargement; elevated liver enzymes have been reported after acute exposure. Oral exposure also produced convulsions, paralysis, and death. The acute oral LD_{50} for rats is 200–700 mg kg^{-1}, while in mice it is 145–430 mg kg^{-1}. Animals experience toxic effects from chlordane similar to those of other organochlorine insecticides except that tremor is absent. CNS

involvement produces hyperexcitability and convulsions.

Human

Acutely, the first symptoms may be convulsions or they may be nausea, vomiting, and gastrointestinal pain. Convulsions usually occur within 0.5–3 h after ingestion or dermal exposure. They are often accompanied by confusion, incoordination, excitability, or, in some cases, coma. Respiratory arrest may result from exposure to high doses. Chlordane alters liver function that can lead to therapeutic drug interactions. Recovery following convulsions has been observed in infants with dosages of 10–28 mg kg^{-1} and in adults at a dosage of 32 mg kg^{-1}. Death has been observed after dermal exposure to 425 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

Rats exhibited decreased body weights, liver lesions, and increased kidney weights. There are also reports of CNS damage including convulsions. Mice prenatally exposed to chlordane have a suppressed immunity as measured by decreased contact hypersensitivity to oxazolone and delayed macrophage activation. No reproductive or teratogenic effects have been observed in animal testing. In rats undergoing a 2 year chronic feeding study, marked liver and kidney damage was noted at 150 and 300 ppm. The incidence of hepatomas is increased in mice, but not rats, fed chlordane.

Human

Chronically, symptoms include anemia, leukopenia, thrombocytopenia, jaundice, and abnormal blood serum chemistry results. Reproductive or teratogenic effects do not appear to be present. Mutagenicity testing generally indicates that chlordane is not mutagenic. Chlordane is classified as group 2B (possibly carcinogenic to humans) by the IARC.

Clinical Management

Treatment is symptomatic. Anticonvulsive treatment with diazepam or phenobarbital is usually effective for control of convulsions. Cholestyramine treatment has been suggested for increased elimination; this treatment has not been proven beneficial for chlordane, although contaminating heptachlor excretion was increased. Activated charcoal administered as a slurry is recommended. Gastric lavage may be useful if performed quickly after ingestion (within 1 h). Emesis is not recommended due to potential CNS depression or seizures.

Environmental Fate

The half-life of chlordane in soil is approximately 4 years. Residues of this highly persistent chemical have been found in excess of 10% of the initially applied amount 10 years or more after application. Chlordane does not chemically degrade nor does it biodegrade in soils. Chlordane binds rapidly to soil particles and stays adsorbed to clay soil. Combined with its insolubility in water, this produces a low potential for ground water contamination. Sandy soil, on the other hand, allows chlordane to pass into ground water.

Evaporation is the major route by which chlordane is removed from soil. Photochemical breakdown by exposure to sunlight plays a very minor role in eliminating chlordane from soil. In water, the major mechanism by which chlordane exits is by volatilization or by adsorption to sediments. Therefore, surface water almost always has very little chlordane while the higher concentrations are found in suspended solids and sediments.

Ecotoxicology

The toxicity of chlordane for fish and fresh water invertebrates is high. Bioaccumulation is a significant factor for chlordane. It has been estimated that bioaccumulation factors for chlordane are in excess of 3000 times background water concentration. The LC_{50} (96 h) for chlordane in bluegill is 0.057–0.075 mg l^{-1} and 0.042–0.090 mg l^{-1} in rainbow trout.

In birds, chlordane is only moderately to slightly toxic. The LD_{50} in bobwhite quail is 83 mg kg^{-1}. The 8 day dietary LC_{50} for chlordane is 858 ppm in mallard ducks, 331 ppm in bobwhite quail, and 430 ppm in pheasant. In addition, chlordane has been shown to be highly toxic in earthworms and in bees.

Other Hazards

Chlordane itself is noncombustible. However, the materials used as carrier solvents (e.g., kerosene) are often flammable making the mixture in use a flammable or combustible mixture. Toxic gases and vapors such as hydrogen chloride, chlorine, phosgene, and carbon monoxide may be released in a fire involving chlordane.

Chlordane is incompatible with strong oxidizers and alkaline reagents. Chlordane will attack some

forms of plastics, rubber, and coatings. It is corrosive to iron and zinc.

Exposure Standards and Guidelines

- ADI is $0.000\,5\,mg\,kg^{-1}\,day^{-1}$.
- MCL is $0.002\,mg\,l^{-1}$.
- RfD is $0.000\,06\,mg\,kg^{-1}\,day^{-1}$.
- PEL is $0.5\,mg\,m^{-3}$ (8 h).

See also: Carbon Monoxide; Charcoal; Chlorine; Chlorine Dioxide; Cyclodienes; Diazepam; Organochlorine Insecticides; Phosgene; Pollution, Soil; Pollution, Water.

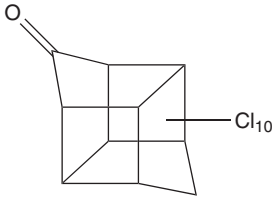
Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicology Profile for Chlordane.
http://extoxnet.orst.edu – Extension Toxicology Network, a collaborative effort of University of California, Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho.
http://toxnet.nlm.nih.gov – Specialized Information Systems, National Library of Medicine. Search for Chlordane.
http://www.osha-slc.gov – US Department of Labor, Occupational Safety and Health Administration.
http://risk.lsd.ornl.gov – Toxicity Summary for Chlordane, Risk Assessment Information System.

Chlordecone

Harihara M Mehendale and Zhengwei Cai

- Chemical Abstracts Service Registry Number: CAS 143-50-0
- Synonyms: 1,3,4-Metheno-2*H*-cyclobuta-(cd)pentaten-2-one; 1,lα,3,3α,4,5,5,5α,5β,6-Decachloroctahydro; Kepone; GC 1189; Ciba 8514; ENT 16,391; NCI-C00191; Decachloroketone; Decachlorotetracyclodecanone; Decachlorotetrahydro-4,7-methanoindeneone
- Chemical/Pharmaceutical/Other Class: Polycyclic chlorinated hydrocarbon
- Chemical Formula: $C_{10}Cl_{10}O$
- Chemical Structure:



Uses

Chlordecone was used as an insecticide and fungicide. Its use was largely terminated following contamination of the James River estuary (Virginia, USA) as a result of improper production practices.

Background Information

Kepone is the registered trade name of Allied Chemical Corporation for chlordecone. Kepone was invented in 1940 by Everett Gilbert, a researcher in the General Chemical division of Allied Chemical Corporation. It was used as an ingredient of several pesticides. Kepone was produced from 1940 to 1965 in small amounts either by Allied itself or by outside firms which sold their product to Allied. In 1966, Allied moved the Kepone project to Hopewell, Virginia. In early 1970s, Allied's international sales of Kepone began to expand and the company started to explore other arrangement for Kepone production. From 1974 to 1975, the Life Science Products, which was associated with Allied, became the sole producer of Kepone in the United States. During the 16 months of operation, over half of the 133 employees in the factory and many residents of the immediate vicinity had evidence of chlordecone intoxication. The illegally discharged chlordecone into the nearby James River by the factory resulted in extensive contamination of the water and marine life throughout the Tide-water Region of Virginia. In July 1975, the Life Science Products plant was officially closed.

Exposure Routes and Pathways

Ingestion is the most common route of exposure to chlordecone. Exposure to this agent may also occur via respiratory and dermal routes in industrial workers.

Toxicokinetics

Chlordecone is readily absorbed (>90%) from the gastrointestinal tract. Absorbed chlordecone rapidly (within 24–48 h) establishes an equilibrium of distribution among most tissues. Absorption of chlordecone may also occur through skin, especially in patients with dermatitis or skin rash. Compared to

other organochlorine pesticides, the ratio of chlordecone concentration in whole blood to that in fat tissue of patients exposed to chlordecone is much higher (1:7) and 75% chlordecone in the blood binds with albumin and high-density lipoprotein. Chlordecone is primarily stored in the liver tissue followed by the adipose tissue in both man and animals. In human, chlordecone is bioreduced to chlordecone alcohol in the liver followed by glucuronide conjugation of the alcohol metabolite. However, this metabolism is quantitatively very minor and not important toxicologically. Chlordecone is not subject to metabolism in animals studied so far, except Mongolian gerbils, which metabolize chlordecone similar to humans.

Fecal excretion is the major route of elimination and only minimal amounts of chlordecone are eliminated through urine. By 84 days, 65% of the dose is excreted in the stool and only 1.6% in the urine. A substantial amount of chlordecone representing as much as 1% of the total body content enters the intestine via biliary excretion. However, the major part of biliary chlordecone (90–95%) is reabsorbed by the intestine and recirculated to the liver (enterohepatic recirculation), while the remaining 5–10% of the biliary chlordecone entering the upper intestine appears in the feces. Elimination of chlordecone from the body is slow. The half-life of chlordecone in the blood and fat tissue is 165 and 125 days, respectively. Lactating women can also excrete substantial amounts of accumulated chlordecone through breast milk.

Mechanism of Toxicity

Chlordecone inhibits brain mitochondrial and synaptisomat membrane-bound Na^+,K^+-ATPase and oligomycin-sensitive Mg^{2+}-ATPase activity and thus may result in blocked cellular uptake and storage of neurotransmitters such as catecholamine and γ-aminobutyric acid, leading to neurotoxicity. Inhibition of Mg^{2+}-ATPase by chlordecone and the consequently deceased hepatic mitochondrial energy production have been postulated for the mechanism of chlordecone-induced hepatic biliary dysfunction. Chlordecone is an inducer of hepatic microsomal drug metabolizing system at high doses. Dietary exposure to nontoxic levels of chlordecone (10 ppm) has been shown to cause a 67-fold increase in lethality of nonlethal dose of carbon tetrachloride in laboratory rats. The mechanism for chlordecone amplification of chloromethane hepatotoxicity and lethality is incapacitated cell division due to decreased energy owing to the disrupted intracellular calcium homeostasis. Because tissue repair cannot occur, limited liver injury progresses unabated, leading to hepatic failure and animal death. Chlordecone impairs the reproductive system by mimicking the effects of excessive estrogen.

Acute and Short-Term Toxicity (or Exposure)

Animal

Exposure to chlordecone in animals may result in increased excitability and liver to body weight ratio, tremor, loss of weight, dermatologic changes, testicular atrophy and increases in hepatocellular carcinoma. Rats, mice, chicks, and quails are sensitive to the toxicity of chlordecone. The LD_{50} for oral administration of chlordecone in corn oil is 71, 126, 250, and 480 mg kg^{-1} for rabbits, rats, dogs, and chicks, respectively. In rabbits, the dermal LD_{50} for chlordecone is 434 mg kg^{-1}. When mice were given daily doses of 50, 25, or 10 mg kg^{-1}, 90% of the animals died within 5, 9, or 24 days, respectively. Chlordecone tremendously potentiates lethal effects and hepatotoxicity of chloromethane in rodents.

Human

There are no reports of death in humans exposed to chlordecone. The major target organs of chlordecone toxicity are the nervous system, the liver, and the testes.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to chlordecone resulted in significant weight loss in rats fed diets containing more than 10 ppm chlordecone for many months. Depressed growth has also been observed in pregnant rats given 2 mg kg^{-1} day^{-1} of chlordecone, in mice fed 40 ppm chlordecone, in laying hen fed 75 ppm and in quail fed 300 ppm chlordecone. Neuromuscular toxicity as evidenced by tremor following chronic exposure to chlordecone was observed in rats, chicks, Japanese quail, and mice. Liver toxicity as evidenced by the increased size of the liver relative to total body weight has been observed in rat, quail mice and dogs chronically treated with chlordecone. Chlordecone exposure through dietary consumption results in blocked and impaired reproductive function in birds and rodents and causes carcinogenesis in rats and mice.

Human

Chlordecone poisoning may cause loss of weight and skin rash. The symptoms of neuromuscular toxicity include an irregular nonpurposive waking tremor (rate, 6–8 Hz) involving the extremities, head, and trunk, and opsoclonus, an unusual oculomotor disorder consisting of chaotic eye movements causing blurred vision. Onset of tremor varies from 5 days to 8 months after initial exposure to chlordecone depending on the duration and intensity of exposure to this compound. Chlordecone poisoning causes liver and spleen enlargement, mitochondrial changes, fatty infiltration of hepatocytes, proliferation of endoplasmic reticulum, and impairment of biliary excretion of selective organic anions. Chlordecone poisoning also causes a decline in sperm number associated with abnormally low percentages of motile sperm. In most cases, these symptoms are reversible upon cessation or exposure. Removal of this chemical from the tissues is accompanied by the disappearance of clinical manifestation of toxicity.

Clinical Management

Removal of chlordecone from the body is the most important measure to take. In cases of ingestion, emesis is indicated as the treatment in other chlorinated hydrocarbon insecticide intoxications, unless the patient is comatose or has lost the gag reflex. Emesis should be followed by administration of activated charcoal and saline cathartics. Oil-based cathartics should be avoided. Administration of cholestyramine, which has been shown to bind chlordecone in the intestinal tract, is an effective way to increase fecal excretion of chlordecone and to accelerate the removal of chlordecone from the blood and other tissues.

Ecotoxicology

Chloredecone is a persistent compound. When it enters water system, marine animals accumulate the residue through the ingestion of plankton which absorb the poison. While these animals may not be killed, they become chlordecone carriers and contribute to the bioaccumulation of the compound in higher animals, including man.

See also: Organochlorine Insecticides. Toxicity, Chronic; Toxicity, Subchronic.

Further Reading

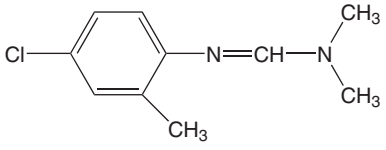
Guzelian PS (1982) Comparative toxicology of chlordecone (kepone) in humans and experimental animals. *Annual Review of Pharmacology and Toxicology* 22: 89–113.

Mehendale HM (1990) Potentiation of halomethane hepatotoxicity by chlordecone: A hypothesis for the mechanism. *Medical Hypotheses* 33: 289–299.

Chlordimeform

Paul R Harp

- Chemical Abstracts Service Registry Numbers: CAS 6164-98-3 (base); CAS 19750-95-9 (salt)
- Synonyms: *N′*-(4-Chloro-*o*-tolyl)-*N*,*N*-dimethylformamidine; Bermat; Chlordimeforme; Chlorodimeform; Chlorophedine; Chlorophenamidine; Fundal; Fundex; Galecron; Spanone; ENT 27335 (base); ENT 27567 (salt); OMS 1209; SHA 059701
- Chemical/Pharmaceutical/Other Class: Organonitrogen acaricide
- Chemical Structure:



Uses

Chlordimeform was used to control mites, ticks, and some members of the Lepidoptera order. Chlordimeform is no longer used in the United States due to its carcinogenic potential and has been withdrawn by the Codex Alimentarius Commission (FAO/WHO).

Exposure Routes and Pathways

The most common accidental exposure pathway was dermal. Inhalation during processing and packaging was also reported as well as suicide attempts through ingestion.

Toxicokinetics

The base formulation readily penetrates the skin but the salt form, which is much more water soluble, does not. Chlordimeform is rapidly demethylated to

demethylchlordimeform and didemethylchlordimeform, both of which are more toxic (based on acute oral LD_{50} values in mice) than the parent compound. Other active but less toxic metabolites include *N*-formyl-4-chloro-*o*-toluidine, 4-chloro-*o*-toluidine, 3-(4-chloro-*o*-tolyl)urea, 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea, and 1-methyl-3-(4-chloro-*o*-tolyl)urea. Neither chlordimeform nor any of its metabolites have been shown to accumulate in any specific tissue.

Studies using animals treated with radiolabeled chlordimeform indicated the majority of the radioactivity to be excreted in the urine within 24 h of the last treatment. Small amounts of radioactivity were detected in the bile and feces.

Mechanism of Toxicity

Animal studies have detected a variety of pharmacological and biochemical changes in response to chlordimeform exposure. The cause of death following acute exposure appears to be cardiovascular collapse. Chlordimeform interacts directly with and inhibits α2-adrenergic receptors in mammalian systems. Lethal doses of chlordimeform cause decreases in cardiac contractility and peripheral resistance resulting in severe hypotension. Respiratory arrest also occurs but is thought to be secondary to the cardiovascular effects. The effects of chlordimeform on the cardiovascular system share similarities with those seen with local anesthetics such as procaine. Chlordimeform also inhibits monoamine oxidase and acts as an uncoupler of oxidative phosphorylation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chlordimeform elicits cardiovascular toxicity. α2-Adrenergic receptors are modulated in rat brain regions following systemic chlordimeform treatment. One study reported that chlordimeform influenced endocrine regulation within the reproductive system (decreased gonadotropin and testosterone levels) by disrupting hypothalamic α-adrenergic activity.

Human

In addition to the cardiovascular and respiratory effects identified in animal studies, severe hemorrhagic cystitis, gross hematuria, proteinuria, swollen liver, decreased appetite, fatigue, vertigo, and dermatitis have been reported in humans following exposure to chlordimeform.

Chronic Toxicity (or Exposure)

Animal

Chlordimeform was negative in the mouse *in vivo* heritable translocation assay when administered by gavage at the maximum tolerated dosage for 7 weeks.

Human

A carcinogenic potential of some chlordimeform metabolites has been demonstrated. The US EPA's Office of Pesticide Programs has classified chlordimeform as a Group B2 – Probable human carcinogen based on findings of malignant hemangioendothelioma in mice.

In Vitro Toxicity Data

Chlordimeform was negative in a number of *in vitro* tests for DNA damage or mutagenicity.

Clinical Management

Ingestion should be treated with gastric lavage or by administration of activated charcoal, either of which is most effective if performed shortly after ingestion. For inhalation exposure, the victim should be removed from the exposure area and observed for signs of respiratory distress. In cases of dermal exposure, contaminated clothing should be removed and discarded. Any exposed areas of skin should be repeatedly washed with soap and water. For eye contact, flush the eyes with generous amounts of lukewarm water for a minimum of 15 min.

Treatment is basically symptomatic and supportive; no specific antidotes are available. Artificial ventilation with 100% humidified oxygen is necessary in cases of respiratory distress. If patient is cyanotic and cyanosis does not respond to oxygen administration, methemoglobin levels should be determined. Methemoglobinemia can be treated by intravenous administration of methylene blue. Support of cardiovascular function may also be required. Bladder damage can be determined by urinalysis. Hypotension may be treated with isotonic intravenous fluids. Dopamine or norepinephrine may be used if hypotension does not respond to infusion of fluids. Convulsions may be treated with intravenous benzodiazepines (diazepam or lorazepam); phenobarbital may be used if the convulsions are recurrent. Because chlordimeform is a monoamine oxidase inhibitor, foods with large amounts of

tryptophan or tyramine should be avoided and sympathomimetic drugs are contraindicated.

Ecotoxicology

Chlordimeform was only slightly toxic in frog tadpoles (48 h $LC_{50} > 35\,mg\,l^{-1}$), catfish (96 h $LC_{50} > 20\,mg\,l^{-1}$), and snails (48 h $LC_{50} > 60\,mg\,l^{-1}$).

See also: Monoamine Oxidase Inhibitors; Pesticides.

Relevant Website

http://www.inchem.org – Chlordimeform (Environmental Health Criteria 199); International Programme on Chemical Safety.

Chlorination By-Products

S Satheesh Anand and Harihara M Mehendale

Drinking water disinfection is a worldwide practice to eliminate the microbial contaminants and is considered to be one of the greatest public health advances in this century. Ever since the use of disinfectants, there is a huge drop in waterborne infectious diseases such as typhoid fever, cholera, hepatitis, and polio, which for many years posed threat to public health. The most widely used disinfectants are chlorine, ozone, chlorine dioxide, and chloramines. Among these, chlorine is the most efficient in removing microbes. The noteworthy biocidal effects of chlorine have been somewhat offset by the formation of potential toxic and carcinogenic chlorination by-products (CBPs). Hence, in order to balance the microbial and chemical risks, it is essential to understand better the chemistry, toxicology, and epidemiology of CBPs.

Formation of CBPs

Chlorine is applied as chlorine gas, powdered calcium hypochlorite ($Ca(OCl)_2$), or liquid sodium hypochlorite (NaOCl; bleach). Chlorine reacts with the organic (natural organic matter, NOM) or inorganic (bromide ion, Br^-) precursors in the water to form chlorine disinfection by-products (CBPs), including trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs), haloketones, chloral hydrate, and chloropicrin. Humic and fulvic acids are the predominant NOMs. When bromine exists, the chlorine oxidizes it to hypobromous acid/hypobromite ion ($HOBr/OBr^-$) to form bromo THMs (bromodichloromethane, BDCM, and dibromochloromethane, DBCM), HAAs, and HANs.

The formation of CBPs is influenced by pH, temperature, ammonia, carbonate alkalinity, chlorine dose, contact time, removal of natural organic matter before chlorine application, etc. Moreover, the composition of these mixtures may change seasonally resulting in higher CBPs during warm season compared to cold season. Generally, chlorinated THM, HAA, and HAN species dominate over brominated species, although the opposite may be true in high-bromide waters. A significant percentage of the total organic halogens still remain unaccounted for. CBPs are rapidly formed during the first 4–8 h, and nearly 90% of the final concentrations are formed within the first 24 h of chlorine addition to waters containing NOM.

Toxicology of CBPs

Since water chlorination produces carcinogenic, mutagenic, or possibly teratogenic by-products, several countries have laid down standards for various CBP levels.

The dominance of chlorine CBP groups generally decreases in the order of THMs, HAAs, and HANs. Among the THMs, chloroform ($CHCl_3$) and BDCM are the first and second most dominant species. Among HAAs, dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are the first and second most dominant species.

Drinking water ingestion is a predominant pathway of CBP exposure. However, exposure via inhalation, dermal contact, and also during showering, bathing, and swimming can occur. Generally, the metabolism of CBPs is higher in mice relative to rats and human metabolism is found to be similar to rats. The toxicity of the CBPs is highly dependent upon the species and strain of the rodents, some of the CBPs, especially THMs, cause kidney damage only in male rats and mice. A brief review of findings relevant to the toxicity of important CBPs follows.

Trihalomethanes

The THMs are volatile liquids at room temperature and a variety of toxic effects have been associated with short-term and long-term exposure of experimental animals at high doses. Each of the four most common THMs – $CHCl_3$, BDCM, DBCM, and

bromoform – has been shown to be carcinogenic to rodents in high-dose chronic studies. $CHCl_3$ is generally the predominant and the most extensively studied chemical of this class. The maximum contaminated limit for the THM is $100\,\mu g\,l^{-1}$. THMs administered by corn oil gavage cause significantly more toxicity than equivalent doses administered in an aqueous emulsion. However, administration via drinking water did not show any signs of toxicity. Nonetheless, bulk of the studies has been conducted using an oil vehicle.

Chloroform

Toxicokinetics $CHCl_3$ absorption is rapid and extensive after oral, dermal, and inhalation routes. $CHCl_3$ appears to distribute widely throughout the body, with high levels in liver and fat and lower levels in blood, brain, muscle, lung, and kidney. However, high levels were found in the kidney of male mice. $CHCl_3$ is rapidly metabolized and it undergoes both oxidative and reductive biotransformation through cytochrome P-450. While the oxidative metabolism produces phosgene, causing toxic effects, the reductive biotransformation forms dichloromethyl radical, which may react with the phospholipids to form adducts. The balance between the oxidative and reductive pathways depends on oxygen and $CHCl_3$ concentrations, animal species, strain, and the site of metabolism. However, oxidative metabolism is predominant. It is metabolized primarily by CYP2E1, whereas at high levels CYP2B1/2 is also involved in the metabolism. $CHCl_3$ biotransformation occurs mainly in the liver and kidney (only in male mice). In humans, $\sim$80% and 90% of the $CHCl_3$ is absorbed under inhalation and oral exposures, respectively. Absorption of dermal exposure was approximately equivalent to inhalation exposure. Human cytochrome P-450 2E1 catalyzes the oxidation of $CHCl_3$.

Acute and Short-Term Toxicity (or Exposure)
Animal The LD_{50} values ranging from 36 to $3245\,mg\,kg^{-1}$ in rats and mice depending upon strain, vehicle, and route of exposure have been reported. Acute and short-term exposures to $CHCl_3$ cause effects on the liver, kidney, central nervous system, and immune system. In male mice, kidney injury followed by cellular proliferation was evident at a dose as low as $60\,mg\,kg^{-1}$ and these effects were seen in liver at $240\,mg\,kg^{-1}$. In rats, such effects were seen at doses above $180\,mg\,kg^{-1}$. Higher lipid peroxidation and depleted GSH were observed following single $CHCl_3$ administration.

Corn oil administration of $CHCl_3$ to male mice (34, 90, 138, or $277\,mg\,kg^{-1}$) and rats (10, 34, 90, or $180\,mg\,kg^{-1}$) for 4 days or 3 weeks caused dose-dependent liver and kidney damage and cell proliferation at 4 days. While these effects were reversed with low doses, the toxic effects were severe with two high doses at 3 weeks. Mice exposed to 49, 147, 490, or $1470\,mg\,m^{-3}$ for 7 days exhibited severe liver damage at two high doses. Kidneys were affected only in the $1470\,mg\,m^{-3}$ group. In rats, the liver and kidney were affected only at highest dose. The extent of $CHCl_3$-induced damage via drinking water is significantly lower than other modes of administration. Drinking water $CHCl_3$ exposure to female mice for 90 days (34, 66, 92, 132, 263, and $400\,mg\,kg^{-1}$) caused central nervous system depression. Mild adaptive response with $66\,mg\,kg^{-1}$ or more was observed. $CHCl_3$ is not teratogenic, but induces fetotoxic effects (decreased fetal body weight). Signs of maternal toxicity (decreased body weight and changes in organ weight) were reported in rats, rabbits, and/or mice.

Human As with animals, $CHCl_3$ anesthesia may result in death in humans due to respiratory and cardiac arrhythmias and cardiac failure. Because of the relatively high frequency of 'late $CHCl_3$ poisoning' (liver toxicity), its use as anesthetic has been abandoned. There are considerable inter-individual differences in susceptibility. Some persons presented serious illness after an oral dose of 7.5 g of $CHCl_3$, whereas others survived a dose of 270 g $CHCl_3$. The mean lethal dose for an adult is estimated to be about 45 g. Chloroform can cause severe toxic effects in humans exposed to $9960\,mg\,m^{-3}$ (2000 ppm) for 60 min.

Chronic Toxicity (or Exposure)
Animal The predominant effects of long-term exposure occur in the liver and kidney, similar to those observed with short-term exposures. Hepatic effects were reported in mice, rats, and dogs administered $15–180\,mg\,kg^{-1}\,day^{-1}$. Reduced fertility, litter size, gestation index, and viability index were reported in a three-generation study in mice. Majority of the long-term exposure studies have been conducted for $CHCl_3$-induced cancer. $CHCl_3$ causes both benign and malignant tumors in experimental animals. $CHCl_3$ in corn oil given to male and female mice for 78 weeks caused liver tumor at 138 and $477\,mg\,kg^{-1}$and 238 and $477\,mg\,kg^{-1}$. However, tumor incidence was negative when $CHCl_3$ was exposed via drinking water or inhalation except liver and kidney tumors in Wistar rats and male Osborne–Mendel rats, respectively. Inhalation exposure to $CHCl_3$ in male BDF mice at 90 ppm for 2 years caused kidney tumor. $CHCl_3$ did not induce cancer in

other strains of rats and mice, though it causes acute and subchronic toxicity.

Human Higher frequency of hepatitis was observed in workers exposed to occupational $CHCl_3$ concentrations of 10–1000 mg m^{-3} for 1–4 years. In another study, workers exposed to 112–1158 mg m^{-3} for 1 or more years, nausea, lassitude, dry mouth, flatulence, depression, and scalding urination were reported without any liver abnormalities. There have been numerous reports over the last 15 years which have evaluated the relationship between chlorinated water and the incidence of bladder and colorectal cancer. Since $CHCl_3$ is the predominant by-product of water chlorination, it is believed to have caused the bladder and colorectal cancers observed in humans. However, there is no conclusive evidence to support this observation. The weight-of-the-evidence evaluation by International Agency for Research on Cancer (IARC) concluded that there is inadequate evidence for the carcinogenicity of chlorinated drinking water in humans. IARC classified $CHCl_3$ as possible carcinogen (group 2B) and EPA classified it as probable carcinogen (group B2).

Mode of Action A substantial body of data demonstrates a lack of direct *in vivo* or *in vitro* genotoxicity of $CHCl_3$. There is, however, compelling evidence that $CHCl_3$ produces cancer in rodents through a nongenotoxic/cytotoxic mode of action, with carcinogenesis resulting from events secondary to $CHCl_3$-induced cytolethality and regenerative cell proliferation. Thus, sustained toxicity would result in tumor development.

Bromodichloromethane

Bromine substitution generally decreases volatility and enhances lipid solubility (uptake into tissues), which increases biotransformation. Among the four THMs commonly found in drinking water, BDCM appears to be the more potent rodent toxicant and carcinogen. However, studies concerning BDCM toxicities are limited.

Toxicokinetics Absorption of BDCM appeared to be rapid and fairly complete. The highest levels were found in the liver, stomach, and kidney. The half-life of BDCM is estimated to be 1.5 h in rat, 2.5 h in mouse, and 0.45–0.63 min for humans. With an aqueous vehicle, the absorption and elimination were rapid as compared to an oil vehicle.

Like $CHCl_3$, BDCM also undergoes P-450-mediated oxidative and reductive metabolism and produces phosgene and dichloromethyl radical, respectively. Cytochrome P-450, CYP2E1, and CYP2B1/2, as well as a theta-class GST, have been implicated in the metabolism of BDCM. CYP2E1 is responsible for BDCM metabolism in humans.

Acute and Short-Term Toxicity (or Exposure)
Animal The oral LD_{50} values ranging from 450 to 969 mg kg^{-1} were reported in mice and rats. Following acute exposure, pathological changes in liver and hemorrhagic lesions in the kidney, adrenals, lung, and brain and clinical observations including ataxia, sedation, and anesthesia were noted. Males appear to be slightly more susceptible than females.

Five consecutive daily BDCM doses to female rats and mice by aqueous gavage proved to be both hepatotoxic and nephrotoxic to female rats (150–300 mg kg^{-1}), but only hepatotoxic to female mice (75–150 mg kg^{-1}). In subchronic studies of 10–14 days in mice and rats, mild effects on liver have been noted at doses as low as 37 mg kg^{-1} and the effects become more pronounced at 125–300 mg kg^{-1}. In the same study, kidney toxicity was noted at 74–148 mg kg^{-1}. Increased incidence of *sternebral* anomalies in fetus in rats at 50–200 mg kg^{-1} during 6–15 days of gestation was noted and the same doses produced maternal toxicity as evidenced by a 40% reduction in body weight gain.

Human Studies are not available for acute or short-term toxicity of BDCM in humans.

Chronic Toxicity (or Exposure)
Animal BDCM administration in drinking water to male rats and mice for 1 year in average daily doses of 4.4, 21, and 39 mg kg^{-1} for rats and 5.6, 24, and 49 mg kg^{-1} for mice caused proximal tubular damage. BDCM administered via corn oil gavage for 102 weeks, 5 days per week, to rats at 50 or 100 mg kg^{-1} and to mice at 25 or 50 mg kg^{-1} (males) and 75 or 150 mg kg^{-1} (females) resulted in non-neoplastic effects in liver and kidney. BDCM exposure to rats in drinking water at 22 and 39 mg kg^{-1} of body weight per day for 52 weeks resulted in decreased sperm motility.

Animal studies provide convincing evidence that BDCM is carcinogenic. BDCM caused cancer at lower doses and at more target sites than for any of the other THMs. In a 2 year bioassay, a corn oil gavage study, compound-related tumors were found in the liver, kidneys, and large intestine in rats. However, only renal and hepatic tumors were evident in mice.

Human No studies were located regarding long-term or carcinogenic effects in humans following exposure to BDCM. IARC concluded that there is sufficient evidence for its carcinogenicity in experimental animals and inadequate evidence for its carcinogenicity in humans and assigned to 2B class. EPA grouped BDCM in B2 class.

Mode of Action BDCM is a relatively weak mutagen, and its conjugation with GSH may lead to genotoxicity. It is proposed that BDCM induces cancer through cytotoxicity leading to regenerative hyperplasia and direct mutation of metabolites. The extent to which each of these processes contributed to the induction of tumors is at present unclear.

Dibromochloromethane

Compared to $CHCl_3$ and BDCM, the toxic potency of DBCM is lower.

Toxicokinetics The pattern of distribution and elimination of DBCM was very similar to that observed with BDCM, but it is the least studied THM. Presumably, metabolism proceeds via the same routes of biotransformation as described for BDCM. Oxidative metabolism of DBCM would be expected to yield a bromochlorocarbonyl rather than phosgene, and reductive dehalogenation would produce a bromochloromethyl radical. Half-lives of DBCM in rats and mice were estimated to be 1.2 and 2.5 h, respectively.

Acute and Short-Term Toxicity (or Exposure)
Animal Acute oral LD_{50} values ranging from 800 to 1200 mg kg^{-1} in mice and rats were reported. A DBCM dose of 500 mg kg^{-1} produced ataxia, sedation, and anesthesia in mice. Acute or repeated administration of DBCM caused decreased response rates and reduced aggressive behavior in mice and hamsters. The administration of DBCM in an aqueous vehicle for 14 days to male and female mice produced hepatotoxicity in both sexes at 250 mg kg^{-1}. Depressed immune function was also observed in both sexes at 125 and 250 mg kg^{-1}. DBCM-induced cardiotoxicity was reported in male rats after 4-week exposure. Corn oil gavage to mice for 13 weeks at 15, 30, 60, 125, or 250 mg kg^{-1} produced dose-dependent lesions and necrosis in kidney, liver, and salivary glands. Exposure on gestational days 6–15 caused a depression of maternal weight gain, but no fetal malformations. Short-term exposure of female mice in drinking water at 685 mg kg^{-1} day^{-1} caused reduction in fertility, reduced litter size, postnatal survival, and postnatal body weight.

Human Reports on acute or short-term effects of DBCM in humans are not available.

Chronic Toxicity (or Exposure)
Animal The chronic oral administration to rats (40 or 80 mg kg^{-1}) and mice (50 or 100 mg kg^{-1}) by corn oil gavage for 104 weeks caused only mild liver and kidney damage. Decreased serum cholesterol at 540 mg kg^{-1} and decreased triglycerides at 20 mg kg^{-1} for 2 years were reported in mice. In a two-generation reproductive study in mice caused reduction in fertility and gestational index in F_1 generation and only fertility was decreased in F_2 generation at 685 mg kg^{-1} in drinking water.

DBCM was not carcinogenic in rats in a 104 weeks corn oil gavage study at 40 or 80 mg kg^{-1}. However, according to NTP, there is equivocal evidence of DBCM carcinogenicity in male mice and some evidence of carcinogenicity in female mice.

Human Clinical case findings resulting from human exposure to DBCM have not been reported. Due to inadequate evidence for its carcinogenicity in humans and limited evidence for its carcinogenicity in experimental animals this compound was assigned to group 3: not classifiable it as to carcinogenicity to humans by IARC and EPA classified it as a possible carcinogen, group C.

Mode of Action The greater propensity for the metabolism of this compound and bromoform as compared with BDCM is difficult to reconcile with its lower carcinogenicity. A possible explanation is less bioavailability resulting from the greater lipophilicity of this compound and the use of corn oil as the vehicle of administration. However, *in vitro* studies showed that DBCM is more potent mutagenic than other THMs.

Bromoform

Toxicokinetics The distribution and elimination of bromoform resembled those of $CHCl_3$. Bromoform (and organic metabolite) elimination via exhaled breath was greater than that for all other THMs in the rat, but less than that for all other THMs in the mouse. The estimated half-life of bromoform was 0.8 h in rats and 8 h in mice. While both oxidative and reductive pathways were involved in bromoform metabolism, oxidative metabolism seems predominant. Bromoform, like DBCM, has a much greater

potential than BDCM to be conjugated by GSH to form a mutagenic intermediate.

Acute and Short-Term Toxicity (or Exposure)
Animal The acute oral LD_{50} values ranging from 1147 to 1550 mg kg^{-1} in mice and rats were noted. Liver is the major target organ. Hepatocelluar vacuolization was reported in mice administered with 300 mg kg^{-1} in drinking water. In rats, lethargy, shallow breathing, and ataxia were observed at 600 and 1000 mg kg^{-1}.

In a 14 day corn oil gavage study, 600 and 800 mg kg^{-1} were found to be lethal to both sexes of rats. Liver and kidney toxicity as well as decreased antibody-forming cells were evident at 250 mg kg^{-1} in male and female mice when administered for 14 days. The magnitude of the effects was less than that observed with the other THMs. Inhalation exposure to 240 or 24 ppm bromoform for 10 or 60 days, respectively, caused effects in liver and kidney. Fetotoxic response was observed after gavage administration at 50, 100, or 200 mg kg^{-1} on gestation days 6–15.

Human Bromoform was used in the late nineteenth and early twentieth centuries as a sedative to children suffering from whooping cough and several deaths due to overdoses have been reported. Hence, its use was discontinued. The principal causes for death were severe central nervous system depression and respiratory failure. No studies are available for short-term bromoform toxicity in humans.

Chronic Toxicity (or Exposure)
Animal Slight liver and kidney damage occurred after chronic (1 or 2 years) exposure to high dose of bromoform. No developmental and reproductive effects were observed. Dose-dependent fatty changes and minimal liver necrosis were observed at 100 or 200 mg kg^{-1} by corn oil gavage, for 103 weeks, to rats and female mice. Rats exposed for 2 years appeared to have decreased resistance to viral infection due to functional impairment of immune system. Two-year exposure to 200 mg kg^{-1} resulted in dose-related incidences of squamous metaplasia of the prostate gland in male rats.

A significant increase in female rats and nonstatistically significant increase in male rats in the incidence of adenomatous polyps or adenocarcinomas was observed at 200 mg kg^{-1} for 2 years. No neoplastic effects were associated with the exposure of mice to bromoform. Bromoform showed positive for *in vitro* mutagenicity tests.

Human Studies are not available to evaluate the chronic human toxicity of bromoform except the deaths reported following overdose of bromoform containing sedative. IARC classified bromoform as group 3 and EPA classified it as B2.

Mode of Action Although bromoform seems to have a greater propensity for metabolism and is a more potent mutagen than BDCM, it appears to be a less potent toxicant and carcinogen. As with DBCM, a possible explanation is less bioavailability resulting from the greater lipophilicity of this compound and the use of corn oil as the vehicle of administration. This concept may be supported by the occurrence of bromoform-induced tumors in the intestinal tract, but not in the liver or kidneys.

Halo Acids

Halo acids are the second most frequently found CBPs after THMs. To date, the chlorinated acetic acids have been more thoroughly characterized toxicologically than their brominated analogs. HAA, unlike THMs, are nonvolatile and they have low dermal absorption (at low concentrations). The dichloroacetates (DCA) and trichloroacetates (TCA) occur in significantly higher concentrations than the monohaloacetates. TCA and DCA are metabolites and ultimate carcinogenic forms of rodent carcinogens, TCE and PERC. The maximum contaminated limit for the haloacetic acid is 60 μg l^{-1}.

Dichloroacetic Acid

This compound exists in drinking water as the salt; however, most of the experiments have been conducted with free acid. Therefore, the applicability of the results of such studies to estimating human risks will be uncertain because of the large pH artifacts that can be expected when administering a strong acid.

Toxicokinetics Absorption of DCA is rapid from the intestinal tract into the bloodstream. Once in the bloodstream, DCA is distributed to the liver and muscles, and then in smaller quantities to the fat, kidney, and other tissues such as the brain and testes. The systemic clearance of DCA is significantly higher. The metabolism of DCA is mediated by a novel GST, GST-zeta found in cytosolic fraction. This enzyme appears to be subjected to autoinhibition by DCA. Although there are substantial species differences in the metabolism of DCA, autoinhibition seems to be true across the species including humans. The half-life of DCA in dogs and rats are between

17.1–24.6 and 2.1–4.4 h, respectively, and 1.5 h in mice. The DCA half-life in humans is much closer to rats.

Acute and Short-Term Toxicity (or Exposure)
Animal DCA is not very toxic when administered acutely to rodents. The LD_{50} values of 4.5 and 5.5 g kg^{-1} in rats and mice, respectively, have been reported for sodium salt of DCA. Increased lipid peroxidation was reported at 300 mg kg^{-1} in rats and mice.

DCA was administered by gavage at 125, 500, or 2000 mg kg^{-1} to rats and at 50, 75, or 100 mg kg^{-1} to dogs for 3 months, dogs were more sensitive than rats. One of three female dogs died at 75 mg kg^{-1}, and one of four male dogs died at 100 mg kg^{-1}. The most overt toxicity in rats was hind limb paralysis at the highest dose and relative liver weights were significantly increased at all doses. Histopathological changes were observed in the brain and testes of both species. Testicular germinal epithelial degeneration was observed in rats at doses of 500 mg kg^{-1} and above and at all doses in dogs, with severity increasing with dose. Repeated short-term exposure to DCA led to higher glycogen accumulation and decreased plasma glucose and lactic acid in rats and mice. The effects such as reduced weights of accessory organs (epididymis, cauda epididymis, and preputial gland), changes in sperm motion, delayed spermiation and formation, and distorted sperm heads have been observed when administered in drinking water. DCA induces soft tissue abnormalities in fetal rats when administered by gavage in a water vehicle at 140 mg kg^{-1} to their dams during gestation days 6–15.

Human DCA was used as a potential orally effective hypoglycemic agent. Only a slight sedation was noted in some patients. More recently, DCA has been evaluated with success in the treatment of lactic acidosis associated with severe malarial mitochondrial myopathy and liver transplantation. Although DCA is used in a variety of medical conditions, it presents little acute risk probably due to the smaller doses.

Reduced plasma triglycerides, increased β-hydroxybutyrate, and increased plasma uric acid were noted over 6 days of administration of DCA as a hypoglycemic agent. Although there is no conclusive evidence, DCA is proposed to cause neurotoxic effects in humans based on the fact that DCA inhibits its own metabolism. These effects are expected to occur at therapeutic doses, 25–100 mg kg^{-1}.

Chronic Toxicity (or Exposure)
Animal DCA produces a severe hepatomegaly in mice at concentrations in drinking water of 1 g l^{-1} and above in 1 year.

DCA produced multiple tumors per animal at 2 g l^{-1} and above for 1 year and only hepatic tumor was reported with 0.5 g l^{-1} for 2 years. In rats, a statistically significant increase in carcinogenicity was observed at 0.5 or 1.6 g l^{-1} after 2 years.

Human The main reason that DCA was not fully developed as a hypoglycemic agent was that long-term administration to patients induced a reversible polyneuropathy. IARC classified DCA as a group 3 compound for its carcinogenicity. EPA classified it as a group 2B: possibly carcinogenic to humans because there is evidence of carcinogenicity in experimental animals, but there is either no evidence or not sufficient evidence of carcinogenicity in humans.

Mode of Action DCA induces tumor by nongenotoxic mechanisms. Most data now suggest that it is the parent compound that is responsible for the effects related to carcinogenicity by interfering with the cellular signaling mechanisms. This, in addition to autoinhibition of its metabolism, suggests that the actual mechanism is by tumor promotion rather than by cytotoxicity and reparative hyperplasia.

Trichloroacetic Acid

Like DCA, TCA exists almost exclusively in the salt form at pH found in drinking water.

Toxicokinetics TCA is readily absorbed from the gastrointestinal tract in experimental animals and humans and its clearance from blood is relatively slow relative to other HAAs. Approximately half of the administered dose was eliminated unchanged. There are substantial differences in the clearance by different species. Clearance is much faster in mice than in rats and human clearance is very slow. The half-life is 5.8 h in mice, 9.3 h in rats, 50 h in humans and approximately 200 h in dogs. TCA produces same metabolites as DCA with or without being converted to DCA.

Acute and Short-Term Toxicity (or Exposure)
Animal The oral LD_{50} of TCA (neutralized to pH 6–7) is found to be 3.32 g kg^{-1} in rats and 4.97 g kg^{-1} in mice when administered in aqueous solution. Acute administration of TCA reduces the blood glucose and increases lipid peroxidation in

rats. The most obvious target organ for TCA is the liver. Repeated administration of TCA in drinking water at a dose as high as $7.5\,g\,l^{-1}$ only produced minimal evidence of liver toxicity. TCA exposure to male rats in drinking water for 90 days caused a small but statistically significant increase in peroxisome proliferation markers. TCA is clearly without substantive cytotoxic effects at doses of less than $300\,mg\,kg^{-1}$. TCA administration at $800\,mg\,kg^{-1}$ via aqueous vehicle from 6 to 15 days of gestation resulted in body weight reductions, soft tissue malformations, and interventricular septal defect.

Human TCA is a strong acid. It is widely recognized that skin contact of TCA has the potential to produce acid burns, and ingestion of TCA has the potential to damage tissues of the gastrointestinal tract or produce systemic acidosis, even though specific studies of these effects do not appear in the literature. TCA is frequently utilized for chemical peeling by physicians practicing dermatologic surgery. The patient developed marked conjunctivitis of the affected eye and abrasions involving 25% of the cornea.

Chronic Toxicity (or Exposure)
Animal While TCA (neutralized) induces cancer in male mice when administered via drinking water at $1–5\,g\,l^{-1}$, such an effect was not observed in rats.

Human Indirectly, it may be presumed that TCA presents little overt hazard to human health because it is a major metabolite of commonly used solvents such as TCE and Perc. Occupational exposures to these solvents have been quite high in the past, but few, if any, effects of the solvents in humans have been attributed to TCA. Therefore, one would surmise that TCA is relatively nontoxic to humans under circumstances of low exposures such as those encountered in chlorinated drinking water. In addition, the mode of tumor induction – peroxisomal proliferation – in animals does not seem to be operating in humans. Hence, humans appear minimally sensitive to the tumorigenic effects of these compounds. IARC has classified TCA as group 3 compound for its carcinogenicity and EPA classified as a group 2B compound.

Mode of Action In the HAA class, significant differences in mode of action have been demonstrated for DCA and TCA. Despite the close structural resemblance of DCA and TCA and their common target organ (liver cancer induction), it is becoming clear that the mechanisms by which they act are different. TCA is a peroxisome proliferator and DCA induced tumor via epigenetic mechanism.

Other By-Products of Chlorination

Brominated HAAs are formed in waters that contain bromide. There are very limited data available on the toxicity of these chemicals. There are no studies on the health effects of brominated HAAs in humans. No formal reports have been made of carcinogenic or mutagenic effects of brominated HAAs. Metabolism of brominated HAAs has received little attention.

Haloaldehydes and haloketones have received very little attention. Members of this class have been identified as key metabolites of chemicals such as TCE, vinyl chloride, and dibromochloropropane. Trichloroacetaldehyde and chloral hydrate are important compounds of this group. Chloral hydrate is primarily known for its depressant effects on the central nervous system and doses of 500–2000 mg produce central nervous system depression in humans. It is also known to cause liver damage. This compound is classified as group 3 by IARC. TCA and DCA are major metabolites of chloral hydrate.

Toxicological data in experimental animals and humans for the haloketones and haloacetaldehydes are extremely limited. Slight liver and CNS effects were observed. Hepatocellular carcinomas in mice were reported, probably due to mutagenic effect. There is a potential carcinogenic hazard associated with halogenated aldehydes.

Epidemiological Studies

Numerous epidemiological studies have attempted to assess the association between cancer and the long-term consumption of disinfected drinking water. In most studies, disease incidence or mortality was compared between populations supplied with chlorinated surface water and those supplied with unchlorinated groundwater. A wide range of cancer sites such as gall bladder, esophagus, kidney, breast, liver, pancreas, prostate, stomach, bladder, colon, and rectum was found to be statistically associated with the use of chlorinated surface water in humans. Additionally, published epidemiological data suggest the possibility that increased spontaneous abortion rates may be related to DBPs in drinking water. The epidemiological evidence is inconclusive and equivocal for an association between cancer and noncancer effect exposure to CBPs in drinking water. The quality of information about water disinfection exposures and potential confounding characteristics differs dramatically between these studies. The confounding factors such as smoking, drinking, exposure to other

chemicals, etc. make the matter more complicated. In addition, occurrence of cancer incidence at one place and nonoccurrence at other places further complicate the interpretation.

It is noteworthy that there is little support in the animal data for certain target organs that are prominently associated with chlorinated drinking water in epidemiological studies (e.g., bladder cancer). Therefore, the possibility has to be left open that the carcinogenic effect of DBPs may be dependent on genetically determined characteristics of a target organ (or tissue) that make it more susceptible than the same organ in test animals.

Conclusions

Chlorination has been the major disinfection process of drinking water in many countries for many years despite the availability of alternative disinfectants. There is a widespread concern about cancer, noncancer and reproductive effects of CBPs based on animal and epidemiological studies. However, most of these studies were conducted with a single chemical, at high doses and using corn oil as vehicle, a potentially confounding factor in toxicological evaluations of drinking water contaminants. These conditions are irrelevant to human exposure. Importantly, carcinogenic effects of individual CBPs may not represent the risk posed by the mixtures, as disinfected drinking water is a very complex mixture of chemicals. Although some epidemiology studies linked CBPs to the incidence of cancer and adverse reproductive effects in humans, there is no scientific basis for the proposed association and none of the chlorination by-products studied individually to date is a potent carcinogen at concentrations normally found in drinking water. In addition, the toxic effects of many CBPs remain largely unknown and many of them remain unidentified. Hence, it is not possible to make sound scientific judgment. It is important to evaluate all CBPs individually or as mixtures in a systematic manner to provide comparative toxicity and to better understand the exposure concentrations in the drinking water based on daily ingestion (approximately $2\,l\,day^{-1}$), inhalation, swimming, bathing, etc. in the risk assessment paradigm. A complicating factor when assessing risk from CBPs is that they occur in complex mixtures that vary by location, disinfection process, distance from the treatment plant, changing conditions of the source water, and even weather conditions. Moreover, the effects may be altered by factors such as coexposure to other compounds, age, lifestyle, etc. Nonetheless, safe drinking water is a substantive health concern and a balance should be achieved between reducing exposure to CBPs and maintaining control of waterborne diseases.

See also: Bromoform; Chlorine; Chlorine Dioxide; Chlorobenzene; Chloroform; Organochlorine Insecticides; Polychlorinated Biphenyls (PCBs); Trihalomethanes.

Further Reading

IPCS (2000) *Environmental Health Criteria, 216: Disinfectants and Disinfectant By-Products*. Geneva: International Programme on Chemical Safety, WHO.

US EPA (1998) National primary drinking water regulations. Disinfectants and disinfection by-products; notice of data availability. Proposed rule. *Federal Registry* 63.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for CBPs.

Chlorine

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 7782-50-5
- Synonyms: Bertholite; Chloor; Chlor; Chlore; Molecular chlorine; Cloro; RTECS FO2100000; UN1017
- Chemical/Pharmaceutical/Other Class: Disinfectant; Bleaching agent

Uses

Chlorine is used to bleach all types of fabric, to disinfect relatively clean impervious surfaces, to purify water, and to control biofouling in cooling systems. It is used in the processing of meat, fish, vegetables, and fruits. It is also used in the manufacturing of synthetic rubber, plastics, pesticides, antifreeze, refrigerants, antiknock compounds, chlorinated hydrocarbons, polyvinyl chloride, and chlorinated lime. Chlorine is also used in detinning

and dezincing iron and as an ingredient in special batteries.

Background Information

For more than 100 years now, industry has exploited this highly reactive chemical produced from one of nature's most plentiful and inexhaustible minerals – common salt. Today, chlorine is used in a vast range of processes to create thousands of often indispensable products that serve our everyday needs at work, home, and play. More than 2 million jobs in European industry are related to this chemical building block. It underpins the manufacture and use of products with an annual value of more than 380 000 million Euros.

Exposure Routes and Pathways

Dermal or ocular contact and inhalation are the most common exposure pathways.

Toxicokinetics

Chlorine persists as an element only at a very low pH (<2), and at the higher pH found in living tissue it is rapidly converted into hypochlorous acid. In this form, it apparently can penetrate the cell and form *N*-chloro-derivatives that can damage cellular integrity.

Mechanism of Toxicity

Chlorine reacts with body moisture to form acids. The acids form acid proteinates.

Acute and Short-Term Toxicity (or Exposure)

Animal

Exposure of cats to a concentration of 900 mg m^{-1} (300 ppm) for 1 h may cause death after a period during which the conjunctiva is inflamed; coughing and dyspnea are also present. Dogs rarely die following a 30 min exposure to 650 ppm and never die following a 30 min exposure to less than 280 ppm. The pulse rate of dogs is retarded during exposure to concentrations of 200 ppm or greater. In guinea pigs, the inhalation of small quantities of chlorine accelerates the course of experimental tuberculosis.

Human

Liquid chlorine causes burns to skin and eyes and will cause frostbite. It may cause lung injury if inhaled. Chlorine causes smarting of the skin and first-degree burns on short exposure; it may cause secondary burns in long exposures. Inhalation of low concentrations causes mild mucous membrane irritation and irritation of the upper respiratory tract. Inhalation of high concentrations of the gas causes necrosis of the tracheal and bronchial epithelium as well as pulmonary edema, atelectasis, emphysema, and damage to the pulmonary blood vessels. Acute exposure may also cause anxiety and vomiting. Exposure to 500 ppm can be lethal over 30 min, while exposure to 1000 ppm can be lethal within a few minutes.

Chronic Toxicity (or Exposure)

Animal

Chlorine gas was not carcinogenic in mice and rats exposed to varying concentrations. Chlorine administered in drinking water produced lymphomas and/or leukemia in rats, but was not carcinogenic in a third study.

Human

Not classifiable as a human carcinogen. Chronic exposure causes permanent, although moderate, reduction in pulmonary function and corrosion of teeth.

In Vitro Toxicity Data

In a series of *in vitro* experiments on a human lymphocyte culture system, it was reported that chlorine induced chromatid and chromosome breaks, translocations, dicentric chromosomes, and gaps.

Clinical Management

Exposure should be terminated as soon as possible by removal of the patient to fresh air. The skin, eyes, and mouth should be washed with copious amounts of water. A 15–20 min wash may be necessary. Contaminated clothing and jewelry should be removed and isolated. Contact lenses should be removed from the eye to avoid prolonged contact of the chemical with the area. Affected areas should not be rubbed. If breathing has stopped, artificial respiration should be given. If breathing is difficult, oxygen should be given.

Environmental Fate

The stability of free chlorine in natural water is very low because it is a strong oxidizing agent and rapidly oxidizes inorganic compounds. It also oxidizes

organic compounds, but more slowly than inorganic compounds.

Ecotoxicology

Chlorine is highly toxic to all forms of aquatic life; there is no potential for bioaccumulation or bioconcentration.

Exposure Standards and Guidelines

Occupational Safety and Health Administration: 8 h time-weighted average (0.5 ppm). Federal drinking water standards (Environmental Protection Agency): 4000 $\mu g\,l^{-1}$.

See also: Detergent; Pollution, Air; Pollution, Soil; Surfactants, Anionic and Nonionic.

Further Reading

Abdel-Rahman MS, Suh DH, and Bull RJ (1984) Pharmacodynamics and toxicity of chlorine in drinking water in the rat. *Journal of Applied Toxicology* 4: 82–86.

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Vetrano KM (2001) Molecular chlorine: health and environmental effects. *Review of Environmental Contamination and Toxicology* 170: 75–140.

Winder C (2001) The toxicology of chlorine. *Environmental Research* 85: 105–114.

Chlorine Dioxide

Zhengwei Cai

- Chemical Abstracts Service Registry Number: CAS 10049-04-4
- Synonyms: Chlorine-oxide; Alcide; Chlorine oxygen acids; Chlorine peroxide; Chloroperoxyl; Doxcide 50; Hatiox E-100; NA 9191; Chlory radical
- Chemical/Pharmaceutical/Other Class: Chlorine dioxide and its by-products are collectively called oxychlorines
- Chemical Formula: ClO_2
- Chemical Structure: O = Cl = O

Uses

Chlorine dioxide is a strong oxidizing agent, bactericide, and antiseptic. It is used in bleaching cellulose, paper pulp, leather, flour, fats and oils, textiles, and beestpwax, and in deodorizing and purifying water. It is currently considered as an alternative to chlorine, as a disinfectant for public water supplies in the United States. It is also used in the manufacture of many chlorite salts.

Exposure Routes and Pathways

Consumption of drinking water is the most probable route of exposure to chlorine dioxide and its by-products. Patients undergoing hemodialysis may be directly exposed to chlorine dioxide through dialysis water disinfected with chlorine dioxide. Chlorine dioxide is a gas; therefore, inhalation is also an exposure pathway.

Toxicokinetics

Chlorine dioxide can be rapidly absorbed through the gastrointestinal tract. Peak blood concentration levels can be reached within 1 h after a single dose administered orally. It can also be slowly absorbed through shaved skin with a half-absorption time of 22 h. Chlorine dioxide is metabolized to chlorite, chlorate, and mostly chloride. Most administered chlorine dioxide and its metabolites remain in plasma followed by kidneys, lungs, stomach, intestine, liver, and spleen. About 43% of orally administered chlorine dioxide is eliminated in the urine and feces within 72 h. It is not excreted via the lungs.

Mechanism of Toxicity

The toxicity of chlorine dioxide is attributed to the oxidative stress caused by this compound and its by-products or metabolites. Animal studies and *in vitro* experiments with human red blood cells indicate that chlorine dioxide and its by-products, especially chlorite, oxidize hemoglobin to methemoglobin by inhibiting methemoglobin reductase, decreasing erythrocyte glutathione levels, stimulating erythrocyte hydrogen peroxide production, and causing hemolytic anemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Delayed death occurred in animals after exposure to 150–200 ppm for less than 1 h. Rats repeatedly exposed to 10 ppm died after 10–13 days of exposure. Rats are more sensitive than mice to the

developmental effects associated with chlorite-treated drinking water.

Human

Chlorine dioxide gas is highly irritating to the skin and mucous membranes of the respiratory tract. Symptoms of exposure by inhalation include eye and throat irritation, headache, nausea, nasal discharge, coughing, wheezing bronchitis, and delayed onset of pulmonary edema. It is explosive in the form of concentrated vapor or solution (10 vol.% in the air). When involved in a fire, chlorine dioxide is a source of oxygen. Daily ingestion of 1 l of water containing 0.7 mg of chlorine dioxide has been reported to cause nausea. Exposure of a worker to 19 ppm for an unspecified time has been reported fatal.

Chronic Toxicity (or Exposure)

Human

The human experience with chlorine dioxide, both in controlled prospective studies and in actual use situations in community water supplies, has failed to reveal adverse health effects. However, glucose-6-phosphati dehydrogenase-deficient individuals and infants are groups thought to be at higher risk to chlorine dioxide toxicity due to their susceptibility to oxidant-induced methemoglobinemia. The chronic toxicity signs are mainly dyspnea and asthmatic bronchitis, and in certain cases irritation of the gastrointestinal tract.

Exposure Standards and Guidelines

The US EPA has recommended standards of $0.06\,mg\,l^{-1}$ for chlorine dioxide and $0.007\,mg\,l^{-1}$ for chlorite and chlorate in drinking water. The exposure limits have been set at TLV–TWA 0.1 ppm ($0.3\,mg\,m^{-3}$) by ACGIH, MSHA, OSHA, and NIOSH; TLV–STEL 0.3 ppm by ACGIH; and IDLH 10 ppm by NIOSH.

See also: Pollution Prevention Act, US; Pollution, Water.

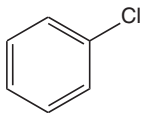
Further Reading

Hose JE, Di Fiore D, Parker HS, and Sciarrotta T (1989) Toxicity of chlorine dioxide to early life stages of marine organisms. *Bulletin of Environmental Contamination and Toxicology* 42(3): 315–319.

Chlorobenzene

Linda A Malley

- Chemical Abstracts Service Registry Number: CAS 108-90-7
- Synonyms: Benzene chloride; Benzene chloro-; Chlorbenzene; Chlorbenzol; Chlorobenzol; Monochlorbenzene; Monochlorobenzene phenyl chloride; NCI-C54886; Caswell No. 183A; EPA Pesticide Chemical Code 056504; MCB, CP 27; I P Carrier T 40; Tetrosin SP
- Chemical/Pharmaceutical/Other Class: Inorganic compounds
- Chemical Formula: C_6H_5Cl
- Chemical Structure:



Uses

Chlorobenzene is used as a solvent for pesticide formulations, in auto parts degreasing, and in the manufacture of adhesives, paints, polishes, waxes, pharmaceuticals, and natural rubber. It is a chemical intermediate in the production of diphenyl oxide, diisocyanates, and nitrochlorobenzene. It has also been used as a fiber-swelling agent and as a dye carrier in textile processing.

Background Information

Chlorobenzene production has been declining since its peak in 1969, and is likely to continue declining due to the substitution of more environmentally acceptable chemicals. Chlorobenzene is produced by chlorination of benzene in the presence of a catalyst, and is produced as an end product in the reductive chlorination of di- and trichlorobenzenes.

Exposure Routes and Pathways

The vapor pressure of chlorobenzene is relatively high (11.8 mmHg); therefore, inhalation is a

potential route of exposure. Since chlorobenzene is soluble in water (448 ppm) and has been detected in wastewater and drinking water, there is potential for oral exposure. In addition, as a result of its solvent and degreasing properties, the potential for accidental skin contact with the material also exists.

Toxicokinetics

Data in rabbits indicate that the toxicity from a single dermal application is minimal with only slight reddening of the skin observed. Continuous skin contact with chlorobenzene for 1 week resulted in moderate erythema and slight superficial necrosis. Absorption in amounts sufficient to cause toxicity can also occur as a result of ingestion or inhalation. Because chlorobenzene is highly lipophilic and hydrophobic, it is thought to be distributed throughout the total body water, with body lipids being a major deposition site.

The kinetics of metabolism and excretion were investigated in rabbits administered a single oral dose of $0.5\,mg\,kg^{-1}$ or doses of 0.5 g twice daily for 4 days. In the single-dose study, 27% of the administered dose was excreted unchanged in the expired air. The majority of the remainder was excreted in the urine as a glucuronide (25%), ethereal sulfate (27%), and mercapturic acid (20%). Similarly, rabbits administered repeated doses of chlorobenzene excreted the majority of the dose in the urine, and only small amounts were detected in the tissues and feces. Rats administered a single, intraperitoneal dose of chlorobenzene also excreted metabolites in the urine which were identified as 4-chlorocatechol, 2-chlorophenol, 4-chlorophenol, and 3-chlorophenol. In addition, chlorobenzene was covalently bound to DNA, RNA, and proteins in the liver, kidney, and lung 22 h following a single intraperitoneal injection. Chlorobenzene is first oxidized to the 3,4-epoxide, which then can follow one of several pathways. One leads to the formation of the I-mercapturic acid conjugate following glutathione conjugation. A second pathway results in the formation of 4-chlorocatechol, and the third pathway ends with the formation of 4-chlorophenol and its conjugates. Data collected from exposed workers and volunteers indicate that for humans, the primary pathways are formation of the *p*-mercapturic acid conjugate and 4-chlorocatechol.

Mechanism of Toxicity

Similar to other volatile organic chemicals, chlorobenzene is a nervous system depressant. In addition, lesions of the liver and kidneys have also been observed following toxic doses.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values for rats, mice, and rabbits were 2290, 2300, and $2830\,mg\,kg^{-1}$, respectively. The approximate inhalation LD_{50} (2 h) is 4300 ppm for mice. Application of chlorobenzene to the skin of rabbits caused slight reddening; prolonged skin contact was irritating. Ocular contact in rabbits caused a transient conjunctival irritation which resolved within 48 h. Tremors, central nervous system depression, and death were observed in cats administered a single inhalation exposure of 3700 ppm and above.

Several repeated-exposure oral studies have been conducted in various species. Although the doses at which effects were observed are variable between species, the primary effects of chlorobenzene were observed in the liver and kidneys. Rats and mice were administered daily doses of 60–$750\,mg\,kg^{-1}$, 5 days per week, for 13 weeks. Survival was lower in rats at $500\,mg\,kg^{-1}$ and above and in mice at $250\,mg\,kg^{-1}$ and above. Pathological changes in the liver and kidneys and changes in the hematopoietic system (spleen, bone marrow, and thymus) were observed in both species at $250\,mg\,kg^{-1}$ and above. In another study, rats were administered doses ranging from 14.4 to $376\,mg\,kg^{-1}\,day^{-1}$, 5 days per week, over a period of 192 days. Doses of $144\,mg\,kg^{-1}\,day^{-1}$ and above caused changes in liver and kidney weights and changes in liver morphology. Doses of $18.8\,mg\,kg^{-1}\,day^{-1}$ and below did not cause any adverse effects. Dogs were administered oral doses ranging from 27.2 to $272.5\,mg\,kg^{-1}\,day^{-1}$, 5 days per week, for 93 days. There were no effects at $54.5\,mg\,kg^{-1}\,day$ and below. At $272.5\,mg\,kg^{-1}\,day^{-1}$, changes in clinical chemistry parameters were observed, four of eight dogs died, and pathological changes were observed in the liver, kidney, gastroenteric mucosa, and hematopoietic tissue.

Repeated-exposure inhalation studies have been conducted in several species. Rats, rabbits, and guinea-pigs were exposed to airborne concentrations ranging from 200 to 1000 ppm for 7 h per day, 5 days per week, for a total of 32 exposures. At 475 ppm and above, organ weight changes and histopathological changes were observed. There were no effects detected at 200 ppm. In another study, changes in hematology parameters and pathological changes in the adrenal cortex, kidney, and liver were observed in rats and rabbits exposed to airborne concentrations of 75 or 250 ppm chlorobenzene vapors for 7 h per day, 5 days per week for 24 weeks.

Exposure of rats to atmospheric concentrations up to 450 ppm did not have any adverse effects on reproductive performance or fertility of male or female rats through two consecutive generations. Chlorobenzene caused minor skeletal alterations in fetuses collected from pregnant rats exposed to atmospheric concentrations up to 590 ppm (a maternally toxic dose) for 6 h per day during the period of organogenesis. Pregnant rabbits exposed to chlorobenzene at concentrations up to 590 ppm did not exhibit evidence of embryotoxicity or teratogenicity.

Human

The human literature primarily consists of case reports. In the industrial environment, symptoms including headache, numbness, skin irritation and redness, eye irritation and redness, irritation and redness of the upper respiratory tract, bronchitis, dizziness, somnolence, loss of consciousness, hematopoietic effects, gastritis, hepatitis, and neuromuscular changes have been reported. Accidental ingestion of 5–10 ml of a cleaning agent containing chlorobenzene caused loss of consciousness, vascular paralysis, and heart failure in a child ($\sim$2 years old).

Chronic Toxicity (or Exposure)

Animal

In a study determining the carcinogenic potential of chlorobenzene, rats were administered daily doses of 0, 60, or 120 mg kg^{-1} day^{-1}, 5 days per week, for 103 weeks, and mice were similarly administered 30 or 60 mg kg^{-1} day^{-1}. No increased tumor incidences were observed in female rats or in male or female mice. Male rats administered 120 mg kg^{-1} day^{-1} had an increased incidence of hepatic neoplastic nodules (8% for untreated control, 4% for vehicle control, 8% for 60 mg kg^{-1}, and 16% for 120 mg kg^{-1}). Based on these results, the US Environmental Protection Agency (EPA) classified chlorobenzene as 'D' (not classifiable as to carcinogenicity in humans).

Human

There were no epidemiology studies in humans regarding long-term exposure to chlorobenzene. However, based on the results of a chronic toxicity study in rats, the US EPA classified chlorobenzene as 'D' (not classificable as to carcinogenicity in humans). In addition, the American Conference of Governmental Industrial Hygienists classified chlorobenzene as 'A3' (confirmed animal carcinogen with unknown relevance to humans).

In Vitro Toxicity Data

Chlorobenzene was not mutagenic in several bacterial strains of *Salmonella typhimurium* or *Escherichia coli* and was negative in rat hepatic DNA repair assays; however, it was weakly positive in a mouse micronucleus assay. Chlorobenzene induced transformation in Fischer 344 adult rat liver cell lines, but was not genotoxic to hepatocytes. In addition, it did not induce DNA repair in the rat hepatocyte primary culture DNA repair assay.

Clinical Management

Treatment is symptomatic and supportive. For ocular contact, the eyes should be irrigated immediately with abundant running water. If the material contacts the skin, the affected areas should be washed with soap and water promptly. If inhalation exposure occurs, the exposed person should be moved to fresh air immediately and provided with respiratory support (oxygen or artificial respiration) if necessary. If the material has been ingested, vomiting should not be induced. For ingestion, gastric lavage (followed by saline catharsis) should be performed or activated charcoal should be administered. The trachea should be protected from aspiration. Renal and hepatic function should be monitored and supported if necessary. Hypotension should be treated symptomatically.

Environmental Fate

In the ambient atmosphere, chlorobenzene will exist as a vapor, and will be degraded by reaction with photochemically produced hydroxyl radicals, with an estimated half-life of 21 days. Photolysis half-lives of 4–18 h were measured in aqueous media. Chlorobenzene is expected to volatilize from soil, and is predicted to have high mobility based on the K_{oc} values. Biodegradation results are variable based on soil type and bacteria type. In river water, the biodegradation half-life was reported to be 150 days, and 75 days in the sediment. Volatilization is expected to occur from water surfaces. Hydrolysis is not predicted to occur.

Ecotoxicology

Chlorobenzene was acutely toxic to rainbow trout, with a 24 h LC_{50} of 1.8 mg kg^{-1}. In addition, the 14 days LC_{50} in guppies was 19 ppm. The 96 h LC_{50} in fathead minnows was 16.9 mg l^{-1} under flow-through conditions.

Table 1 Summary of exposure criteria for chlorobenzene

Agency	*Criteria*	*Averaging time*
ACGIH	TLV – TWA, 10 ppm	8 h/40 h week
NIOSH	IDLH, 1000 ppm	NA
OSHA	PEL (TWA), 75 ppm (350 mg m^{-3})	8 h/40 h week

Conversion: 1 ppm = 3.19 mg m^{-3}. OSHA, Occupational Safety and Health Administration; NIOSH, National Institute of Occupational Safety and Health; ACGIH, American Conference of Governmental Industrial Hygienists; TLV – TWA, threshold limit value – time-weighted average; IDLH, immediately dangerous to life or health; PEL, permissible exposure limit; NA, not applicable.

Other Hazards

Chlorobenzene is highly flammable, and the vapors are heavier than air. They will spread along the ground and collect in low or confined areas. The lower flammable limit is 1.8%, the upper flammable limit is 9.6%, and the flash point is 85°F (29.2°C closed cup). Combustion of chlorobenzene can form phosgene and hydrogen chloride gases. Chlorobenzene reacts with strong oxidizing materials, powdered sodium, and phosphorus trichloride and sodium.

Exposure Standards and Guidelines

Based on the results of a chronic toxicity study in rats, the US EPA classified chlorobenzene as 'D' (not classifiable as to carcinogenicity in humans). In addition, the American Conference of Governmental Industrial Hygienists classified chlorobenzene as 'A3' (confirmed animal carcinogen with unknown relevance to humans).

The current exposure standards and guidelines are summarized in **Table 1**.

See also: Carcinogen Classification Schemes; Chlorophenols; Pesticides.

Further Reading

DHHS/NTP Toxicology and Carcinogenesis Studies of Chlorobenzene in F344/N Rats and B6C3F1 Mice (Gavage Studies) Technical Report Series No. 261 (1985) NIH Publication No. 86-2517.

US EPA Ambient Water Quality Criteria Document; Chlorinated Benzenes (1980) EPA 440/5-80-028.

US EPA Health Assessment Document; Chlorinated Benzenes (1985) EPA 600/8-84-015.

US EPA Health Criteria Document for Chlorobenzene NTIS/PB89-192116 (June 1988).

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chlorobenzene.

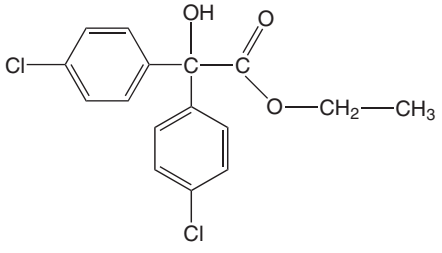
Chloroethyl Sulfide, Bis-2 *See* Mustard Gas.

Chlorobenzilate

David Janz

This article is a revision of the previous print edition article by Tamal Kumar Chakraborti, volume 1, pp. 300–301, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 510-15-6
- Synonyms: Ethyl-4,4-dichlorobenzilate; Ethyl-4, 4-dichlorodiphenylglycollate; Akar; Folbex; Acaraben
- Chemical/Pharmaceutical/Other Class: Chlorinated hydrocarbon
- Chemical Formula: $C_{16}H_{14}Cl_2O_3$
- Chemical Structure:



Uses

The primary use of chlorobenzilate is as an acaricide for mite control on citrus crops and in beehives. Historically, it was used as a synergist for DDT. Although classified as a Restricted Use Pesticide in

the United States and not registered for use in Canada, chlorobenzilate is believed to be used on crops other than citrus in other countries.

Exposure Routes and Pathways

Occupational exposure to chlorobenzilate may occur through inhalation or dermal contact during its production and use as an acaricide. Exposure to the general population may occur via contaminated food and drinking water.

Toxicokinetics

Chlorobenzilate is readily absorbed from the gastrointestinal tract. Dermal absorption occurs following exposure to commercial (oil-based) formulations. No significant storage of chlorobenzilate in adipose tissue of dogs was reported following daily oral administration of 12.8 mg kg^{-1} for 35 weeks. Dichlorobenzilic acid, dichlorobenzyhydrol, chlorobenzoic acid, and dichlorobenzophenone were the major metabolites produced when chlorobenzilate was incubated in the presence of rat liver homogenates. Urinary excretion of these metabolites in addition to significant excretion of unchanged chlorobenzilate in the feces was reported in dogs and rats after oral administration. Although structurally similar to DDT, chlorobenzilate is much more rapidly excreted following absorption.

Mechanism of Toxicity

Similar to other organochlorine pesticides in this structural class, chlorobenzilate causes disruption of normal flow of Na^+ and K^+ across axonal membranes in the central (CNS) and peripheral nervous systems, and may also antagonize GABA-mediated inhibition in CNS. The net result is a hyperexcitable state of neurotransmission.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for chlorobenzilate in rats is 2784–3880 mg kg^{-1}. The dermal LD_{50} is greater than 10 000 mg kg^{-1} in rats and rabbits.

Human

Symptoms of acute poisoning following ingestion, inhalation, or dermal absorption of chlorobenzilate are similar and include nausea, dizziness, vomiting, incoordination, confusion, and muscle weakness or pain. Death may result from respiratory collapse or arrythmias. Chlorobenzilate is a severe eye irritant and causes conjunctivitis following chronic exposure.

Chronic Toxicity (or Exposure)

Animal

A three-generation reproductive study in rats reported testicular atrophy but no effect on reproduction. No mutagenic or teratogenic effects have been reported in animals. Chlorobenzilate has produced hepatocellular carcinoma in mice but the evidence for carcinogenicity in rats is equivocal.

Human

Chronic skin exposure may cause skin inflammation. Chlorobenzilate is considered a possible human carcinogen.

In Vitro Toxicity Data

Chlorobenzilate did not inhibit human placental CYP 19 aromatase activity and did not express estrogen receptor activation *in vitro*.

Clinical Management

Only symptomatic treatment is available. An airway should be established and if necessary assisted ventilation provided. The cardiac rhythm should be monitored and treatment for arrhythmia should be given if required. For eye exposure eyes must be flushed immediately with water or saline and irrigation maintained during transport. For ingestion, oral administration of activated charcoal is indicated. For skin contamination, the exposed area should be washed with soap and water.

Environmental Fate

If released to soil, chlorobenzilate is expected to have low mobility, and therefore unlikely to leach into groundwater. Volatilization from soils is not expected to be a significant fate process. The half-life of chlorobenzilate in fine sandy soils was estimated to be 10–35 days, and degradation was primarily microbial. In silty clay loam and clay soils, the half-life of chlorobenzilate was estimated to be 10.8–15.1 and 29.5–169.1 days, respectively. If released into water, chlorobenzilate is expected to adsorb to particulate matter and sediment. Bioconcentration factors in carp were 224–709, indicating the potential for moderate to high accumulation in aquatic organisms. If released into air, chlorobenzilate will exist in both vapor and particulate phases. The half-life of

vapor-phase chlorobenzilate in ambient air was estimated to be 3.2 days.

Ecotoxicology

Chlorobenzilate is only slightly to practically nontoxic in birds. The 7 day dietary LC_{50} for chlorobenzilate is 3375 ppm bobwhite quail. The 5 day dietary LC_{50} in mallard ducks is greater than 8000 ppm. The LC_{50} (96 h) in rainbow trout is $0.7\,mg\,l^{-1}$ and in bluegill it is $1.8\,mg\,l^{-1}$. Chlorobenzilate is practically nontoxic to bees.

Exposure Standards and Guidelines

The reference dose and the acceptable daily intake for chlorobenzilate is $0.02\,mg\,kg^{-1}\,day^{-1}$.

See also: Carbamate Pesticides; DDT(Dichlorodiphenyltrichloroethane); Organochlorine Insecticides.

Further Reading

Smith AG (2001) DDT and its analogs. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1305–1355. San Diego, CA: Academic Press.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Chloroform

Anna M Fan

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 67-66-3
- Synonyms: Trichloromethane; Formyl trichloride; Methane trichloride; Trichloroform; Freon 20; COBEHN spray-cleaner; Methenyl trichloride; Methyl trichloride; NCI-C02686; R 20; RCRA waste No. U044; UN1888
- Chemical/Pharmaceutical/Other Class: Chlorinated aliphatic hydrocarbon
- Chemical Formula: $CHCl_3$

Uses

Chloroform is a volatile, low-molecular weight, lipophilic compound and a chlorinated trihalomethane. Most of the chloroform produced in the United States is used to make fluorocarbon 22 (HCFC 22) and the rest is produced for export and miscellaneous uses. In the past it was used as an inhalation anesthetic and as an extraction for, fats, oils, greases and other products, as a dry cleaning spot remover, in fire extinguishers, and as a fumigant. It is available as emulsions, spirits, tinctures, and chloroform water. Chloroform is also formed as a by-product of chlorination of water, wastewater, and swimming pool. Other sources include pulp and paper mills, hazardous waste sites, and sanitary landfills.

Exposure Routes and Pathways

Inhalation, ingestion, and dermal contact are the most common routes of exposure. Inhalation exposure can be from indoor or outdoor air, especially in the workplace related to its manufacture and use. Other possible sources of exposure include drinking water, beverages, and food using water containing chloroform and swimming pool water, but chronic poisoning is unlikely by this route. Acute poisoning may be by accidental or deliberate ingestion.

Toxicokinetics

Chloroform is readily absorbed through the lungs when inhaled and through the gastrointestinal tract when ingested. It is readily distributed throughout the body and is highly fat-soluble. The rate of pulmonary uptake in humans is initially rapid, but it decreases as the concentration reaches equilibrium. The total quantity absorbed through the lungs is directly proportional to the following: the concentration in the inspired air; the exposure time; the blood/air Ostwald solubility coefficient; the solubility in the various body tissue; and physical activity. The basic kinetic parameters of chloroform absorption by inhalation and its equilibrium in the body apply equally to both low and high concentrations. Chloroform may be absorbed through intact skin in both humans and animals, but absorption is slow and limited by the moderate lipophilicity of the chemical. It can readily cross the human placenta.

Liver is the principal site of chloroform metabolism which involves two major pathways, both of which are catalyzed by the cytochrome P-450 enzymes in the presence of NADPH. The oxidative pathway produces phosgene and the reductive pathway produces the dichloromethyl free radical. Other metabolites of chloroform include chloromethanol, hydrochloric acid, hydrogen chloride, and digluathionyl dithiocarbonate, with carbon dioxide as the predominant end product of metabolism.

Chloroform is rapidly eliminated from the body. It is primarily excreted by the lungs as carbon dioxide. The half-life for elimination following a single oral dose of 500 mg in two subjects was 1.5 h.

Various physiologically based pharmacokinetic models for chloroform have been described using physiological and metabolic parameter values for rats, dogs, and humans to exercise the models.

Mechanism of Toxicity

Chloroform causes progressive depression of the central nervous system (CNS), ultimately producing deep coma and respiratory center depression. The exact mode of action for chloroform-induced toxicity in the liver and the kidneys is not certain, but metabolism to toxic metabolites by the cytochrome P-450-dependent pathways is likely to play a critical role. Chloroform is converted to chloromethanol, which rapidly dechlorinates to produce hydrochloric acid and phosgene. Phosgene is a poisonous gas that can cause injury to tissues. Phosgene reacts with water to produce carbon dioxide and chloride ion. These by-products can bind to glutathione to produce diglutathione dithiocarbonate. The hepatotoxicity and nephrotoxicity of chloroform is thought to be due largely to phosgene.

For the carcinogenicity of chloroform, there is increasing yet not conclusive evidence that it is due to an epigenetic process, that is, that the mode of carcinogenic action of chloroform is largely due to oxidative metabolism leading to cytotoxicity and cell proliferation in the liver and probably in the kidneys. The hypothesis for this mechanism is based on the theory that chloroform acts as a promoter of previously initiated cells by virtue of regenerative hyperplasia which occurs in response to renal and hepatic toxicity. Some noted that mechanisms of carcinogenicity have not been sufficiently developed to discount carcinogenic effects observed in rodents from predicting cancer hazards to humans, and that other possible mechanisms for chloroform have not been studied. Genotoxicity studies have shown both negative and positive findings. Thus current scientific information suggests that cytotoxicity/cell proliferation appears to be a major factor in chloroform-induced carcinogenesis, but this may not be sufficient to explain the underlying mechanism(s), and multiple mechanisms may be operating concurrently. In recognition that this mode of action may not be exclusive, in the absence of definitive evidence, it would be premature to draw a conclusion on a single, genetic or epigenetic, mechanism.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chloroform in animals is known to cause acute toxicity similar to that in humans. The following lethal dose (LD) values have been reported:

Oral LD_{50} in rats	300, 1194 mg kg^{-1}
Oral LD_{50} in guinea pigs	820 mg kg^{-1}
Oral LD_{Lo} in dogs	100 mg kg^{-1}
Inhalation LC_{Lo} in cats	35 g m^{-3} (4 h)$^{-1}$
Inhalation LC_{50} in cats	47 g m^{-3} (4 h)$^{-1}$
Subcutaneous LD_{50} in mouse	704 mg kg^{-1}
Intravenous LD_{Lo}	75 mg kg^{-1}
Skin LD_{50} in rabbits	$>$20 000 mg kg^{-1}

Acute and subchronic exposures result in toxicity to the liver, kidneys, respiratory system and CNS.

Human

Chloroform causes similar toxicity in humans and animals. Chloroform is an irritant and a CNS and cardiovascular system depressant. Exposure to chloroform can cause liver and kidney toxicity.

Inhalation and ingestion are harmful and may be fatal. The major effect from acute inhalation is CNS depression. It produces dizziness, tiredness, headache at lower concentrations ($<$1500 ppm), anesthesia in the range of 1500–3000 ppm, and may cause death at high levels (e.g., 40 000 ppm). A dose of 10 ml (14.8 g) of chloroform can cause CNS depression and death due to respiratory and cardiac arrest. The oral lethal dose is estimated to be between 0.5 and 5 g kg^{-1} (1 oz to 1 pint) for an average 70 kg man. Short-term inhalation of chloroform at 900 ppm can cause dizziness, fatigue, and headache. Skin contact may result in irritation and redness and high levels can cause sores. Eye contact with liquid chloroform may result in painful irritation of the superficial eye structures, burns, and may cause corneal necrosis and ulcers.

Chronic Toxicity (or Exposure)

Animal

As is with acute and subchronic exposures, chronic exposures result in toxicity to the liver, kidneys, respiratory system and CNS. The majority of the animal data are based on oral exposures, with limited data on inhalation. In rats, liver toxicity consisted of degenerative and foamy vacuolization and necrosis, and increased liver weights in males. Kidney toxicity showed cloudy swelling and nephritis. A chronic reference dose (RfD) of $0.01\,mg\,kg^{-1}\,day^{-1}$ was developed by United States Environmental Protection Agency (US EPA) based on observation of moderate/marked fatty cyst formation in the liver and elevated serum glutamic pyruvic transaminase (SGPT), in a dog chronic oral bioassay, with an LOEL of $15\,mg\,kg^{-1}\,day^{-1}$ (converted to $12.9\,mg\,kg^{-1}\,day^{-1}$), and an uncertainty factor of 1000.

Inhalation exposures to chloroform in animals have shown developmental effects, such as decreased fetal body weight, fetal resorptions and malformations in the offspring. Reproductive effects following inhalation exposures included decreased conception rates, decreased ability to maintain pregnancy, and abnormal sperms. Oral exposures have shown decreased fetal weight, increased fetal absorptions but not birth defects.

Cancer of the liver and kidneys were observed in rats and mice following administration of chloroform by the oral route. These include the following studies: (1) a long-term study by the National Cancer Institute in Osborne–Mendel rats and B6C3F1 mice treated with chloroform by gavage in corn oil; (2) a carcinogenicity study conducted in male Osborne–Mendel rats and female B6C3F1 mice administered chloroform in drinking water; and (3) a series of three studies using ICI, CBA, C57BL, and CF/1 mice administered chloroform in toothpaste. Kidney tumors were found in male rats and liver tumors in male and female mice.

Human

Chloroform may cause dry mouth, headache, hallucinations, dysarthria, ataxia, loss of reflexes, gastrointestinal distress, hepatotoxicity, and psychotic behavior. Inhalation has been associated with liver effects, including hepatitis and jaundice, and CNS effects, such as depression and irritability. Oral exposure can lead to effects on the blood, liver and kidneys. Prolonged or repeated skin contact may cause dermatitis.

There are no epidemiologic data available that attribute human cancer to exposure to chloroform *per se*. Epidemiologic studies suggest an association between cancer of the large intestine, rectum and/or bladder and the constituents of chlorinated water. Chloroform is listed as a group 2B probable human carcinogen by the International Agency for Research in Cancer. It is classified by the US EPA as a Group B2 chemical, a probable human carcinogen, based on 'sufficient evidence' of carcinogenicity in animals. Under the US EPA's Proposed Guidelines for Carcinogen Risk Assessment, chloroform is considered by the Agency as likely to be carcinogenic to humans by all routes of exposure under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues. Chloroform is also considered by the US EPA as not likely to be carcinogenic to humans by any route of exposure under exposure conditions that do not cause cytotoxicity and cell regeneration. Therefore, US EPA has not derived an oral carcinogenic potency slope or an inhalation unit risk for chloroform.

In Vitro Toxicity Data

Chloroform has shown both negative and positive results in genotoxicity studies. The studies included gene mutation in bacteria, yeast, and mammalian cells; DNA damage; sister chromatid exchange; micronuclei induction, recessive lethality, and sperm abnormalities. Positive findings included dose-dependent increases in sister chromatid exchange in mouse (*in vivo*) bone marrow and in chromosomal aberrations in rats (*in vivo*) treated orally or i.p. with chloroform, positive mouse micronuclei assay, induction of intrachromosomal recombination in yeast and reduction in recombination in the presence of a free radical scavenger, DNA binding *in vivo*, and a threefold increase in micronucleated kidney cells in rats exposed orally to a high dose of chloroform. This last finding is of particular interest because of damage at the chromosome level in the animal species (rat) and tissue (kidney cells) most relevant to the carcinogenicity assessment of chloroform.

Clinical Management

Features of chloroform poisoning following ingestion include headache, impaired consciousness, convulsions, respiratory paralysis, dizziness, abdominal pain, nausea, vomiting, and diarrhea. Inhalation may result in dizziness and shortness of breath.

In cases of ingestion, ipecac-induced emesis is not recommended. Activated charcoal slurry with or without saline cathartic or sorbitol can be given in cases of oral exposures. Exposed skin should be decontaminated by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of water at room temperature for at least 15 min.

Management includes early decontamination, supportive and symptomatic treatment with respiratory and cardiac monitoring (respiratory assistance, defibrillation, possible fluid replacement), avoiding catecholamine drugs and treatment of liver and/or kidney failure (renal dialysis) if they occur. No specific antidote is available.

Environmental Fate

Chloroform evaporates easily into the air where it breaks down slowly to form products including phosgene and hydrogen chloride. It dissolves well in water where it may form breakdown products. It does not adsorb well onto soil and can travel through soil to groundwater where it persists for a long time.

Ecotoxicology

Scenedesmus subspicatus	48 h	EC_{10} 225 mg l^{-1} (biomass)
		EC_{50} 560 mg l^{-1} (biomass)
Chlamydomonas angulosa		EC_{50} 382 mg l^{-1} (cell count, biomass, carbon dioxide update)
Selenastrum capricornutum		EC_{50} > 1000 mg l^{-1} (cell count, biomass, carbon dioxide update)
Daphnia magna	48 h	LC_{50} 28.9–353 mg l^{-1}
Rainbow trout		LC_{50} 1.24–2.03 mg l^{-1} (200–50 mg calcium carbonate l^{-1})
Bluegills	96 h	LC_{50} 18.2 mg l^{-1} (flow through)

Amphibians appear to be quite sensitive to the effects of chloroform. Very little information is available on terrestrial microorganisms and vertebrates. No information was identified on the toxicity of chloroform to birds or wild animals.

Other Hazards

Persons with higher risk are those who have concurrent exposure to chemical that induce liver cytochrome P-450, and those with underlying liver, kidney, or neurologic conditions.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration has set a maximum allowable concentration of chloroform of 50 ppm in workroom air during an 8 h work day in a 40 h work week.

The US EPA has set a federal drinking water standard (called maximum contaminant level, or MCL) of 100 μg l^{-1} for total trihalomethanes (a class of drinking water disinfectant by-products).

The US EPA requires that spills or accidental releases of 10 pounds or more of chloroform into the environment be reported.

In 2001, US EPA set an RfD of 0.01 mg kg^{-1} day^{-1} based on an increase in the incidence of moderate to marked hepatic fatty cyst formation in the liver of dogs and SGPT in a chronic oral study. The same RfD value was obtained using the lowest-observed-adverse-effect level (NOAEL)–lowest-observed-adverse-effect level (LOAEL) approach and the Benchmark Dose approach. A point of departure (POD or LED_{10}) of 23 mg kg^{-1} day^{-1} was calculated using quantitative modeling of kidney tumor dose–response data in a drinking water bioassay. When compared to the RfD of 0.01 mg kg^{-1} day^{-1} for noncancer end point based on kidney toxicity, a margin of exposure (MOE) of 2000 is obtained. Therefore, the RfD for non-cancer effect is also considered by US EPA as adequately protective of public health for cancer effects by the oral route, on the basis of the nonlinear dose response for chloroform and the mode of action for both cancer and noncancer effects having a common link through cytotoxicity. No cancer slope factor or unit risk values were developed.

The Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, has developed an inhalation reference exposure level of 50 ppb (300 μg m^{-3}) based on a whole-body inhalation study in rats, 7 h day^{-1}, 5 days $week^{-1}$, for 6 months. The critical effects are pathological changes in the liver and kidney, with an (average experimental exposure) LOAEL of 5.3 ppm, and a total uncertainty factor of 300.

The Agency for Toxic Substances and Disease Registry has established an acute inhalation minimal risk level (MRL) of 0.5 mg m^{-3} (0.1 ppm) based on liver effects in mice, an intermediate inhalation MRL of 0.2 mg m^{-3} (0.05 ppm) based on liver effects in workers, and a chronic inhalation MRL of 0.1 mg m^{-3} (0.02 ppm) based on liver effects in humans.

Miscellaneous

An assessment by the World Health Organization for swimmers exposed to chloroform while using indoor

pools disinfected with chlorine showed that pool users could exceed the tolerable daily intake when concentrations of chloroform in the water and air are relatively high.

See also: Carcinogen Classification Schemes; Carcinogenesis; Cytochrome P-450.

Further Reading

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World Health Organization (WHO) (2004) *Chloroform*. Concise International Chemical Assessment Document 58. Geneva: World Health Organization.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chloroform.

http://193.51.164.11 – International Agency for the Research on Cancer (IARC).

http://www.who.int – World Health Organization (WHO).

Chloromethyl Ether, Bis-

C Vaman Rao

- Chemical Abstracts Service Registry Number: CAS 542-88-1
- Synonyms: BCME; *sym*-Dichloromethyl ether; Dichloromethyl ether
- Chemical/Pharmaceutical/Other Class: Alkyl organic synthetic compound with a strong unpleasant odor
- Chemical Formula: $C_2H_4OCl_2$
- Chemical Structure: $ClCH_2—O—CH_2Cl$

Uses

Bis(choromethyl) ether (BCME) is primarily used in the synthesis of polymers, ion exchange resins, and plastics. It used as a chemical intermediate for the synthesis of other complex organic alkyl compounds as well as chloromethylating (cross-linking) reaction mixture in anion exchange resins. It is used as a dental restorative material.

In textile industry it is used in laminating and as adhesive in the flocking of fabrics and in the finishing product of the fabrics as a mixture with formaldehyde containing reactants and resins. Nonwoven textile industry uses it as binder and thermosetting of acrylic emulsion.

Exposure Routes and Pathways

Primary routes of human exposure to BCME are inhalation and dermal contact, which might occur in chemical plants that make or use BCME. Also, some BCME may exist in chemical waste sites, which may be inhaled by breathing the air containing BCME vapors. The risk of potential occupational exposure to BCME is greatest for chemical plant workers, ion exchange resin makers, laboratory workers, and polymer makers. BCME is highly unstable in water, quickly breaking down into formaldehyde and hydrochloric acid. Therefore, exposure through water pollution is limited.

Toxicokinetics

BCME is rapidly absorbed through skin and lung surface. On contact with body fluids, it is quickly broken down to formaldehyde and hydrochloric acid, and interacts with cells and tissues at various levels. Absorption by the body depends on the proximity to the source of production at the industry and at the waste dump site. BCME is mainly metabolized in the liver but to some extent it is also metabolized in the lung tissue. On metabolism, BCME is converted into an epoxide, which is a reactive species of free radical capable of reacting with any organic substance. Metabolites of BCME cause alkylation of DNA leading to mutagenesis and carcinogenesis.

Glutathione *S*-transferase, sulfotransferase, and glucuronidation help in removal of toxic metabolites.

Mechanism of Toxicity

In humans, acute exposure to BCME may cause skin, mucous membrane, and respiratory tract irritation. Lung irritation, congestion, edema, and hemorrhage have been observed in rats and hamsters following acute inhalation exposure. BCME is irritating to the skin of mice and rabbits. Corneal opacity has been observed in rabbits. Acute animal tests in rats, mice, hamsters, and rabbits have demonstrated BCME to have extreme acute toxicity via inhalation and high acute toxicity via oral and dermal exposure. Chronic bronchitis, chronic cough, and impaired respiratory function have been observed in humans following chronic inhalation exposure. However, exposure to BCME usually occurs concurrently with exposure to chloromethyl methyl ether, which itself is a lung irritant. Chronic inhalation exposure of mice to BCME has been reported to cause respiratory distress.

Chronic Toxicity (or Exposure)

Animal

The International Agency for Research on Cancer (IARC) (1974, 1979, 1982, 1987) reported that there is sufficient evidence of carcinogenicity of BCME. When BCME is administered through subcutaneous route to mice of both sexes, it induced pulmonary tumors, papillomas, and firbrosarcomas; local sarcomas in female mice; and fibromas and fibrosarcomas in female rats. BCME is also an initiator of skin tumors in mice. It produced low incidence of tumors of respiratory tract in rats and hamsters after exposure by inhalation. When administered by inhalation, BCME induced lung tumors in mice and squamous cell carcinoma of the lung and esthesioneuroepitheliomas of the nasal cavity in rats. When applied topically, BCME induced papillomas, most of which developed into squamous cell carcinoma in female mice.

Human

BCME is known to be a human carcinogen based on sufficient evidence of carcinogenicity in humans. Numerous epidemiological studies and case reports from around the world have documented that workers exposed to BCME have an increased of lung cancer. Two studies of workers exposed to BCME showed an increased risk of lung cancer, mainly small cell carcinoma. Two subsequent studies have shown a positive association between atypical cells in bronchial excretion on exposure to BCME. Among heavily exposed workers, the relative risk of cancer is 10-fold or greater. Risks increase with duration and cumulative exposure. Maximal relative risks appear to occur 15–20 years after first exposure, and latency is shortened among workers with heavier exposure. Excess respiratory cancer mortality was most markedly increased in workers less than 55 years of age.

The American Conference of Governmental Industrial Hygienists time-weighted average threshold limit value is 0.001 ppm ($0.0047\,mg\,m^{-3}$) with the notation that material is a confirmed human carcinogen.

Clinical Management

There is no antidote recommended for poisoning by BCME. Administration of free radical scavengers should alleviate the toxicity.

Environmental Fate

No information is available on the transport and partitioning of BCME in the environment. Due to the relatively short half-life in both air and water, it is unlikely that significant partitioning between media or transport occurs. Primary process for BCME degradation in air is believed to be reaction with photochemically generated hydroxyl radicals to yield chloromethyl formate ClCHO, formaldehyde, and HCl. Atmospheric half-life due to reaction with hydroxyl radicals is estimated to be 1.36 h. Hydrolysis in the vapor phase is found to be slower with an estimated half-life of 25 h.

BCME is rapidly hydrolyzed in water to yield formaldehyde and HCl, and the hydrolysis rate constant is estimated to be $0.018\,s^{-1}$ at 20°C, which is equal to a half-life of ~ 35 s.

No information is available on the fate of BCME in soil. It is probable that BCME would rapidly degrade upon contact with moisture in soil. Due to its high volatile nature, it is not expected that BCME would persist in soil for significant periods.

Exposure Standards and Guidelines

The US Environmental Protection Agency (EPA) recommends that levels in lakes and streams should be limited to 0.0000038 parts per billion (ppb) parts of water to prevent possible health effects from drinking water or eating fish contaminated with BCME. Any release to the environment greater than 10 lbs of BCME must be reported to the EPA.

The EPA calculated an inhalation unit risk estimate of $0.062\,\mu g^{-1}\,m^3$. The EPA estimates that, if an

individual were to continuously breathe air containing BCME at an average of $0.000016\,\mu g\,m^{-3}$ ($1.6 \times 10^{-8}\,mg\,m^{-3}$) over his or her entire lifetime, that person would theoretically have no more than a 1 in 10^6 increased chance of developing cancer as a direct result of breathing air containing this chemical. Similarly, the EPA estimates that breathing air containing $0.00016\,\mu g\,m^{-3}$ ($1.6 \times 10^{-7}\,mg\,m^{-3}$) would result in not greater than a 1 in 10^5 increased chance of developing cancer, and air containing $0.0016\,\mu g\,m^{-3}$ ($1.6 \times 10^{-6}\,mg\,m^{-3}$) would result in not greater than a 1 in 10^4 increased chance of developing cancer.

The EPA has calculated an oral cancer slope factor of $220\,mg^{-1}\,kg\,day$. The Occupational Safety and Health Administration (OSHA) has set a limit of 1 ppb as the highest acceptable level in workplace air, and strict controls have been established to minimize exposure to this chemical.

The Agency for Toxic Substances and Disease Registry (ATSDR) has established an intermediate inhalation minimal risk level (MRL) of $0.0014\,mg\,m^{-3}$) (0.0003 ppm) based on respiratory effects in rats. The MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. It is not a direct estimator of risk but rather a reference point to gauge the potential effects. At exposures increasingly greater than the MRL, the potential for adverse health effects increases.

Further Reading

American Conference of Governmental Industrial Hygienists (ACGIH) (1999) *1999 TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents. Biological Exposure Indices.* Cincinnati, OH: ACGIH.

Budavari S (ed.) (1989) *The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals*, 11th edn. Rahway, NJ: Merck and Co. Inc.

International Agency for Research on Cancer (IARC) (1974) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous Alkylating Agents*, Vol. 4. Lyon: World Health Organization.

International Agency for Research on Cancer (IARC) Monographs (1974, 1979, 1982, 1987).

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Bis(chloromethyl) ether.

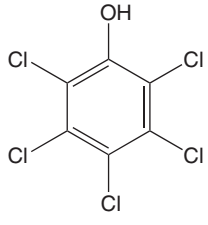
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Bis(chloromethyl) Ether.

Chlorophenols

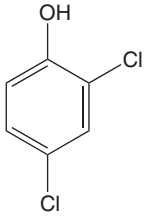
Murali Badanthadka and Harihara M Mehendale

This article is a revision of the previous print edition article by Stephanie E Foster and Paul W Ferguson, volume 1, pp. 312–313, © 1998, Elsevier Inc.

- REPRESENTATIVE CHEMICALS: Pentachlorophenol; 2,4-Dichlorophenol
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aromatic alcohols
- CHEMICAL STRUCTURES:



Pentachlorophenol



2,4-Dichlorophenol

Uses

Chlorophenols are used in dye synthesis, fungicides, herbicides, wood preservatives, and as ingredients in alcohol denaturants.

Exposure Routes and Pathways

Exposure to chlorophenols may occur through ingestion, inhalation, or dermal contact.

Toxicokinetics

Absorption of pentachlorophenol is rapid through oral, dermal, or inhalation exposure. The major tissue deposits vary somewhat between species. In humans, liver, kidney, brain, spleen, and fat are the major deposition sites. In the mouse, the gall bladder is a principal storage site. In the rat, it is the kidney. The primary route of elimination is by the kidneys in

unchanged form. Labeled pentachlorophenol given to rats by injection or oral route yielded 41–43% unchanged pentachlorophenol in the urine. One metabolite, tetrachlorohydroquinone (5–24%), was identified. Elimination half-life for pentachlorophenol may be up to 20 days in chronically exposed individuals.

In a study, a single dose of 15 mg pentachlorophenol per kg was administered intravenously and orally to B6C3F1 mice. After intravenous administration, the values of clearance and volume of distribution were $0.057 \pm 0.007 \,l\,h^{-1}\,kg^{-1}$ and $0.43 \pm 0.06 \,l\,h^{-1}\,kg^{-1}$, respectively. The elimination half-life was 5.2 ± 0.6 h. After oral administration, peak plasma concentration ($28 \pm 7 \,\mu g\,ml^{-1}$) occurred at 1.5 ± 0.5 h and bioavailability (1.06 ± 0.09) was complete. The elimination half-life was 5.8 ± 0.6 h. Only 8% of the pentachlorophenol dose was excreted unchanged in the urine. Pentachlorophenol was primarily recovered in urine as glucuronide and sulfate conjugate metabolites. A portion of the dose was recovered in urine as tetrachlorohydroquinone (5%) and its conjugates (15%). For both pentachlorophenol and tetrachlorohydroquinone, sulfates accounted for 90% or more of the total conjugates.

There is marked gender difference in biological half-life in non-human primates. Biological half-life for excretion in the Rhesus monkey was 41 and 92 h in males and females, respectively.

Mechanism of Toxicity

Chlorophenols block adenosine triphosphate (ATP) production, without blocking the electron transport chain. They inhibit oxidative phosphorylation, which increases basal metabolic rate and increases body temperature. As body temperature rises, heat-dissipating mechanisms are overcome and metabolism is accelerated. Adenosine diphosphate (ADP) and other substrates accumulate, and stimulate the electron transport chain further. This process demands more oxygen in a futile effort to produce ATP. Oxygen demand quickly surpasses oxygen supply and energy reserves of the body become depleted.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} for pentachlorophenol in laboratory animals ranges from 30 to $100 \,mg\,kg^{-1}$.

Human

Most prevalent signs and symptoms after ingestion of 30–250 ml of chlorophenols are corrosion of tissue, profuse sweating, intense thirst, nausea, vomiting, diarrhea, convulsions, pulmonary edema, cynosis, and coma. If death from respiratory failure is not immediate, jaundice and oliguria or anuria may occur.

Chronic Toxicity (or Exposure)

Human

Repeated exposure may cause symptoms of acute poisoning. Skin sensitivity reactions occur occasionally. Prolonged skin contact with chlorophenols may cause bladder tumors, hemolytic anemia, and lens opacities.

Pathologic findings in deaths by chlorophenols include necrosis of mucous membranes, cerebral edema, and degenerative changes in the liver and kidneys.

Clinical Management

Upon exposure by ingestion, where corrosive injury is absent, the decontamination to prevent further absorption may be achieved by use of activated charcoal. Emesis by syrup of ipecac may be considered, but not preferred. Next, milk should be given to drink. Gastric lavage and emesis are contraindicated in the presence of esophageal injury. In the case of dermal exposure, the poison should be removed by washing the affected skin or mucous membrane with copious amounts of water for at least 15 min.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is $0.5 \,mg\,m^{-3}$ for 8 h time-weighted average (TWA). The threshold limit value is $0.5 \,mg\,m^{-3}$ for 8 h TWA. The National Institute for Occupational Safety and Health recommended exposure limit is $0.5 \,mg\,m^{-3}$ for 10 h TWA.

See also: Chlorophenoxy Herbicides; Drugs of Abuse; Dyes.

Further Reading

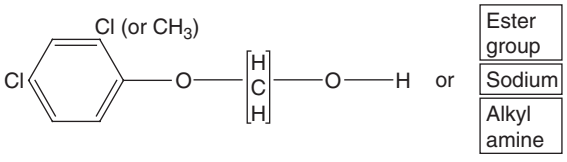
Jensen J (1996) Chlorophenols in the terrestrial environment. *Reviews of Environmental Contamination and Toxicology* 146: 25–51.

Chlorophenoxy Herbicides

Subramanya Karanth

This article is a revision of the previous print edition article by Thuc Pham, volume 1, pp. 313–315, © 1998, Elsevier Inc.

- Representative Chemicals: 2,4-D (2,4-dichlorophenoxy acetic acid); 2,4-DP (2-(2,4-Dichlorophenoxy)propionic acid); 2,4,5-T (2,4,5-Trichlorophenoxy acetic acid); Dicamba (3,6-Dichloro-*o*-anisic acid); MCPA (4-Chloro-2-methyl-phenoxy acetic acid); MCPP (2-(4-Chloro-2-methylphenoxy)propionic acid); Silvex (2-(2,4,5-Trichlorophenoxy)propionic acid)
- Chemical Abstracts Service Registry Numbers: CAS 94-75-7 (2,4-D); CAS 120-36-5 (2,4-DP); CAS 93-76-5 (2,4,5-T); CAS 94-76-4 (MCPA); CAS 93-65-2 (MCPP); CAS 93-72-1 (Silvex); CAS 1918-00-9 (Dicamba)
- Chemical/Pharmaceutical/Other Class: Herbicides
- Chemical Structure (General):



Uses

Chlorophenoxy herbicides are commonly used for controlling broadleaf weeds in agriculture. They are extensively used for the control of vegetation along highways, maintenance of parks, golf courses, home lawns, and gardens.

Background Information

Agent Orange, a mixture of the chlorophenoxy herbicides 2,4-D and 2,4,5-T, was extensively used by US military during Vietnam War in order to destroy forest and other vegetation from the premises of US bases. About 19 million gallons were used on ~3.6 million acres of land in Vietnam and Laos during the period from 1962 to 1971. Some lots of 2,4,5-T were contaminated with dioxins formed during manufacturing. Because dioxins resist degradation and remain in the environment for years, they are considered persistent organic pollutants. The particular dioxin present in Agent Orange, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or TCDD, is notoriously known as the most acutely toxic synthetic chemical. TCDD levels were found to be higher among veterans serving in Vietnam compared to those serving elsewhere at the same time. Concerns that these and other health problems may have been associated with exposure to Agent Orange stimulated a series of scientific studies, health care programs, and compensation programs directed in support of veterans. A large class-action lawsuit filed in 1979 was settled out of court in 1984 resulting in the Agent Orange Settlement Fund, which distributed nearly $200 million to veterans between 1988 and 1996.

A number of studies were conducted to evaluate a possible link between Agent Orange and cancer. The Air Force Health Study compared ~1200 Ranch Hand veterans directly involved in herbicide distribution to 1300 veterans not involved. Periodic physical exams, medical records reviews, and blood dioxin measurements were taken over a 20 year period. About a dozen states, mostly in the Midwest and Northeast, conducted health studies of Vietnam veterans, some of which included cancer information. A series of studies of Australian Vietnam veterans also evaluated cancer incidence. Because of some limitations of the Vietnam veteran studies, other studies were used to draw conclusions on Agent Orange and cancer. No association with soft tissue sarcoma was seen in the Ranch Hand study, in a study of over 10 000 Marines who had served in Vietnam, a large study of sarcoma patients in VA hospitals, the Selected Cancers Study, or studies of veterans in several US states. Most studies have not reported an increase in non-Hodgkin lymphoma (NHL) or Hodgkin disease and respiratory cancers (lung, trachea, bronchus, and larynx). While the VA and Ranch Hand studies did not show an excess of prostate cancer, the Australian studies showed an increased incidence of this type of cancer. Three studies demonstrated an association between paternal Agent Orange exposure and acute myeloid leukemia in children. There were no links established between Vietnam service and cancers of the gastrointestinal tract or brain. Thus, a number of studies were conducted on the possible association of Agent Orange exposure and cancer incidence, with considerable variation in outcome across studies.

The 'Agent Orange Act of 1991' required the US Secretary of Veterans Affairs to request an evaluation of the health effects of Agent Orange in Vietnam Veterans by the National Academy of Sciences. The Institute of Medicine (IOM) formed a 'Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides'. The Committee initiated a series of studies to evaluate cancer and noncancer

health effects in veterans. The IOM concluded that there was sufficient evidence of an association between Agent Orange and soft tissue sarcomas, NHL, Hodgkin disease, and chronic lymphocytic leukemia. Regulatory agencies have relatively similar conclusions regarding carcinogenicity of chlorophenoxy herbicides and TCDD. The US National Toxicology Program (NTP) does not list chlorophenoxy herbicides (including Agent Orange) as carcinogens, but lists 2,3,7,8-TCDD as 'known to be a human carcinogen'. The International Agency for Research on Cancer has not rated Agent Orange itself, but the chlorophenoxy herbicides including 2,4-D and 2,4,5-T are categorized as 'possibly carcinogenic to humans' while 2,3,7,8-TCDD is categorized as 'known to be carcinogenic to humans'. In contrast, the US Environmental Protection Agency has not classified either chlorophenoxy herbicides or TCDD as to carcinogenicity.

Exposure Routes and Pathways

Chlorophenoxy herbicides can be absorbed into the body by inhalation of its aerosol, through the skin, and by ingestion. The most common exposure pathway is accidental or intentional ingestion.

Toxicokinetics

Chlorophenoxy herbicides are readily absorbed through the gastrointestinal tract and distributed throughout the body. They are excreted unchanged mainly in the urine and are generally not stored in the body. Studies in laboratory rats given 1, 5, or $10\,mg\,kg^{-1}$ of ^{14}C 2,4-D have shown that 94–99% is eliminated from the body unchanged within 72 h. Biological half-life ranged from 10 to 33 h. Metabolic conversions may occur more with higher doses.

Mechanism of Toxicity

The mechanism of action of chlorophenoxy herbicides in mammals is not clearly known. They are believed to elicit toxicity by cell membrane damage, uncoupling of oxidative phosphorylation, or disruption of acetylcoenzyme A metabolism. Myotonia (stiffness and incoordination of hind extremities) is commonly observed following overdose of 2,4-D. In addition, high doses can cause significant metabolic acidosis and renal failure in humans. Formulations of chlorophenoxy herbicides were often contaminated by complex chlorinated hydrocarbons, e.g., dibenzodioxins. TCDD, which is highly toxic to mammals, was one of the common dioxin pollutants in Agent Orange.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chlorophenoxy herbicides exhibit low mammalian toxicity. The acute oral LD_{50} for 2,4-D in rats is $>300\,mg\,kg^{-1}$. Dogs are more susceptible to 2,4-D poisoning with an oral LD_{50} of $100\,mg\,kg^{-1}$.

Human

Ingestion of high doses can lead to burning of the mouth, vomiting, abdominal pain, and gastrointestinal hemorrhage. Acute exposure may also cause severe metabolic acidosis, myotonia, and muscle weakness, which can persist for a long period of time.

Chronic Toxicity (or Exposure)

Animal

Long-term feeding studies in rats have revealed that exposure to a daily dosage of $300\,mg\,kg^{-1}\,day^{-1}$ does not cause any adverse effects while exposure to higher doses can result in body weight loss and liver changes.

Human

Chronic exposure to some common chlorophenoxy herbicides such as 2,4-D through drinking water can potentially cause damage to the nervous system, kidneys, and liver. Chronic exposure to 2,4-D has also been linked to immune system suppression and endocrine disruption. Carcinogenic potential of these herbicides is not clear. 2,4-D and MCPA, which are commonly used in wheat production, have been linked to birth defects.

Clinical Management

Chlorophenoxy compounds are moderately irritating to skin. In case of dermal or eye exposure, the contaminated area should be bathed or flushed with copious amounts of water for $\sim$15 min and if irritation persists a physician should be contacted. Ingestion of substantial amounts of these chemicals results in spontaneous emesis. If the patient is fully alert and there are no apparent signs of emesis, emesis is induced with syrup of ipecac (adults, 30 ml; children $<$12 years, 15 ml), followed by one to two glasses of water. In order to limit the absorption of the herbicide in the gut, 30–50 g of activated charcoal is administered in $\sim$6–8 ounces of water. Severe intoxication with chlorophenoxy compounds may result in renal failure. To avoid

toxicant buildup in the kidney and to accelerate excretion, intravenous fluids (saline or dextrose) are administered and serum electrolytes monitored. Early initiation of forced alkaline diuresis with sodium bicarbonate may be useful in the management of acute poisoning.

Environmental Fate

Chlorophenoxy herbicides are generally not persistent in the environment. Common herbicides such as 2,4-D, MCPP, and dicamba are readily biodegraded by soil and aquatic microorganisms. 2,4-D and dicamba are commonly found in public drinking water systems. Typical half-life in water ranges from 10 to 15 days.

Ecotoxicology

Chlorophenoxy herbicides are moderately toxic to nontarget species. Some of the commercial products are highly toxic to aquatic invertebrates and other beneficial nontarget plants.

See also: Carcinogen Classification Schemes; Carcinogenesis.

Relevant Websites

http://www.cancer.org – American Cancer Society.
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://infoventures.com – US Department of Agriculture, Forest Service.

Chloropicrin

Priya Raman

- Chemical Abstracts Service Registry Number: CAS 76-06-2
- Synonym: Trichloronitromethane
- Chemical/Pharmaceutical/Other Class: Fumigant insecticide
- Chemical Formula: CCl_3NO_2

Uses and Background Information

Chloropicrin is a widely used fungicide that is primarily used for preplant soil fumigation. Chloropicrin is used to fumigate stored grain and to treat soil against fungi, insects, and nematodes. It is also a tear gas agent for military use. Chloropicrin was first synthesized in 1848. It was patented as an insecticide in 1908 and has been extensively used since then as a soil fumigant at high application rates of $18\,lb\,acre^{-1}$. Chloropicrin, which has a total US production of ~10–11 million pounds per year was also used as a war gas agent during World War I.

Exposure Routes and Pathways

Chloropicrin has strong lacrimatory properties and is a potent skin irritant. Thus, dermal and eye exposures are the most common routes of chloropicrin toxicity. It is also an inducer of vomiting, bronchitis, and pulmonary edema in humans. As a fumigant, the respiratory tract is the principal target of chloropicrin toxicity. The primary lesion following ingestion of chloropicrin is manifested by corrosive effects on the forestomach tissue. The intraperitoneal LD_{50} in mice is $25\,mg\,kg^{-1}$. Human exposure to chloropicrin also occurs from trace levels in drinking water disinfected by chlorination.

Toxicokinetics

The toxic effects of chloropicrin occur very rapidly. The liver is the primary site of metabolism of this compound. Reductive dechlorination of chloropicrin serves as the basis for its multiple types of toxic action. Following an intraperitoneal or oral administration of chloropicrin, urine is the major route for excretion of its metabolites, mostly (43–47%) within the first 24 h. The urinary metabolites at 24 h are polar and nonvolatile.

Mechanism of Toxicity

Recent studies identify a new metabolic pathway for chloropicrin involving a rapid dechlorination to $CHCl_2NO_2$ and conversion of glutathione (GSH) to GSSG plus possible adduct formation with thiol proteins. In this newly discovered pathway, chloropicrin is metabolized to thiophosgene, characterized as the cyclic cysteine adduct (raphanusamic acid) in mice urine. The initially formed GS-CCl_2NO_2 metabolite is proposed to either react further with GSH or is cleaved by cysteine-β-lyase, ultimately leading to raphanusamic acid, which is excreted. Chloropicrin is an

SN_2 alkylating agent with an activated halogen group and reacts with sulfhydryl groups, 'fixing' enzymes. Oxidation of protein thiols with chloropicrin is accompanied with the formation of internal and cross-linked disulfide bonds leading to the suggestion that inhibition of pyruvate dehydrogenase (PDH) and succinate dehydrogenase (SDH) with critical SH groups in their active sites, are involved in acute mammalian toxicity. Both PDH and SDH complexes are inhibited *in vitro* by chloropicrin with moderate potency ($IC_{50} = 4–13\,\mu mol\,l^{-1}$). Chloropicrin also has the additional toxic effect of interfering with oxygen transport by its reaction with SH– groups in hemoglobin. Thus, chloropicrin toxicity in mice is linked to the accumulation of oxyhemoglobin in tissues, particularly the liver. Chloropicrin may also undergo a photochemical transformation to phosgene.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chloropicrin is 10 times more potent than its dehalogenated metabolites. It has been reported to inhibit porcine heart pyruvate and mouse liver succinate dehydrogenase complexes with IC_{50} values of 4 and $13\,\mu mol\,l^{-1}$ respectively. Mice treated intraperitoneally with choloropicrin at $50\,mg\,kg^{-1}$ showed a dose-dependent increase in liver oxyhemoglobin, hemoprotein, and total hemoglobin levels. Acute toxicity of chloropicrin in mice is due to the parent compound or metabolites other than $CHCl_2NO_2$ or CH_2ClNO_2. In rats, the respiratory tract is the primary target on inhalation exposure. Chloropicrin is intensely irritating with an intraperitoneal LD_{50} of $25\,mg\,kg^{-1}$ in mice. Rabbits exposed to an intravenous injection of chloropicrin at a dosage of $15\,mg\,kg^{-1}$ died within 15–240 min; clinical and autopsy findings were typical of acute pulmonary edema. Chloropicrin induces lesions in the lower respiratory tract with an RD_{50} (concentration which elicits a respiratory rate decrease of 50%) values of 8 ppm. The no-observed-adverse-effect level (NOAEL) for systemic toxicity following chloropicrin exposure is reported to be 1.0 ppm and greater than 1.5 ppm for developmental toxicity and reproductive parameters. The NOAEL for maternal toxicity has been established to be 0.4 ppm and the NOAEL for fetal toxicity is 1.2 ppm suggesting that the developing fetus is not a target tissue for chloropicrin toxicity.

Human

Exposure to chloropicrin causes eye and respiratory tract irritation accompanied by vomiting and diarrhea. The primary signs and symptoms following inhalation exposure to chloropicrin include coughing, nasal and pharyngeal mucosal edema, and erythema, lacrimation, and rhinorrhea. Fatal pulmonary edema has been reported with an onset of 3 h post-exposure. Chloropicrin is a strong eye irritant, producing ocular burning, eye pain, and lacrimation following eye exposure. These effects may last up to 30 min or longer. Redness and edema may be noted 1 or 2 days following exposure. Dermal exposure to chloropicrin produces severe skin irritation.

Chronic Toxicity (or Exposure)

Animal

Following an oral gavage for 90 days, the no-effect-dose is $8\,mg\,kg^{-1}\,day^{-1}$, with severe forestomach tissue lesions characterized by inflammation, necrosis, acantholysis, hyperkeratosis, and epithelial hyperplasia. Mice exposed to 8 ppm chloropicrin vapor for $6\,h\,day^{-1}$ for 5 days developed moderate to severe degeneration of the respiratory and olfactory epithelium as well as fibrosing peribronchitis and peribronchiolitis of the lung.

Human

Following exposure to chloropicrin vapor in an agricultural chemicals facility, persistent chest wall pain as well as an increase in creatine phosphokinase levels has been reported. Severity of symptoms and degree of biochemical abnormalities were reported to occur in a dose-dependent pattern. Inhalation exposure to very high levels of chloropicrin can lead to pulmonary edema, unconsciousness, and even death.

In Vitro Toxicity Data

Chloropicrin is a bacterial mutagen and induces sister chromatid exchanges in cultured human lymphocytes, but is not considered as carcinogenic. Mutagenicity assays establish chloropicrin to be toxic but not mutagenic at 500 nmol per plate.

Clinical Management

Following an eye exposure to chloropicrin, the affected eyes should be irrigated with copious amounts of tepid water for at least 15 min. If irritation persists following decontamination, ophthalmic corticosteroids or local anesthetic ointments may be used. In case of an inhalation exposure, the patient should be monitored for respiratory distress. Emergency airway support and 100% humidified supplemental oxygen with assisted ventilation may be needed.

Following dermal exposure to chloropicrin, the exposed area must be washed thoroughly with soap and water. If dermatitis persists, topical treatment with wet dressings of Burow's solution 1:40, followed by corticosteroid creams or calamine lotion, may be given. Secondary infection may necessitate antibiotic therapy. Oral antihistamines may be useful for pruritis.

Environmental Fate

The half-life of chloropicrin in sandy loam soil was 8–24 h and 4.5 days with carbon dioxide being the terminal breakdown product. Chloropicrin can be produced during chlorination of drinking water in the presence of nitrated organic contaminants. Chloropicrin is efficiently photolyzed in the atmosphere. The half-life of chloropicrin in air exposed to stimulated sunlight is reported to be 20 days, the photoproducts being phosgene, nitric oxide, chlorine, nitrogen dioxide, and dinitrogen tetroxide.

Ecotoxicology

Chloropicrin is highly toxic to fish, the 96 h LC_{50} for trout and bluegill being 0.0165 and 0.105 mg l^{-1} respectively. However, little information is available about the effects of chloropicrin on birds.

Exposure Standards and Guidelines

Chloropicrin is a class I toxicity, restricted use pesticide (RUP), labeled with the signal word 'Danger'. The only exposure guidelines available for chloropicrin are that of permissible exposure level and threshold limit value, both having a value of 0.1 ppm. It has an inhalation reference exposure level of 0.4 μg m^{-3}.

Miscellaneous

Chloropicrin is a clear, colorless oily liquid with a sharp, highly irritating odor. It has a molecular weight of 164.38 and a water solubility of 1.6 g l^{-1} at room temperature. Chloropicrin is miscible with most organic solvents, and has a melting point of −64°C. Some trade names for products containing chloropicrin include Chlor-O-Pic, Metapicrin, Timberfume and Tri-Chlor.

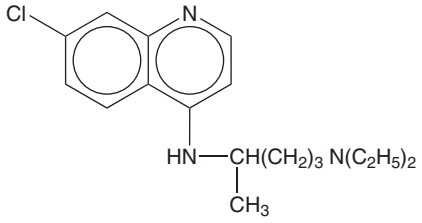
See also: Pesticides.

Chloroquine

F Lee Cantrell

This article is a revision of the previous print edition article by Michael Shannon, volume 1, pp. 316–317, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 54-05-7
- Synonyms: SN 7618; Sanoquin; Tresochin; Silbesan; Artichin; Bipiquin; Avlocluor; Tanakan; Resochin; Resoquine; Aralen
- Chemical/Pharmaceutical/Other Class: Aminoquinoline antimalarial/antirheumatic
- Chemical Formula: $C_{18}H_{26}ClN_3$
- Chemical Structure:



Uses

Chloroquine is used as an anti-inflammatory and antimalarial drug.

Exposure Routes and Pathways

Chloroquine is available in oral and intravenous forms.

Toxicokinetics

Chloroquine is absorbed rapidly and almost completely from the gut; peak serum concentrations are attained within 1 or 2 h. Chloroquine plasma protein binding is ~55%. Its volume of distribution is 116–285 l kg^{-1}. The drug may be found in 500 times greater concentration within the liver, spleen, kidneys, lungs, and leukocytes (compared with plasma). Chloroquine appears to cross the placenta readily. A very small amount is transmitted into breast milk.

The primary route of metabolism is deethylation, producing desethylchloroquine. Elimination is significantly reduced in the presence of hepatic disease.

Nearly 50% of chloroquine is recovered in urine as unchanged drug. The terminal half-life of chloroquine varies from 12 to 60 days.

Mechanism of Toxicity

The mechanism of action of chloroquine is not completely understood but involves inhibition of DNA and RNA polymerase. Chloroquine is also a direct myocardial depressant that impairs cardiac conduction through membrane stabilization.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chloroquine is not used therapeutically in domestic animals. Toxic manifestations of overdose in animals are undefined.

Human

Symptoms of overdose include nausea, vomiting, transient visual or auditory deficits, drowsiness, and seizures followed by severe cardiac arrhythmias, shock, or cardiorespiratory arrest. Hypotension may be severe and intractable, producing metabolic acidosis and end-organ failure. Cardiac conduction disturbances include complete atrioventricular dissociation, QRS and QT prolongation, severe bradycardia, and ventricular fibrillation. Acute ingestions of 30–50 mg kg^{-1} of chloroquine in adults and as little as 300 mg in children are potentially fatal.

Chronic Toxicity (or Exposure)

Animal

Rats chronically administered chloroquine in food for up to 2 years demonstrated dose related inhibition of growth compared with controls. High-dose (from 100 up to 1000 mg kg^{-1} diet over 2 years) studies in rats showed myocardial and other muscle damage centrilobular liver necrosis and testicular damage.

Human

Chronic use of chloroquine may produce cinchonism, a syndrome characterized by headache, visual changes, and gastrointestinal disturbances. Visual disturbances are associated with retinal artery spasm. Ototoxicity may also occur. Dermatologic reactions, particularly a lichenoid skin eruption, may result from chronic chloroquine use.

In Vitro Toxicity Data

Studies in cultured chick brains demonstrated inhibition of retinal pigment epithelium viability at concentrations similar to those seen *in vivo* for patients experiencing chloroquine-induced retinopathy.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. In patients presenting within 1 h of ingestion, activated charcoal should be administered. In the event of depressed consciousness or seizures, airway protection should first be secured. Sodium bicarbonate, epinephrine, and high-dose diazepam should be used to treat cardiotoxicity. Diazepam is recommended for the treatment of seizures. Methods of extracorporeal drug removal, such as hemoperfusion and hemodialysis, are ineffective.

See also: Charcoal; Diazepam.

Further Reading

CDC (1988) Childhood chloroquine poisoning – wisconsin and washington. *Morbidity and Mortality Weekly Report* 37: 437–439.

Piette JC, Guillevin L, and Chapelon C (1987) Chloroquine cardiotoxicity. *New England Journal of Medicine* 317: 710–711.

Relevant Website

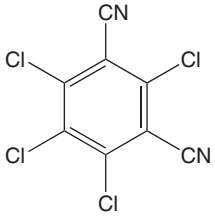
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Chloroquine.

Chlorothalonil

Priya Raman

- Chemical Abstracts Service Registry Number: CAS 1897-45-6
- Synonyms: 2,4,5,6-Tetrachlorobenzenedicarbonitrile; Tetrachloroisophthalonitrile
- Chemical/Pharmaceutical/Other Class: Industrial, agricultural, and horticultural fungicide

- Chemical Structure:



Uses and Background Information

Chlorothalonil is an extensively used pesticide in agriculture, silviculture, and urban settings. This is an important broad-spectrum nonsystemic fungicide that has been widely used for more than 30 years as an effective disease management tool for potatoes, peanuts, turf, and vegetable and fruit crops. Typical chlorothalonil application rates are $1\,kg\,ha^{-1}$ with four to nine applications per growing season. Chlorothalonil can enter surface waters through rainfall runoff, spray drift, or atmospheric deposition, subsequently having an impact on the aquatic biota. It is also used as a fungicide, bactericide, and nematocide. It is used as a wood preservative in some countries. Chlorothalonil is also used as a mildew-preventing agent in paints.

Exposure Routes and Pathways

Dermal and eye exposure are the most common routes of accidental exposure to chlorothalonil. It may also be ingested or inhaled.

Toxicokinetics

Chlorothalonil is rapidly absorbed both orally and via inhalation. However, the amount of material absorbed is limited and dose related. Thus, while ~30% of an administered dose of up to $50\,mg\,kg^{-1}$ is absorbed, absorption at high doses decreases relatively. There is also a marked species-dependent variation in chlorothalonil absorption. Chlorothalonil is only poorly absorbed by the dermal route of exposure. Absorbed chlorothalonil undergoes rapid distribution among body tissues in rats, tissue concentration being in the order of kidney > liver > whole blood. Conjugation with glutathione constitutes the primary route of metabolism of chlorothalonil. Liver represents the major site for chlorothalonil conjugation. In the enzymatic reaction, 4-(glutathione-*S*-yl)-2,5,6-trichloroisophthalonitril is formed initially. This is also a substrate for glutathione-*S*-transferases (GSTs), resulting in the substitution of a second chlorine atom to give 4,6-bis(glutathione-*S*-yl)-2,5-dichloroisophthalonitril. Hydrolysis studies indicated that the metabolism of chlorothalonil is pH dependent. Thus, 4-hydroxy-2,5,6-trichloroisophthalonitrile and 3-cyano-2,4,5,6-tetrachlorobenzamide are formed at pH 9 but not at pH 7. The metabolism of chlorothalonil was recently investigated in liver and gill cytosolic and microsomal fractions from channel catfish using high-performance liquid chromatography. The reports indicate that chlorothalonil is detoxified *in vitro* by GST-catalyzed glutathione (GSH) conjugation. However, no human data are currently available for the biotransformation of chlorothalonil. Chlorothalonil is primarily eliminated via the kidneys. Following administration of $1\,mg\,kg^{-1}$ chlorothalonil endotracheally, orally, or dermally to rats, less than 6% was recovered in blood or urine within 48 h. The major route of elimination following oral administration to rats is in the feces (>80%) with 5.4–11.5% being excreted in the urine as the dose increases from 5 to $200\,mg\,kg^{-1}$. Marked species differences exist in the pharmacokinetic behavior of chlorothalonil. Thus, following oral administration of $50\,mg\,kg^{-1}$ chlorothalonil, dogs and rhesus monkeys excrete up to 98% and 92% of the dose, respectively, in the feces compared with ~82% in rats.

Mechanism of Toxicity

Glutathione conjugation represents a bioactivation reaction for chlorothalonil resulting in the formation of *S*-conjugates toxic to the kidney. Chlorothalonil acts as an alkylating agent and reacts with cellular sulfhydryl compounds. Alkylation of biological molecules results in effects on cellular function and viability. Chronic damage to the proximal tubular epithelium may be involved in the mechanism of chlorothalonil tumorigenicity to the kidneys.

Animal Toxicity

Forestomach and the renal proximal tubule are the primary target tissues of chlorothalonil toxicity in Sprague–Dawley rats. Toxicity is characterized by hypertrophy, hyperplasia, vacuolization, and degeneration of renal tubular epithelium and acanthosis, hyperkeratosis, and hyperplasia of the squamous epithelium of the forestomach. Chlorothalonil is a well-known skin and eye irritant. Sustained contact with the squamous epithelium of the forestomach can lead to an inflammatory response. The earliest observation following chlorothalonil administration at $175\,mg\,kg^{-1}\,day^{-1}$ to rats for varying periods of time for up to 91 days has been characterized by multifocal ulceration and rosion of the mucosa,

subsequently progressing to hyperplasia and hyperkeratinosis. These lesions have been observed in subchronic and chronic studies in rats and mice (no-observed-effect level (NOEL) $\sim 2\,mg\,kg^{-1}\,day^{-1}$) and in chronic studies appear to be closely related with incidence of neoplasia (NOEL ~ 4–$21\,mg\,kg^{-1}\,day^{-1}$). Undiluted chlorothalonil is a strong irritant and produces irreversible corneal, iridal, and conjunctival effects in rabbits. Weakness and sedation precedes death in animals given acute toxic doses intraperitoneally. Chronic oral administration to rats results in ataxia. Hematuria, vaginal bleeding, and epistaxis are seen in rats following chronic oral exposure. In chronic dermal exposures to chlorothalonil dissolved in acetone, the no-effect level for irritation is 0.001%. The 0.01% concentration is a mild irritant and 0.1% a moderate irritant. Prolonged exposure of rodents to chlorothalonil results in nephrotoxicity and renal tubular hyperplasia and these effects, if sustained, can lead to a tumorigenic response. Chlorothalonil produced a dose-related increased incidence of renal tubular adenomas and adenocarcinomas in rats. The oral LD_{50} in rats is greater than $10\,g\,kg^{-1}$. Chlorothalonil is predicted to be a rodent carcinogen via a nongenotoxic mechanism.

Human Toxicity

Facial dermatitis has been reported in occupational exposures and can occur in the absence of direct skin contact, presumably due to the high volatility of chlorothalonil. Chlorothalonil is a strong primary skin irritant and may also cause allergic contact urticaria and anaphylaxis. Patch testing with concentrations greater than 0.01% may produce primary irritant reactions. Hypersensitivity reactions characterized by facial erythema, periorbital erythema and edema, eczema, and pruritis have been observed following chlorothalonil exposure. Photosensitivity reactions were seen in some individuals. High concentrations of chlorothalonil produce delayed irritant reactions. Delayed dermal irritant effects have also been noted 48–72 h after cessation of exposure. Immediate respiratory reactions such as tightness of chest and throat, may occur following inhalation exposure to chlorothalonil. A recent review of the potential cancer risks of chlorothalonil to operators and consumers conducted in the United Kingdom for the Pesticide Advisory Committee (UK, 1994) provided evidence that chlorothalonil is not genotoxic. The primary potential for human exposure to chlorothalonil is to forest service applicators applying the fungicide.

Clinical Management

One of the primary forms of treatment is to support respiratory and cardiovascular function. Dilution and dermal/eye decontamination are primary considerations. Following oral exposure, immediate dilution with 4–8 oz of milk or water is recommended. Vomiting must be induced if victim is conscious. If inhaled, victim must be immediately moved to fresh air. And if not breathing, artificial respiration should be provided. In case of dermal exposure to chlorothalonil, the exposed area should be thoroughly washed with soap and water. Allergic contact dermatitis may be treated with antihistamines, topical steroids, and/or systemic steroids. Following an eye exposure, the affected eyes should be irrigated with copious amounts of tepid water for at least 15 min. Immediate medical attention for the eyes is also recommended. Exposure may cause temporary allergic side effects. Symptoms include redness of the eyes, mild bronchial irritation, and redness or rash on exposed skin. Temporary allergic reactions can be treated with antihistamines or steroid creams and/or systemic steroids upon consultation with the physician.

Environmental Fate

Chlorothalonil is moderately persistent in soil, having a half-life of up to 3 months in moderately moist soil. The principal breakdown product of chlorothalonil in the soil is 4-hydroxy-2,5,6-trichloroisophthalonitrile, which is slightly toxic to aquatic organisms and moderately toxic to birds and mammals. Chlorothalonil is almost insoluble in water and does not evaporate easily.

Ecotoxicology

Chlorothalonil is highly toxic to fish and other aquatic invertebrate animals. However, it is relatively nontoxic to birds, mammals, and bees.

Exposure Standards and Guidelines

Chlorothalonil is classified as a general use pesticide (GUP) by the US Environmental Protection Agency (EPA). Owing to its potential for causing eye irritation, chlorothalonil is also classified as a toxicity class II-moderately toxic chemical. Based on the increased incidence of renal tumor in female rats, EPA currently lists chlorothalonil as a group B2 (probable human) carcinogen; the Q^* value being $0.00766\,mg\,kg^{-1}\,day^{-1}$. Chlorothalonil has an acceptable daily intake and a reference dose value of 0.03 and $0.015\,mg\,kg^{-1}\,day^{-1}$, respectively.

Miscellaneous

Chlorothalonil is an aromatic halogen compound that appears as a grayish to colorless crystalline solid that is odorless or has a slightly pungent odor. It has a molecular weight of 265.92, water solubility of $0.6\,mg\,l^{-1}$ at room temperature, and a melting point of 250–251°C. Chlorothalonil is only slightly soluble in acetone, dimethyl sulfoxide, cyclohexane, and xylene. Some popular trade names for chlorothalonil include Bravo, Daconil 2787, Echo, Exotherm Termil, Nopcocide, Repulse, and Tuffcide. This compound can be found in formulations with many other pesticide compounds.

See also: Nematocides.

Further Reading

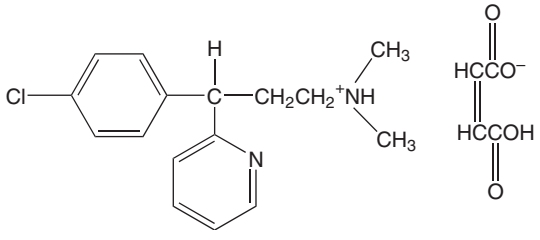
Andre V, Lebailly P, Pottier D, *et al.* (2003) Urine mutagenecity of farmers occupationally during a 1-day use of chlorothalonil and insecticides. *International Archives of Occupational and Environmental Health* 76(1): 55–62 (Epub Oct 2002).

Sherrard RM, Murray-Gulde CL, Rodgers JH Jr., and Shah YT (2003) Comparative toxicity of chlorothalonil: *Ceriodaphnia dubia* and *Pimephales promelas*. *Ecotoxicology and Environmental Safety* 56(3): 327–333.

Chlorpheniramine

Brenda Swanson-Biearman

- Chemical Abstracts Service Registry Number: CAS 132-22-9
- Synonym: Chlortrimeton®
- Chemical/Pharmaceutical/Other Class: A propylamine-derivative (alkylamine) H-1 receptor antagonist
- Chemical Formula: $C_{16}H_{19}ClN_2$
- Chemical Structure:



Uses

Chlorpheniramine, like other antihistamines, is most often used to provide symptomatic relief of allergic symptoms caused by histamine release. It is often included in multisymptom cold preparations.

Exposure Routes and Pathways

Ingestion and injection are the routes of both accidental and intentional exposures to chlorpheniramine.

Toxicokinetics

Chlorpheniramine is well absorbed following oral administration with peak plasma levels occurring within 2–6 h. The drug undergoes substantial metabolism in the gastrointestinal mucosa during absorption and first pass through the liver. The volume of distribution of chlorpheniramine is $3.4–7.5\,l\,kg^{-1}$ and ~69–72% of the drug is bound to plasma proteins. Chlorpheniramine and its metabolites (desmethylchlorpheniramine and didesmethylchlorpheniramine) are excreted almost completely in the urine. Urinary excretion is enhanced with an acidic urine pH, but this is not a viable treatment option. The elimination half-life is more rapid in children than adults, 9.5–13 and 14–24 h, respectively.

Mechanism of Toxicity

The toxicity of antihistamines is related to their anticholinergic (antimuscarinic) activity. The action of acetylcholine at the muscarinic receptors is blocked resulting in signs and symptoms of anticholinergic poisoning.

Acute and Short-Term Toxicity (or Exposure)

Animal

Absorption in dogs is rapid and complete from the gastrointestinal tract, reaching peak plasma concentrations 30–60 min after oral administration. Plasma half-life is ~24 h. Central nervous system (CNS) changes, including sedation or hyperexcitability, salivation, and vomiting, have occurred in dogs and cats following acute low-dose exposures to antihistamines. Seizures and cardiac effects have occurred following acute high-dose exposures. Symptomatic and supportive care, followed by appropriate gastrointestinal decontamination procedures, is the mainstay of treatment.

Human

The alkylamine derivatives are among the most potent antihistamines producing more CNS stimulation and less drowsiness than other antihistamines. In overdoses, CNS stimulation is more common in children. Adult overdose usually causes CNS depression with drowsiness or coma followed by excitement, seizures, and postictal depression. Anticholinergic symptoms including fixed and dilated pupils, flushed skin, dry mouth, fever, urinary retention, hallucinations, and seizures. Cardiovascular effects including tachycardia (prolonged QTc and QRS intervals and nonspecific ST and T-wave changes), hypertension or hypotension, dysrhythmias, and cardiovascular collapse may occur. Severe toxicity may result in cerebral edema, deep coma, cardiorespiratory collapse, or death. Onset of symptoms may occur within 30 min to 2 h after ingestion; death may occur several days after onset of toxic symptoms.

Chronic Toxicity (or Exposure)

Animal

Chronic animal feeding models designed to test for carcinogenicity and mutagenicity have so far proved negative.

Human

Chronic dosing studies in adults and children have demonstrated predictable side effects of drowsiness and sedation in therapeutic doses.

In Vitro Toxicity Data

Ames *Salmonella* and mouse lymphoma tests for mutagenicity have been negative.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Appropriate time-dependent gastrointestinal decontamination should be performed based on the history of the ingestion and the patient's level of consciousness. Electrocardiogram monitoring should be considered in patients who have taken large overdoses. Sinus tachyarrhythmias rarely require treatment. In agitated patients, sedation with benzodiazepines will generally control tachycardia. The use of class Ia antidysrhythmics (quinidine, disopyramide, procainamide, aprindine) and most class III antidysrhythmics (*N*-acteylprocainamide, sotalol) should be avoided since they may further prolong the QT interval and have been associated with torsades de pointes. Intravenous benzodiazepines are recommended for the treatment of seizures. Physostigmine administration may be necessary in a limited number of patients suffering from severe central and peripheral anticholinergic symptoms refractory to conventional therapy.

See also: Benzodiazepines.

Further Reading

Wogoman H, Steinberg M, and Jenkins AJ (1999) Acute intoxication with guaifenesin, diphenhydramine, and chlorpheniramine. *American Journal of Forensic Medicine and Pathology* 20: 199–202.

Wyngaarden JB and Seevers MH (1951) The toxic effects of antihistaminic drugs. *Journal of the American Medical Association* 145: 277–282.

Chlorpromazine

Linda A Malley

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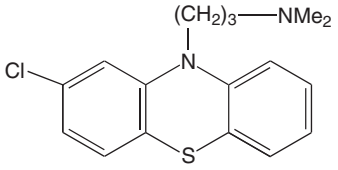
- Chemical Abstracts Service Registry Number: CAS 50-53-3
- Synonyms: 10H-Phenothiazine-10-propanamine; 2-Chloro-*N*,*N*-dimethyl-(9CI CA index name); Phenothiazine; 2-Chloro-10-[3-(dimethylamino)propyl]-(7CI, 8CI); 2-Chloro-10-[3-(dimethylamino)propyl]phenothiazine; 2-Chloropromazine; 4560 R. P.; Aminazin; Aminazine; Ampliactil; Amplictil; BC 135; Chlorpromanyl; Chlordelazin; Chlorderazin; Chlorpromados; Contomin; CPZ; Elmarin; Esmind; Fenactil; Fenaktyl; Fraction AB; HL 5746; Largactil; Largactilothiazine; Largactyl; Megaphen; Novomazina; Phenactyl; Proma; Promactil; Promazil; Propaphenin; Prozil; Sanopron; SKF 2601-A; Thorazin; Thorazine; Torazina; Wintermin
- Chemical/Pharmaceutical/Other Class: Phenothiazine antidepressants
- Chemical Formula: $C_{17}H_{19}ClN_2S$

- Chemical Structure:



Uses

Chlorpromazine is a phenothiazine type of antidepressant and is used as a medication for both humans and animals. In humans, it is employed primarily in the treatment of psychiatric patients as an effective treatment for the management of psychotic disorders, manic depressive illness, apprehension, and anxiety, as well as for the treatment of severe behavioral problems in children. It is also used for short-term treatment of hyperactive children who exhibit excessive motor activity with accompanying conduct disorders. Chlorpromazine is also used to control nausea and vomiting, intractable hiccups, for reduction of choreiform movement in Huntington's disease, and, prior to surgery, acute intermittent porphyria. It is also used as adjunct in the treatment of tetanus, amphetamine toxicity, and migraines. In animals, chlorpromazine is recommended in excitable sows following farrowing, especially in those reluctant to accept their newborn; to capture African lions; as an adjunct to restraint and anesthesia; and as a neuroleptanalgesia (inducing a state of quiescence) in bears, and in reptiles prior to the administration of barbiturate anesthesia.

Background Information

Chlorpromazine is manufactured by heating 2-chloro-phenothiazine and 3-chloropropyl dimethylamine in the presence of sodomide, followed by reaction with hydrogen chloride.

Exposure Routes and Pathways

Chlorpromazine is administered orally, intravenously, intramuscularly, and via suppository. Pharmacists, physicians, and nurses dispensing or administering chlorpromazine could be exposed through dermal or inhalation contact.

Toxicokinetics

Absorption

Absorption of orally administered chlorpromazine is dependent on dosage form, with the elixir giving highest plasma concentration. Absorption of chlorpromazine tablets is erratic. The presence of food in the stomach changes the absorption rate. Antacids decrease the absorption of chlorpromazine.

Distribution and Excretion

Peak plasma levels are reached at 2–4 h, although wide variations (at least 10-fold) in plasma concentrations occur among individuals. Chlorpromazine is 92–97% bound to plasma proteins, principally albumin, at plasma chlorpromazine concentrations of 0.01–1 $\mu g\,ml^{-1}$. Concentrations in the brain are four to five times higher than the plasma. Following a single oral chlorpromazine dose of 120 $mg\,m^{-2}$ to four healthy men, $<1\%$ of the dose was excreted unchanged in the urine within 72 h. Following continuous oral administration of chlorpromazine to a limited number of psychiatric patients in dosages ranging from 0.1 to 1.4 g daily, an average of 37% of the dose was excreted in urine, principally as metabolites. Although intestinal absorption is complete, oral bioavailability is 32% because of variable metabolism in intestinal wall and liver. Intramuscular administration avoids much of the first-pass metabolism in the liver (and possibly also gut) and provides measurable concentrations in plasma within 15–30 min; bioavailability may be increased up to 10-fold.

Chlorpromazine is strongly bound to protein, crosses blood–brain barrier, and concentrates in the brain against plasma gradient. More than 90% of the drug in plasma is bound to proteins, is metabolized in the liver, and is excreted in both urine and feces. There is some evidence that chlorpromazine can cause hepatic microsomal enzyme induction, which indicates that it may accelerate its own metabolism.

Rapid placental transfer was reported in goats and mice. In goats, the fetal plasma levels approached 50% of maternal values within 10 min of the mother receiving an intravenous dose, and the fetal/maternal plasma ratio remained at 0.5 for 1 h, whereas ratios in the liver, kidney, heart, and brain all approached 1 and showed a marked effect on fetal heart rate. In pregnant mice, radiolabeled chlorpromazine rapidly crossed the placenta and accumulated in the eyes of both fetuses and mothers. Marked radioactivity remained in tissues of the eye for 5 months after the drug had been eliminated from other tissues.

Markedly variable half-life values reported for chlorpromazine vary from a relatively short plasma half-life of 2–6 to 18 h. Disappearance of chlorpromazine from plasma includes a rapid distribution phase (half-life $\sim$2 h) and slower early elimination phase (half-life $\sim$30 h). One study reported that after

an oral dose of 120 mg m^{-2} to human volunteers, chlorpromazine displayed a mean elimination half-life of ~18 h (range, 6–119 h). Half-life of elimination from human brain is unknown. The pharmacokinetics of chlorpromazine was investigated in 25 pediatric patients (aged 0.3–17 years) who received an intravenous infusion of 1 mg kg^{-1} chlorpromazine with intravenous metoclopramide administered concomitantly. Compared with previously reported values for adults, the pharmacokinetics of chlorpromazine appeared to be accelerated in children. This was especially evident for half-life and clearance values.

Metabolism

Chlorpromazine undergoes considerable metabolism during absorption (in gastrointestinal mucosa) and first-pass through the liver. As many as 10 or 12 chlorpromazine metabolites have been found to occur in appreciable quantities in humans. Quantitatively, the most important of these are nor2-chlorpromazine (doubly methylated), chlorphenothiazine (removal of entire side chain), methoxy and hydroxy products, and glucuronide conjugates of hydroxylated compounds. In urine, 7-hydroxylated and dealkylated (nor2) metabolites and their conjugates predominate.

In patients, chlorpromazine and various metabolites may be detected in urine 6–18 months after termination of treatment. Little or no chlorpromazine is eliminated in urine of the dog. The primary excretory product in the dog is chlorpromazine sulfoxide, but only 10–15% of the dose is eliminated as such. In horses, metabolites have been detected in urine up to 96 h following intramuscular injection. Following oral administration, metabolites are no longer detected after 80–96 h. The percentage of the dose recovered in equine urine is low, with the average being 10% after intramuscular administration and 27% after oral administration. Unconjugated metabolites excreted by the in horse represented only 1–1.5% of dose after either route of administration; these were excreted entirely as sulfoxide derivatives. Glucuronide-conjugated metabolites are predominantly excreted by the horse in a ratio to unconjugated metabolites of ~7:1 after intramuscular injection and 18:1 after oral administration. Sulfate-conjugated metabolites make up ~5% of the total after oral administration but are detected only in trace amounts after intramuscular injection. Phenothiazine derivatives were not detected in feces with spectroscopic analytical methodology.

Chlorpromazine and its metabolites were found in the maternal plasma and urine, in the fetal plasma and amniotic fluid, and in neonatal urine after doses of 50–100 mg of chlorpromazine were given intramuscularly to pregnant women shortly before delivery.

Mechanism of Toxicity

The action of chlorpromazine upon the brain stem reticular system is to increase reticular activity and stimulate filtering mechanisms in the reticular formation that act to reduce inflow of stimuli in a selective manner. Low doses of chlorpromazine also depress vasomotor reflexes mediated by either the hypothalamus or the brain stem. It inhibits the release of growth hormone, perhaps by action on the hypothalamus; may antagonize the secretion of prolactin release-inhibiting hormone; and appears to cause the reduction in a secretion of corticotropin-regulatory hormone in response to certain stresses. Chlorpromazine has significant adrenergic antagonistic activity and can block the pressor effects of norepinephrine. Extrapyramidal reactions appear to be mediated by blockade of central dopaminergic receptors involved in motor function.

Chlorpromazine also inhibits the reuptake of norepinephrine and 5-hydroxy-tryptamine in rat cerebral cortex but does not affect reuptake of gamma-aminobutyric acid. It is also a potent competitive inhibitor of the stimulatory effects of dopamine on adenylate cyclase.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} value for rats was 225 mg kg^{-1}. Both single and repeated oral exposures to chlorpromazine have been conducted in several species including rats, dogs, cats, and horses. The primary effects of chlorpromazine were the reduction in hematocrit, onset of cardiac arrhythmias, ocular lesions, photosensitization, stimulation of hepatic microsomal enzyme activity, delay in fetal bone ossification, effects on the nervous system, histological changes in lungs and kidneys, ocular lesions, reduction in bile flow, and hormonal changes.

A single dose of 2–4 mg kg^{-1} chlorpromazine caused tachycardia, hypotension, and depression in horses and reduced the number of red blood cells in hemoglobin for up to 2 weeks in repeated dose studies. Clinical signs of instability, lunging forward in an uncoordinated manner, stumbling, and falling were also observed. Parenteral doses of 2.5–5 mg kg^{-1} chlorpromazine caused cardiac arrhythmias in both

unanesthetized and anesthetized dogs, and dose levels of 30 mg kg^{-1} day^{-1} caused ocular lesions in two strains of dogs after 73 days. Subcutaneous doses of 1.5 mg kg^{-1} produced no signs of toxicity, but when administered intravenously caused moderate depression and ataxia which lasted between 6 and 12 h. Marked CNS depression and ataxia were noted for 24–48 h when the intravenous dose was increased to 3 mg kg^{-1}.

When a single dose of chlorpromazine hydrochloride was administered to CF rats on the 14th day of gestation, the ischium and pubis remained unossified until the 20th day of gestation; ossification of skull bones was also delayed. Ossified vertebral bodies and arches were less affected. Ossification was delayed by 1–3 days in long bones of extremities, by 1 day in scapula, and by 2 or 3 days in the ilium. The ribs were also late in maturing.

Fetal rats collected by cesarean section from dams treated with 0, 5, 15, 30, or 45 mg kg^{-1} day^{-1} chlorpromazine during gestation days 6–15 were evaluated on gestation day 21. Fetuses in the 5, 15, 30, and 45 mg kg^{-1} day^{-1} groups had reduced body weight. Body weight and/or weight gain of the dams in the 5, 15, 30, and 45 mg kg^{-1} day^{-1} groups was also reduced, and absolute maternal liver weight was reduced in the 30 and 45 mg kg^{-1} day^{-1} groups. The proportion of litters with one or more resorptions or nonlive fetuses was increased at 15 mg kg^{-1} day^{-1} and above. Pregnant mice were treated with 0, 2.5, 5, 15, or 30 mg kg^{-1} day^{-1} chlorpromazine during gestation days 6–15. Maternal mortality was increased in the 30 mg kg^{-1} day^{-1} group, and maternal weight/weight gain was reduced in dams administered 15 or 30 mg kg^{-1} day^{-1}. The percentage per litter of resorbed and nonlive fetuses was increased in the 30 mg kg^{-1} day^{-1} group, and fetal weight was reduced at 15 mg kg^{-1} day^{-1} and above. Fetal malformations were increased in the 30 mg kg^{-1} day^{-1} group and included open eye, cleft palate, hydronephrosis, missing ribs, and fused ribs. Offspring that were born and matured through weaning from rats treated with chlorpromazine during gestation had impaired basal body temperature control, and impaired performance in behavioral tests.

Human

The minimum lethal or toxic dose is not well established in the literature. The acute fatal dose is thought to be in the range of 15–150 mg kg^{-1} depending on the formulation However, adults have survived ingestion of 9.7 g, and a 350 mg dose was lethal in a 4-year-old child. Four deaths were reported in children following ingestion of 20–74 mg kg^{-1} chlorpromazine.

Adverse Reactions Adverse reactions may accompany the use of products containing chlorpromazine. A representative sample is provided below; however, the *Physician's Desk Reference* should be reviewed for specific product information.

Some adverse effects of chlorpromazine may be more likely to occur, or occur in greater intensity, in patients with preexisting medical conditions. Adverse effects include drowsiness/sedation, jaundice, dermatoses, icterus, hepatotoxicity, hematological disorders, blood dyscrasias, agranulocytosis, hypertension, neurological disorders (dystonias, motor restlessness, pseudo-parkinsonism, tardive dyskinesia), endocrine disorders (inhibition of ovulation and lactation; suppression of menstrual cycle, infertility; growth hormone release, reduction in secretion of corticotropin regulatory hormone, galactorrhea, mastalgia, gynecomastia), autonomic disorders, changes in skin pigmentation, ocular changes (blurred vision, corneal opacities, deposits in the cornea or lens), peripheral anticholinergic effects (decreased sweating, decreased gastric secretion and motility), hypotension, central nervous system (CNS) depression to the point of somnolence and coma (monitoring should be performed especially for neck/upper airway problems), agitation, restlessness, seizures, pyrexia (impairs the body's ability to regulate temperature), ECG changes (including widened QRS complexes) arrhythmias, ventricular tachycardia, possibly ventricular fibrillation, photosensitivity, itching, chromosomal breaks, sudden death, and immune system dysfunction (lupus).

Interactions with Other Agents Chlorpromazine may inhibit or enhance the effects of other agents or medications. Chlorpromazine has potentiated the effects of the following classes of compounds: oral hypoglycemic agents, beta blockers, dopamine, appetite suppressants, antiemetics, barbiturates, thiazide diuretics, lithium, hypotensives, tricyclic antidepressants, anticholinergics, antithyroid medication, monoamine oxidase inhibitors, ophthalmics, anticonvulsants, antidyskinetics, antihistamines, cold remedies, and opioid analgesics. Concurrent use of chlorpromazine with methoxsalen, trixsalen, or tetracycline can potentiate intraocular photochemical damage. Concurrent use of metrizamide can lower the seizure threshold. Concurrent use of chlorpromazine with medications known to alter hepatic microsomal enzyme activity may result in increased hepatotoxicity. In addition, concurrent use of chlorpromazine with the following substances altered the effects of both chlorpromazine and the agent: epinephrine, ethanol, nicotine, dopamine, levodopa, probucol, phenylephrine, mephentermine,

metaraminol, amantadine, maprotiline, physostigmine, dichlorvos, paraquat, and ithium. Chlorpromazine should not be taken or administered in combination with any other prescription or over-the-counter medications or herbal agents without approval from the physician.

Chronic Toxicity (or Exposure)

Animal

Chronic studies in animals were not reported for chlorpromazine.

Human

The human literature consists primarily of case reports. A woman treated for 6 years with chlorpromazine developed multifocal tics and vocalizations following discontinuation of the chlorpromazine therapy. These symptoms suggest that chronic receptor site blockade can result in hypersensitivity of dopamine receptor sites.

Pigment granules have been reported on the anterior lens surface following large doses over long periods of time. The granules became incorporated into the lens and caused loss of transparency.

Lupus was reported to develop in some patients administered chlorpromazine.

In Vitro Toxicity Data

Chlorpromazine was mutagenic in the Ames assay with activation.

Clinical Management

Any suspected overdose patient should be transported to a health care facility as soon as possible. Any patient with clinical signs of phenothiazine overdose should be admitted.

A careful patient history should be taken by physicians to determine the patient's previous and present exposure to neuroleptic agents. Emesis should not be induced. Gastric lavage should be considered; activated charcoal may be administered with or without a cathartic. Positioning, fluids, and dopamine may be useful in the treatment of hypotension. Treatment of seizures may be necessary in patients with CNS excitation. Ventricular tachyarrhythmias should be treated with lidocaine followed by pacing if needed. Neuroleptic malignant syndrome should be treated with dantrolene or bromocriptine along with conservative treatment. Acute renal failure may need to be addressed in patients who develop rhabdomyolysis. Extrapyramidal symptoms may be relieved by barbiturates, methylphenidate hydrochloride, or benztropine. Symptoms resembling withdrawal may occur if administration of chlorpromazine is stopped abruptly.

Environmental Fate

In the ambient atmosphere, chlorpromazine is expected to exist as a vapor and a particulate. The vapor would be degraded by photochemical-produced hydroxyl free radicals, with an estimated half-life of 2 h. Particulates would be expected to have no mobility, and chlorpromazine is not expected to volatilize. In water, chlorpromazine is expected to absorb to suspended particulates. There is a high potential for bioconcentration.

Ecotoxicology

There is no aquatic toxicity information available for chlorpromazine.

Other Hazards

When heated to decomposition, fumes of hydrogen chloride, nitroxides, and sulfoxides are emitted.

Exposure Standards and Guidelines

There are no exposure standards for chlorpromazine.

See also: Tricyclic Antidepressants.

Further Reading

Booth NH and McDonald LE (eds.) (1982) *Veterinary Pharmacology and Therapeutics*, 5th edn. Ames, IA: State University Press.

Department of Health and Human Services/National Institute of Environmental Health Sciences. National Toxicology Program; Chlorpromazine Hydrochloride (CAS No. 69-09-0) Administered to Fischer 344 Rats on Gestation Days 6 through 15, NTP Study No. TER82055 (March 7, 1983).

Hatanaka T, Sato S, Endoh M, Katayama K, Kakemi M, and Koizumi T (1988) Effect of chlorpromazine on the pharmacokinetics and pharmacodynamics of pentobarbital in rats. *Journal of Pharmacobiodynamics* 11(1): 18–30.

Jain AK, Kelwala S, and Gershon S (1988) Antipsychotic drugs in schizophrenia: Current issues. *International Clinical Psychopharmacology* 3(1): 1–30.

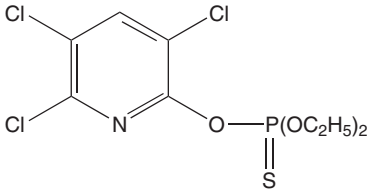
Klawans HL, Falk DK, Nausieda PA, and Weiner WJ (1978) Gilles de la Tourette syndrome after long-term chlorpromazine therapy. *Neurology* 28(10): 1064–1066.

Saillenfait AM and Vannier B (1988) Methodological proposal in behavioural teratogenicity testing: Assessment of propoxyphene, chlorpromazine, and vitamin A as positive controls. *Teratology* 37(3): 185–199.

Chlorpyrifos

Anuradha Nallapaneni and Carey N Pope

- Chemical Abstracts Service Registry Number: CAS 2921-88-2
- Synonyms: Dursban; Lorsban
- Chemical/Pharmaceutical/Other Class: Synthetic organophosphorus insecticide in the phosphorothionate class
- Chemical Structure:



Uses

Chlorpyrifos is a broad-spectrum insecticide useful in controlling a variety of agricultural, urban, and household insects. In the United States, the household use of chlorpyrifos has been recently eliminated but it is still used considerably in agriculture.

Exposure Routes and Pathways

Oral and inhalation routes are primary exposure pathways.

Toxicokinetics

Chlorpyrifos is absorbed rapidly and effectively by the oral route but less effectively by the dermal route. Chlorpyrifos undergoes activation via the P-450–mixed-function oxidase pathway to the oxygen analog, chlorpyrifos oxon. Metabolites include diethylphosphoric acid, 3,5,6-trichloro-2-pyridyl phosphate, and 3,5,6-trichloro-2-pyridinol. Differences in serum A esterase activity appear to contribute significantly to species differences in sensitivity to chlorpyrifos. Major conjugates include glucuronide (80%) and glycoside (4%) metabolites. An $-SCH_3$ addition on the pyridinol ring has also been reported. The water-soluble glucuronide and glycoside conjugates are eliminated primarily via the urine (90%). A trace amount of the parent compound is also eliminated via the urine.

Mechanism of Toxicity

Similar to other organophosphorothionate insecticides, the toxicity of chlorpyrifos is due to inhibition of acetylcholinesterase following its oxidative metabolism to the oxon (i.e., chlorpyrifos oxon). Extensive inhibition of this enzyme results in stimulation of the central nervous system (CNS), the parasympathetic nervous system, and the somatic motor nerves. The IC_{50} values for the insecticide and its oxon are approximately 5×10^{-3} and 5×10^{-9} mol l^{-1}, respectively.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chlorpyrifos is moderately toxic in rodents, with oral LD_{50} values in rats of 95–270 mg kg^{-1} and 60 mg kg^{-1} in mice. Young rodents are markedly more sensitive than adults to chlorpyrifos and a number of other organophosphorus insecticides. Rabbits are relatively resistant to chlorpyrifos, with LD_{50} values $\sim$1 g kg^{-1}. In contrast, many birds are highly sensitive to chlorpyrifos, e.g., the LD_{50} in chickens is 32 mg kg^{-1}. Resistance in rabbits and sensitivity in birds is likely due to differences in A-esterase mediated detoxification. The dermal LD_{50} in rats is $>$2 g kg^{-1}. The 4 h inhalation LC_{50} for chlorpyrifos in rats is $>$0.2 mg l^{-1}.

Human

Exposure to chlorpyrifos can affect the central nervous system, heart, and respiratory system. Symptoms of acute exposure to chlorpyrifos may include paresthesia, incoordination, headache, dizziness, tremor, nausea, cramping, excessive sweating, blurred vision, dyspnea, and bradycardia. Very high exposures may lead to unconsciousness, convulsions, and death. Chlorpyrifos is a skin and eye irritant. Ocular contact may cause pain, moderate eye irritation, and slight temporary corneal injury. Prolonged skin exposure may cause skin irritation. Toxicity is moderate for a single oral dose; swallowing larger amounts may cause serious injury, even death. If aspirated, chlorpyrifos formulations may cause airway damage or even death due to chemical pneumonia.

Chronic Toxicity (or Exposure)

Animal

While young rats are more sensitive than adults to cholinergic toxicity following acute chlorpyrifos exposures, some results suggest that low-level repeated chlorpyrifos exposures elicit lesser degrees of age-related differences in toxic response. A number

of studies suggest, however, that chlorpyrifos may adversely affect neurodevelopmental processes through interaction with a number of macromolecular targets other than acetylcholinesterase.

Human

Cholinesterase inhibition can sometimes persist for weeks; thus, repeated exposures to small amounts of this material may result in accumulation of acetylcholinesterase inhibition with possible sudden-onset acute toxicity. Chlorpyrifos may be capable of causing organophosphate-induced delayed neurotoxicity in humans; a massive overdose resulted in signs characteristic of delayed neurotoxicity. Animal studies generally indicate, however, that doses several times higher than the LD_{50} would be required to initiate delayed neurotoxicity.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered, followed by 2–4 mg (in adults) or 0.015–0.05 mg kg^{-1} (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Environmental Fate

Chlorpyrifos is moderately persistent in soils, with a half-life from 2 weeks to 1 year depending on soil type, climate, pH, and other conditions. Half-life was not affected by organic matter content, however. Chlorpyrifos undergoes degradation by UV light, chemical hydrolysis, and by microbial processes. In moist conditions, 62–89% of the chlorpyrifos remained in soil after 36 h. Chlorpyrifos adsorbs strongly to soil particles and has low water solubility, thus there is little potential for contaminating groundwater. The breakdown product, trichloropyridinol, adsorbs only weakly to soil particles and is more mobile and persistent. Concentrations of chlorpyrifos diminish rapidly in water following application as emulsiable preparations but less so when applied as granules and controlled release formulations. Volatilization is likely the principle mode of chlorpyrifos loss from water. Chlorpyrifos is relatively unstable in water. Hydrolysis is constant in acidic or neutral conditions higher in alkaline water.

Ecotoxicology

Chlorpyrifos is moderately to very highly toxic to birds, with oral LD_{50} values from 8 to 112 mg kg^{-1} in a number of species. Reduction in eggs was noted in mallard ducks at dietary exposure of 125 ppm chlorpyrifos but no effect on egg laying in hens given 50 ppm. Chlorpyrifos is very highly toxic to aquatic vertebrate and invertebrate organisms. Application of chlorpyrifos at concentrations as low as 0.01 pounds per acre can lead to death in aquatic organisms. The 96 h LC_{50} for chlorpyrifos was 0.009 mg l^{-1} in rainbow trout, 0.098 mg l^{-1} in trout, 0.806 mg l^{-1} in goldfish, 0.01 mg l^{-1} in bluegill, and 0.331 mg l^{-1} in fathead minnow. One study reported deformities in fathead minnow at 0.002 mg l^{-1} exposure for 30 days. Chlorpyrifos bioaccumulates in tissues of aquatic organisms with concentration factors of 58–5100. Chlorpyrifos can pose hazard to other wildlife species including bees.

Exposure Standards and Guidelines

The chronic reference dose for chlorpyrifos is 0.005 mg kg^{-1} day^{-1}; the population adjusted dose is 0.0005 mg kg^{-1} day^{-1}. The 8 h dermal permissible exposure limit is 0.02 mg m^{-3}.

See also: A-Esterases; Cholinesterase Inhibition; Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphates.

Further Reading

Cochran RC (2002) Appraisal of risks from nonoccupational exposure to chlorpyrifos. *Regulatory Toxicology and Pharmacology* 35(1): 105–121.

Meyer A, Seidler FJ, Cousins MM, and Slotkin TA (2003) Developmental neurotoxicity elicited by gestational exposure to chlorpyrifos: When is adenylyl cyclase a target? *Environmental Health Perspectives* 111: 1871–1876.

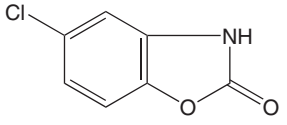
Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Chlorzoxazone

Kashyap N Thakore and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 95-25-0
- Synonyms: Chlorozaxone; 5-Chloro-2(3*H*)-benzoxazolone; 5-Chloro-2-benzoxazolol; 5-Chloro-2-hydroxybenzoxazole; 2-Hydroxy-5-chlorobenzoxazole; Paraflex; Biomioran; Solaxin
- Chemical/Pharmaceutical/Other Class: Strong oxidizing agent
- Chemical Formula: $C_7H_4ClNO_2$
- Chemical Structure:



Uses

Chlorzoxazone is used as a centrally acting skeletal muscle relaxant and as an analgesic. It is also a strong oxidizing agent.

Exposure Routes and Pathways

When heated to decompose, chlorzoazone emits acrid smoke and irritating fumes.

Toxicokinetics

The oral suspension is rapidly absorbed and eliminated. After intravenous administration, the decay of the plasma concentration is rapid. The half-life for an oral dose is ~ 1 h.

Chlorzoxazone is rapidly metabolized in the liver by carbon hydroxylation at position 6 mediated by CYP1A2 as well as by CYP2E1 and the 6-hydroxychlorzoxazone formed is conjugated with glucuronide. The concentration in fat is two times the plasma levels. Chlorzoxazone is eliminated mainly as the glucuronide conjugate by urine.

Mechanism of Toxicity

The toxic metabolite 6-hydroxychlorzoxazone is formed by the action of CYP1A2 and CYP2E1.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the oral LD_{50} is $763\,mg\,kg^{-1}$ body weight (bw) and the intraperitoneal LD_{50} is $150\,mg\,kg^{-1}$ bw. In the mouse, the oral LD_{50} is $440\,mg\,kg^{-1}$ bw, the intraperitoneal LD_{50} is $50\,mg\,kg^{-1}$ bw, and the subcutaneous LD_{50} is $170\,mg\,kg^{-1}$ bw. In the hamster, the oral LD_{50} is $662\,mg\,kg^{-1}$ bw and the intraperitoneal LD_{50} is $166\,mg\,kg^{-1}$ bw.

Human

Chlorzoxazone is harmful if swallowed, inhaled, or absorbed through skin. It causes drowsiness; central nervous system effects such as headache, dizziness, and blurred vision; nausea and vomiting; and eye, skin, and mucous membrane irritation. The target organs are the liver, nerves, and skeletal muscles. Although morbidity and mortality are low in pure compound ingestion, they may be increased in multiple ingestions.

Workers exposed to this compound should wear personal protective equipment and their work should be carried out only in restricted areas. Technical measures should prevent any contact with the skin and mucous membranes. Clothing and equipment after use should be placed in an impervious container for decontamination or disposal.

Chronic Toxicity (or Exposure)

Information on animal and human data is not available.

Clinical Management

In case of contact, the eyes and skin should be flushed immediately with water for at least 15 min. If the victim is not breathing, artificial respiration should

be administered; if breathing with difficulty, oxygen should be given. If patient is in cardiac arrest, cardiopulmonary resuscitation should be provided. If swallowed, the mouth should be washed out with water provided the person is conscious. Life-support measures should be continued until medical assistance has arrived. An unconscious or convulsing person should not be given liquids nor induced to vomit.

See also: Skeletal System.

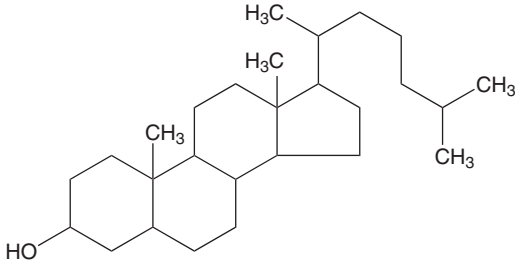
Further Reading

Powers BJ, Cattau EL Jr., and Zimmerman HJ (1986) Chlorzoxazone hepatotoxic reactions: An analysis of 21 identified or presumed cases. *Archives of Internal Medicine* 146(6): 1183–1186.

Cholesterol

Brad Hirakawa

- Chemical Abstracts Service Registry Number: CAS 57-88-5
- Synonyms: (−)-Cholesterol; 5,6-Cholesten-3β-ol; 5-Cholesten-3β-ol; Cholest-5-en-3β-ol; Cholesterin; Cholesterine; Cholesterol base H; Cholesteryl alcohol; Cordulan; D [5](-Cholesten-3β-ol); Dusoline; Dusoran; Dythol; β-Hydroxycholest-5-ene; Hydrocerin; Kathro; Lanol; Nimco; Nimco cholesterol base No. 7l2; Provitamin D; Super hartolan; Tegolan
- Chemical Class: Endogenous sterol
- Chemical Formula: $C_{27}H_{46}O$
- Chemical Structure:



Uses

A natural product, cholesterol is a fat-soluble compound that is present in our daily diet. It can form esters with fatty acids. Most of the cholesterol found in the plasma is in the form of cholesterol esters. It can also be endogenously formed in the cells of the body. The majority of the cholesterol is produced by the liver cells; however, all other cells also form cholesterol. The membrane structure of the cells is partially made up of cholesterol. It is synthesized from multiple molecules of acetyl-CoA. The major use of cholesterol in the body is to form cholic acid in the liver, which conjugates with other substances to form bile salts. Bile salts help in digestion and absorption of fats. Cholesterol is used in small amounts to form adrenocorticoid hormones, progesterone, estrogens, and testosterone. Cholesterol is also used in a multitude of pharmaceutical and cosmetic preparations.

Exposure Routes and Pathways

Cholesterol is an endogenous compound. Oral exposure is the most common exposure pathway.

Mechanism of Toxicity

Cholesterol is transported to and from the cells by special carriers called lipoproteins. There are two types, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). It is believed that excess LDL cholesterol can clog arteries, increasing risk of heart attack and stroke. Conversely, studies suggest that higher levels of HDL cholesterol reduce risk of heart attack. That is, the ratio of LDL to HDL appears to be important. The adverse effects of a prolonged (i.e., chronic) history of excess LDL relative to HDL is of much greater concern than acute exposures to cholesterol.

Chronic Toxicity (or Exposure)

Animal

Evaluations of the carcinogenicity of cholesterol to experimental animals did not result in clear positive results.

Human

LDL cholesterol levels above 100 mg dl^{-1} and HDL cholesterol levels below 40 mg dl^{-1} are risk factors for cardiovascular disease.

In humans, the association between cholesterol and cancer risk contains some apparent contradictions. Depending on whether cholesterol is measured in the diet, blood, or feces, its association with cancer risk may tend to be either positive or negative. Therefore, there is inadequate evidence from scientific studies that cholesterol is carcinogenic to humans.

Clinical Management

Statins are a class of cholesterol-lowering drugs that affect serum cholesterol levels by a variety of mechanisms. Examples include Lipitor, Zocor, Pravachol, Lescol, and Mevacor. Recent evidence suggests regular consumption of soluble fibers (via diet or supplementation) can lower serum cholesterol levels.

See also: Cardiovascular System; Liver.

Relevant Websites

http://www.americanheart.org – American Heart Association.
http://www.inchem.org – International Programme on Chemical Safety INCHEM.
http://www.nhlbi.nih.gov – US National Heart, Lung, and Blood Institute, National Institutes of Health – National Cholesterol Education Program.

Choline

Brad Hirakawa

- Chemical Abstracts Service Registry Numbers: CAS 62-49-7; CAS 123-41-1 (choline hydroxide)
- Synonyms: Bursine; Ethanaminium; Fagine; Gossypine; Luridine; Sincaline; Sinkalin; Sinkaline; Vidine; (2-Hydroxyethyl)trimethylammonium hydroxide
- Chemical/Pharmaceutical/Other Class: Cholinergic agonist
- Chemical Formula: $C_5H_{15}NO_2$
- Chemical Structure: $[HOCH_2CH_2N^+(CH_3)_3]$

Uses

Choline is used as a direct cholinergic agonist in therapeutics and as a research tool.

Exposure Routes and Pathways

Dermal and oral contacts are the most common exposure pathways.

Toxicokinetics

Choline is metabolized to trimethylamine, which is excreted in skin, lungs, and kidney.

Mechanism of Toxicity

Choline is a cholinergic agonist, choline acetyltransferase substrate; therefore, it exerts toxicity by directly hyperstimulating the postganglionic cholinergic receptors. This may lead to stimulation of gastrointestinal, urinary, uterine, bronchial, cardiac, and vascular receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute choline overexposure results in hyperstimulation of the cholinergic nervous system.

Human

The estimated oral lethal dose for humans is 200–400 g. Oral doses of 10 g produce no obvious effect. Vital signs may include bradycardia (decreased heart rate), hypotension, hypothermia, miosis (small pupils), salivation and lacrimation (tearing), ocular pain, blurred vision, bronchospasm, muscle cramps, fasciculation (muscle twitching), weakness, nausea, vomiting, diarrhea, and involuntary urination.

Chronic Toxicity (or Exposure)

Little is known about the chronic toxicity of choline in laboratory animals or humans.

Clinical Management

Atropine sulfate is the drug of choice. Epinephrin may assist in overcoming severe cardiovascular or bronchoconstriction. Diazepam, phenytoin, and phenobarbital may be given in cases of seizures. Induction of emesis is not necessary due to spontaneous vomiting. Activated charcoal slurry with or without

saline cathartic may be used. Sorbitol should not be used because it may contribute to the nausea and diarrhea. Skin decontamination should be accomplished by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min.

Other Hazards

Choline base solutions are corrosive and are extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin.

See also: Anticholinergics; Cholinesterase Inhibition; Neurotoxicity.

Relevant Website

http://www.cdc.gov – National Institute for Occupational Safety and Health (NIOSH) website.

Cholinesterase Inhibition

Barry W Wilson

Introduction and History

Cholinesterases (ChEs) are a ubiquitous group of enzymes that hydrolyze esters of choline. A well-known example is acetylcholinesterase (AChE, acetyl choline hydrolase, EC 3.1.1.7), the enzyme responsible for hydrolyzing the important neurotransmitter acetylcholine (ACh). Another ChE is butyrylcholinesterase (BuChE, acylcholine acylhydrolase, EC 3.1.1.8), also known as nonspecific cholinesterase. The preferred substrate for AChEs is ACh; BuChEs prefer to hydrolyze esters like butyrylcholine and propionylcholine. Both AChE and BuChE are inhibited by some organophosphate (OP) and carbamate (CB) esters and also by other chemicals.

Many ChE inhibitors act at the catalytic site of the enzyme, forming enzyme–inhibitor complexes that are slow to hydrolyze. The use of ChE inhibitors as insecticides and as chemical warfare agents, their toxicity to humans, and their impact on wildlife have made them important to toxicology researchers, public health and environmental health officials.

This entry focuses on ChE inhibitions by OPs and CBs. Other chemicals, such as tacrine, cocaine, and succinylcholine, are briefly discussed.

One of the first ChE inhibitors to be studied was a CB, physostigmine (eserine), an alkaloid from the calabar bean (*Physostigma venenosum*) used in a 'trial by ordeal' in West Africa. The accused were forced to eat the poisonous beans; survivors were proclaimed innocent. The drug has been used as a treatment for glaucoma since 1877. In 1931, Englehart and Loewi showed it blocked ChE activity. Soon after, neostigmine, an analog, was shown to be effective in the symptomatic treatment of myasthenia gravis.

OPs with high toxicity were synthesized as chemical warfare agents in the late 1930s and early 1940s. During this period Schrader discovered the insecticidal properties of OPs resulting in the synthesis of tetraethyl pyrophosphate in 1941 and of parathion in 1944. Synthetic CBs developed as pesticides have been in commercial use since the 1950s. Some OPs and CBs exhibit toxicities in addition to their direct inhibitions of ChEs. These include long-term and short-term damage to nerves and muscles, mutagenicity, and effects on reproduction.

Acetylcholinesterase, Butyrylcholinesterase, and Other Esterases

AChEs and BuChEs are specialized carboxylic ester hydrolases that preferentially hydrolyze choline esters. They are classed among the B-esterases, enzymes that are inhibited by OPs. Another B-esterase is neuropathy target esterase (NTE), an enzyme associated with organophosphate-induced delayed neuropathy (OPIDN). Enzymes that actively hydrolyze OPs are known as A-esterases. They provide an important route of detoxification. Examples are paraoxonase and DFPase (**Table 1**). The tertiary structure and amino acid sequences of several AChEs and BuChEs have been elucidated.

ChEs are widely distributed in the body. AChEs regulate excitation at cholinergic synapses by destroying the neurotransmitter ACh. These enzymes are some of the most active known, cycling within a few milliseconds. AChEs are found in excitable tissues at synapses, neuromuscular junctions, myotendinous junctions, central nervous system (CNS) neuron cell bodies, axons, and muscles (**Table 2**). AChEs are also found in the erythrocytes (red blood cells (RBCs)) of mammals, in the serum of some birds and mammals, and in the blood platelets of

Table 1 Esterase classes

A-esterases
Hydrolyze OPs to inactive products
Found in liver and HDL in plasma
High activity in mammals
Lower activity in birds
Examples: Paraoxonase and DFPase
B-esterases
Widely distributed in cells and tissues
Inhibited by OPs and CBs
Slow hydrolysis of OP–enzyme complex
Relatively rapid hydrolysis of CN–enzyme complex
Examples: AChE, BuChE, CaE, and NTE

OP, organophosphate ester; HDL, high-density lipoprotein; CB, carbamate; AChE, acetylcholinesterase; BuChE, butyrylcholinesterase; CaE, carboxylesterase; NTE, neuropathy target estarase.

Table 2 Cholinesterase properties

All
Hydrolyze ACh and other choline esters
AChE
Prefers ACh, is inhibited by excess substrates
Found at neural junctions and in mammal RBCs and plasma and platelets of some vertebrates
BuChE
Prefers butyrylcholine, propionylcholine
Widely distributed in vertebrate tissues and plasma

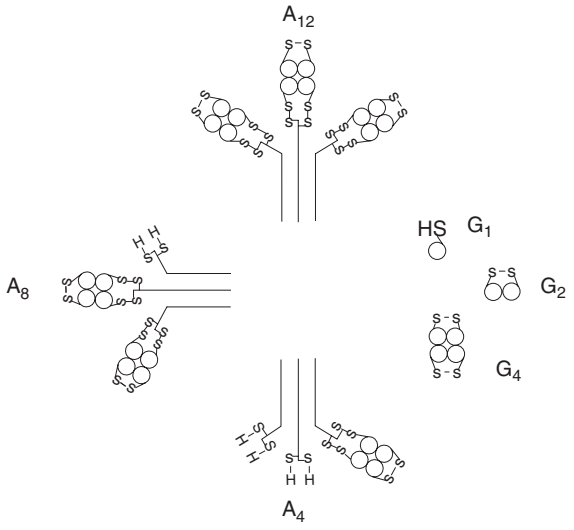


Figure 1 Subunit structure of the multiple molecular forms of ChEs. G, globular forms; A, asymmetric forms with collagen-like tails. Each circle is a catalytic subunit; disulfide bridges indicated by S–S as found in the electric organ of the electric eel. (Modified from Brimijoin WS (1992) US EPA Workshop on Cholinesterase Methodologies.)

rodents (rats and mice) and ruminants (sheep). (For example, the serum ChE activity of the American Kestrel, a small falcon, consists almost entirely of AChE and the serum ChE of the laboratory rat is high in both AChE and BuChE activities.) The AChE activity of human blood is localized to its RBCs. AChE activity occurs in the serum of developing mammals and birds and in precursors of formed blood elements in some species; it decreases to adult levels after birth.

BuChEs are also widely distributed. They are found at synapses, motor endplates, and muscle fibers together with AChE. BuChE activity in blood is restricted to serum.

Substrate preferences of AChE and BuChE enzymes vary with species. For example, although both mammal and bird AChEs rapidly hydrolyze ACh and its thiocholine analog acetylthiocholine (AcTh), avian AChEs also readily hydrolyze acetyl β-methylcholine and acetyl β-methylthiocholine, while mammalian AChEs do not. AChEs and BuChEs respond differently to increasing substrate concentration. AChEs are inhibited by excess substrate (often above 2 mM); BuChEs are less sensitive to substrate inhibition.

AChEs and BuChEs have multiple molecular forms and complicated life histories (**Figures 1** and **2**). Some of the forms move from site to site within cells, others are secreted into body fluids. AChEs consist of asymmetric and globular forms. The asymmetric forms tend to be localized at synapses and motor endplates; they have glycosylated heads joined together by sulfhydryl groups containing the active sites, and collagen tails that attach the enzymes to cell surfaces. The globular forms lack collagen tails; they are made up of the catalytic subunits.

AChE and BuChE subunits are synthesized within cells (e.g., nerve, muscle, liver, and some megakaryocytes), glycosylated within the Golgi apparatus, and secreted. Collagen-tailed forms become attached to the cell surface at specific binding sites. Globular forms are released into body fluids or bind to cell surfaces by ionic bonds. Antibodies have been prepared to several purified AChEs and BuChEs, and protein and nucleic acid sequences have been determined.

The three-dimensional structure of AChE from the electric organ of *Torpedo californica* has been established. One interesting feature is that the active site is embedded in a 'gorge' of $\sim$20 Å that reaches halfway into the protein. The postulated 'anionic site', theoretically invoked to bind the quaternary ammonium ion of ACh, appears to be represented by aromatic amino acids in the gorge itself; these and charges in the active center are believed to stabilize the choline group. In addition, some inhibitions, such as that due

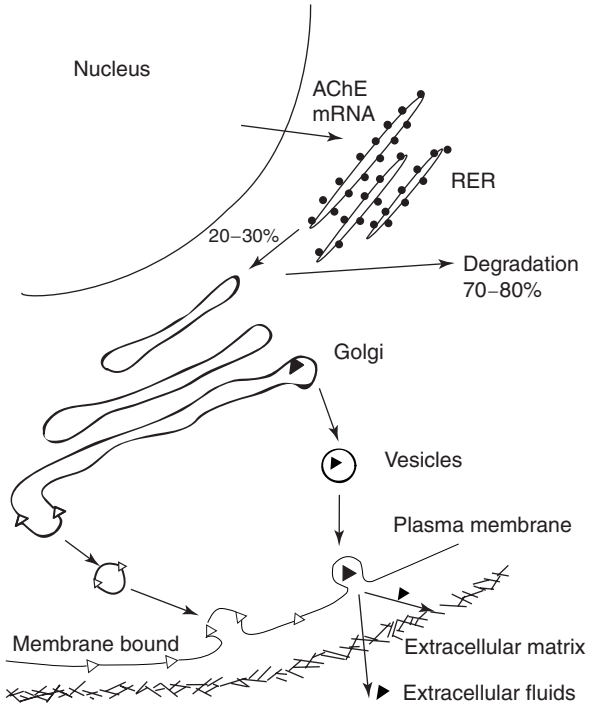


Figure 2 Life cycle of ChEs. AChE is synthesized as a monomer globular form (G_1). Up to 80% is degraded by intracellular processes. Secretory forms are separated from membrane-bound forms, collagen tails are added to asymmetric forms, and the enzyme is glycosylated and becomes enzymatically active. After secretion, globular forms may escape into the body fluids, while asymmetric forms are bound to the synaptic basal lamina. (Modified from Brimijoin WS (1992) US EPA Workshop on Cholinesterase Methodologies.)

to excess substrate, are believed to be due to a 'peripheral site'. Elucidation of the structure of ChE molecules may open the way to a new generation of 'designer' anti-ChE agents with improved specificities of action.

Functions of Cholinesterases

$$E + AX \underset{k_{-1}}{\overset{k_{+1}}{\longleftrightarrow}} EAX \overset{k_2}{\longleftrightarrow} EA \overset{k_3}{\longleftrightarrow} E + A$$

where E is the enzyme, AX is the substrate (ACh) or inhibitor, EAX is the reversible enzyme complex, and *k*s are reaction rate constants.

A 100 years of research has established that a major function of AChE is to hydrolyze the ACh released by cholinergic neurons, regulating the course of neural transmission at synapses, motor endplates, and other effector sites. The reaction is multistep: first is the formation of a reversible enzyme–substrate complex (EAX); second is the acetylation of the catalytic site of the enzyme (EA); and third is the hydrolysis of the enzyme–substrate complex yielding acetic acid, choline, and the regenerated enzyme (E + A). The generally accepted mechanism has been (1) an electrostatic attraction between the positive charge on the quaternary nitrogen atom of Ach and the negative charge on the so-called 'anionic site' on the enzyme forms the enzyme–substrate complex, (2) a basic imidazole moiety (histidine) and an acidic moiety (tyrosine hydroxyl) at the active site catalyze the acetylation of a serine hydroxyl, followed by (3) a rapid deacetylation restoring the enzyme and cleaving acetylcholine into acetate and choline. A similar reaction scheme is believed to apply to BuChEs. The new information on the conformation of these molecules should result in a greater understanding of the biophysical mechanisms underlying their catalytic actions.

In contrast to the functional information available for the roles of ACh and AChE, the function or functions of RBC and serum ChEs are still matters for speculation. One idea is that they protect the body from natural anti-ChE agents (e.g., phyosostigmine) encountered during the evolution of the species; another idea is that they have specific but still unknown roles in tissues. For example, there are reports that inhibition of BuChE activity blocks adhesion of neurites from nerve cells in culture and that AChE promotes outgrowth of neurites as if the enzymes had roles in cell adhesion and differentiation.

Toxicities

The toxicities of OPs and CBs often roughly parallel their effectiveness as inhibitors of brain AChE. For example, **Figure 3** shows the relationship between the toxicity *in vivo* of directly acting OPs and their inhibition of AChE *in vitro*, plotting intraperitoneal LD_{50} versus the PI_{50} in mice. (The LD_{50} is the dose resulting in 50% mortality; the PI_{50} is the negative logarithm of the concentration of toxicant resulting in 50% inhibition of the enzyme.) Only two of the chemicals tested did not 'fit' the curve.

In general, many of the physiological effects of anti-ChEs are those attributable to excess ACh at junctions in the nervous system. The precise symptoms and the time course of ChE inhibition depend on the chemicals and the localization of the receptors affected. The properties of some cholinergic receptors are listed in **Table 3**. Cholinergic junctions are classified into several categories based on their pharmacological sensitivities to nicotine, muscarine, atropine, and curare. Early symptoms of cholinergic poisoning represent stimulation of neuroeffectors of the parasympathetic system. These effects are termed muscarinic – stimulated by muscarine and are blocked by atropine. Such effects include slowing

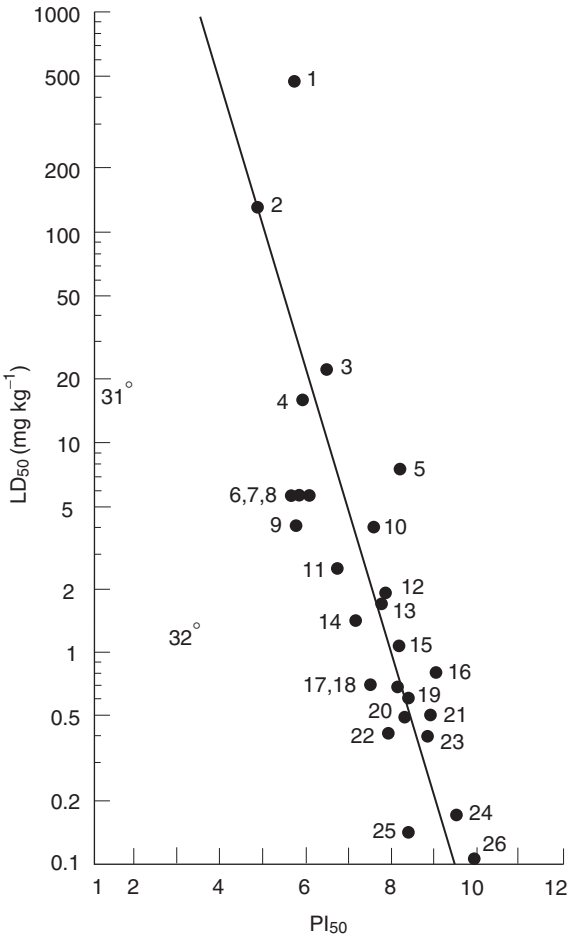


Figure 3 Relationship of the toxicity *in vivo* (LD_{50}) of directly acting OPs to AChE inhibition *in vivo* (PI_{50}). 1, dipterex; 2, *O*,*O*-diethyl-4-chlorophenylphosphate; 3, *O*,*O*-diethyl-bis-dimethylpyrophosphoramide(*sym*); 4, TIPP; 5, *O*,*O*-diethylphosphostigmine; 6, isodemeton sulfoxide; 7, isodemeton; 8, isodemeton sulfone; 9, DFP; 10, diethylamidoethoxy-phosphoryl cyanide; 11, *O*,*O*-dimethyl-*O*,*O*-diisopropyl pyrophosphate (asym); 12, diethylamidomethoxyphosphoryl cyanide; 13, tetramethyl pyrophosphate; 14, *O*,*O*-diethylo phosphorocyanidate; 15, *O*,*O*-dimethyl-*O*,*O*-diethyl pyrophosphate (asym); 16, soman; 17, TEPP; 18, *O*-isopropyl-ethylphosphonofluoridate; 19, tabun; 20, amiton; 21, diethylamido ispropoxyphosphoryl cyanide; 22, *O*,*O*-diethyl-*S*-(2-diethylaminoethyl)phosphorothioate; 23, sarin; 24, *O*,*O*-diethyl-*S*-(2-triethylammoniumethyl)thiophosphate iodide; 25, echothiophate; 26, methylfluorophosphorylcholine iodide; 27, methylfluorophosphoryl-*B*-methylcholine iodide; 28, *O*-ethyl-methylphosphorylthiocholine iodide; 29, methylfluorophosphoryl-homo-choline iodide; 31, schradan; 32, dimefox (27–30, LD_{50} values 0.03–0.07). (Adapted from Gallo L (1991) Organophosphorus insecticides. In: Hayes WJ Jr. and Laws ER Jr. (eds.) *Handbook of Pesticide Toxicology*, vol. 2, p. 932. San Diego, CA: Academic Press.)

of the heart (bradycardia), constriction of the pupil of the eye (miosis), diarrhea, urination, lacrimation, and salivation. Actions at skeletal neuromuscular junctions (motor endplates) are termed nicotinic – stimulated by nicotine, blocked by curare, but not by atropine. Overstimulation results in muscle fasciculation (disorganized twitching) and, at higher doses, muscle paralysis. A third site of action of anti-ChEs is the cholinergic junctions of the sympathetic and parasympathetic autonomic ganglia. These junctions are also nicotinic – stimulated by nicotine but not affected by muscarine, atropine, or curare, except at high concentrations. Their actions affect the eye, bladder, heart, and salivary glands, with one set often antagonizing the actions of another. Finally, there are the junctions of the CNS: some are stimulated by nicotine, and some are affected by atropine. They are not responsive to muscarine or curare. CNS symptoms include hypothermia, tremors, headache, anxiety, convulsions, and coma. Death generally occurs when the agents extensively affect the respiratory centers in the brain. Whether or not there are consistent behavioral effects at low dose levels of OPs and CBs, such as deficits in learning and memory, is a matter of current research, especially on drugs under development to treat Alzheimer's disorder.

Table 3 Properties of cholinergic receptors

Muscarinic peripheral NS
Parasympathetic nervous system
Muscarine stimulates
Atropine blocks
Nicotinic peripheral NS
Skeletal muscle motor endplates
Nicotine stimulates
Curare blocks
Atropine has no effect
Nicotinic CNS
Autonomic NS antagonist
Sympathetic and parasympathetic NS
Nicotine stimulates respiratory center

NS, nervous system; CNS, central nervous system.

The excess ACh produced at the motor endplate brings about a transient myopathy in experimental animals. Experiments *in vivo* and *in vitro* of Dettbarn, Wecker, Salpeter, and others using cholinergic drugs and ACh receptor blockers indicate that excess ACh leads to an influx of Ca^{2+} ions and other cations into the postsynaptic cell, resulting in regions of necrosis in the muscle fiber around the motor endplates. From 10% to 30% of the fibers may be damaged and recovery may take several weeks or more. A disorder known as intermediate syndrome in humans involves prolonged muscle weakness and some muscle damage lasting several weeks or longer after exposure to high levels of some OPs, including methyl parathion, fenthion, and dimethoate.

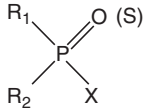
Although most of the effects of OPs and CBs are considered to be caused by AChE inhibition, there is evidence that anti-ChEs directly affect ACh receptors in the CNS and PNS and that some anti-AChE pesticides depress the immune system in experimental animals.

A few OPs such as tri-ortho cresyl phosphate (TOCP), leptophos, mipafox, methamidophos, isofenphos, and chlorpyrifos, cause OPIDN, a neuropathy that results in the death of some motor and sensory neurons in humans and experimental animals. Some, such as chlorpyrifos and isofenphos, require very high dose levels to be neuropathic – higher levels than could occur if the chemicals were used as directed. TOCP, an industrial chemical, has been responsible for the paralysis of thousands of people since the turn of the century. Inhibition of ~70% or more of the carboxylesterase NTE is often associated with the disorder. It is known as a 'delayed' neuropathy because onset of the disorder is usually 10 days to several weeks after exposure. Discussion of this neuropathy is beyond the scope of this article, except to note that neuropathic chemicals that are the most dangerous often are those that are better NTE inhibitors than AChE inhibitors, permitting a higher dose of the chemical to be reached before cholinergic symptoms or death occurs. Agricultural chemicals are routinely screened for OPIDN using hens since chickens are sensitive to the disorder.

The action of many toxicants, including anticholinergic compounds, often involves specific sites on molecules and cells. Such finely tuned molecular events suggest the possibility of discovering 'genocopies', genetic abnormalities that mimic chemically induced disorders. For example, patients have been reported with smaller than normal motor endplates, defective in AChE, and suffering from muscle weakness. There are no reported human AChE-less mutants; it is likely that such a genetic disaster would be lethal. There are humans with inherited differences in their serum BuChEs with decreased activity of the enzyme in their blood. Possessors of these genotypes usually are symptomless, unless they are given succinylcholine (or a similar drug) during surgery to bring about muscle relaxation. Lack of sufficient blood BuChE to speedily destroy the drug intensifies and prolongs the activity of succinylcholine, sometimes with fatal consequences. BuChEs may also play a detoxifying role in cocaine intoxication by hydrolyzing the drug. Several studies on experimental animals indicate that depressing ChEs with anti-ChEs intensifies the toxic effect of cocaine. A 'knock-out' genetically manipulated mouse lacking AChE studied by Oksana Lockridge and colleagues seems to use BuChEs as a substitute to destroy excess ACh.

Organophosphorus Cholinesterase Inhibitors

OP inhibitors are substituted phosphoric acids of the form



where R_1 and R_2 are usually alkyl or aryl groups linked either directly or via O or S groups to the P atom. According to one classification, X, termed the leaving group, may be (1) a quaternary nitrogen; (2) a fluoride; (3) a CN, OCN, SCN, or a halogen other than F; or (4) other groups. (See **Figure 4** for representative organophosphorus cholinesterase inhibitors.)

1. OPs containing quaternary nitrogen (phosphorylcholines) are strong inhibitors of ChEs and directly acting cholinergics. One, ecothiophate iodide, is used in the treatment of glaucoma.
2. Fluorophosphates are also highly toxic and relatively volatile. Sarin and soman are chemical warfare agents. Diisopropyl fluorophosphate (DFP) is often used by biochemists to study serine–active enzymes. Mipafox and DFP cause OPIDN in humans and experimental animals.
3. An example of a CN-containing nerve gas is Tabun.
4. Most OP pesticides are in the fourth and largest category. Many are dimethoxy or diethoxy compounds. OPs used in agriculture tend to be manufactured in the relatively stable P = S form. They are less toxic than OPs with the P = O (oxon) group. (Phosphates lack a sulfur atom, phosphorothioates have a single sulfur atom, and phosphorodithioates have two sulfur atoms.) Many pesticides such as parathion, methyl parathion, diazinon, and chlorpyrifos are phosphorothionates.

Three important chemical reactions that underlie ChE inhibitions are hydrolysis, desulfuration, and alkylation.

- *Hydrolysis*: The rate of hydrolysis is a function of the acid and alcohol groups, pH, and temperature. It usually increases with increasing pH, temperature, and UV light.
- *Desulfuration*: An important oxidation is the conversion of the P = S group of phosphorothionates to P = O, the oxon form, increasing the intensity of ChE inhibition.

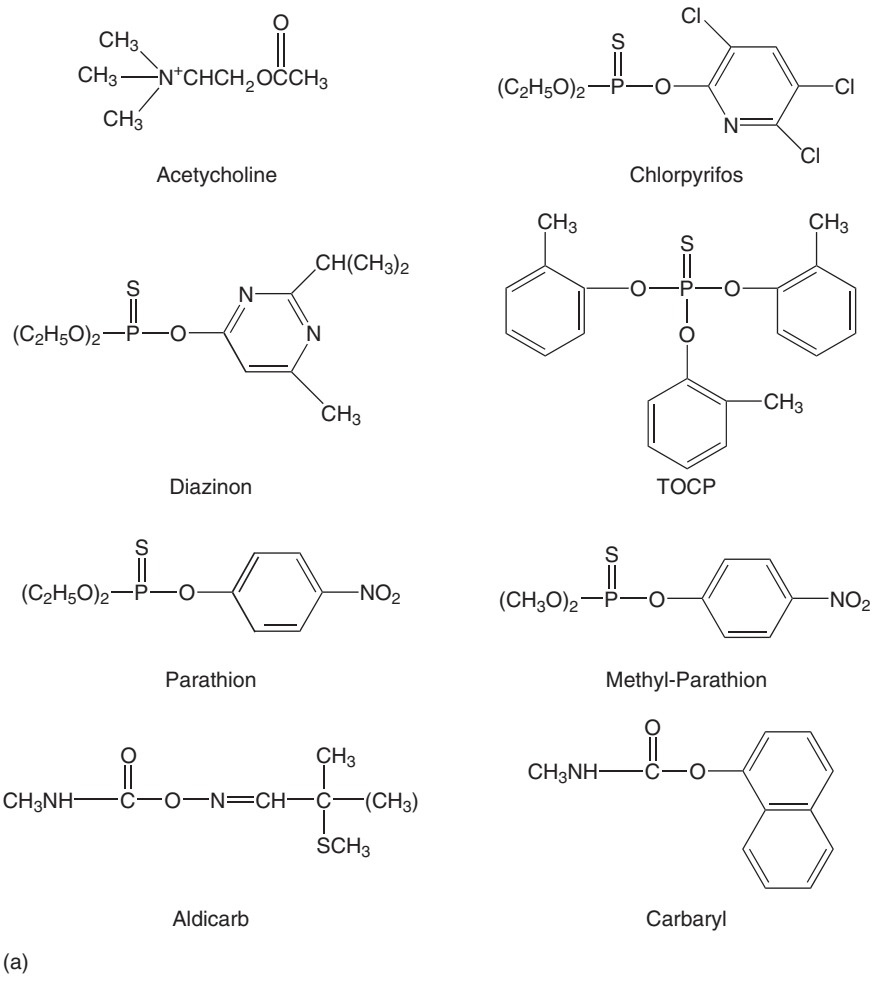


Figure 4 Representative organophosphorus and organocarbamate cholinesterase inhibitors.

- *Alkylation*: Alkyl substituents, especially methoxy groups, may act as alkylating agents. They are capable of altering nucleic acids, leading some to be concerned about OPs as mutagens.

Carbamate Cholinesterase Inhibitors

The CBs used as pesticides are N-substituted esters of carbamic acid. CBs developed in the 1950s as insect repellents were found to have insecticidal activity, leading to the development of the napthyl CBs with high anti-ChE activity and selective toxicity against insects. One example is carbaryl; it is widely used because of its low toxicity to mammals and its degradability. Aldicarb, a plant systemic, is more toxic than carbaryl. A few years ago aldicarb was associated with a July 4th holiday incident when West Coast residents complained of anticholinergic symptoms after eating aldicarb-contaminated watermelon.

Most *N*-methyl and *N*,*N*-dimethyl carbamates are better AChE inhibitors than BuChE inhibitors. However, *N*-carbamylated AChE spontaneously reactivates faster than *N*-carbamylated BuChE. AChE activity may recover as rapidly as 30 min following exposure – much faster than after exposure to OPs.

Although phosphorylation of AChE by OPs is heavily influenced by the electron-withdrawing power of the leaving group, carbamylation by methyl carbamates is also greatly dependent on molecular complementarity with the conformation of the enzyme as well as reactivity of the molecule. In general, phenolic and oxime moieties are more reactive than benzyl alcohol groups.

N-methyl carbamates do not need activation to inhibit ChEs. However, at least in the case of aldicarb, inhibition increases with metabolism. Aldicarb is rapidly oxidized to the relatively stable aldicarb sulfoxide, which in turn is more slowly metabolized to aldicarb sulfone, a stronger AChE inhibitor. These products are then detoxified by conversion to oximes and nitriles, which in turn are degraded to aldehydes, acids, and alcohols. Procarbamate derivatives were

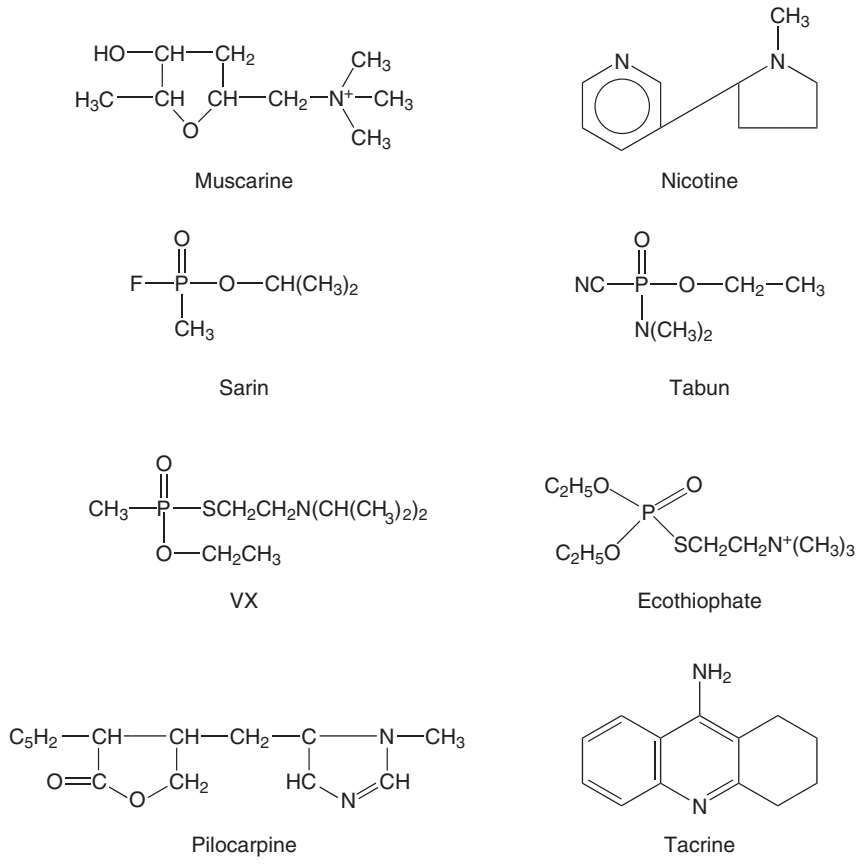


Figure 4 Continued.

developed to reduce the toxicity of CBs to mammals; the hydrogen atom on the carbamate nitrogen is replaced by a wide variety of nucleophiles – many with a sulfur atom – causing reduction in anti-ChE activity. The bond is rapidly broken in insects, restoring the activity and toxicity of the parent compound.

The rapid spontaneous reactivation of carbamates can be a problem in determining ChE activity. For example, some testing routines require that animals be put on a control diet for 24 h before sampling. With CBs, the inhibitions may have disappeared by the time the assays are performed. In addition, the dilutions specified in some assays may reduce the inhibition and high concentrations of substrate may compete with the carbamate to further reactivate the enzyme.

Chemical Warfare Anticholinesterase Agents

Anticholinesterase chemical warfare agents have been stockpiled since their development immediately before and during World War II. Several countries have active research programs into their toxicity and control. P = O groups confer potent anti-ChE inhibition properties and, in addition, the toxicity of agents such as soman and VX may be due in part to their actions on receptors and perhaps other proteins too. The toxicity of the nerve agents is greater than that of agricultural chemicals. For example, the dermal LD_{50} of agent VX is estimated to be 0.04–0.14 mg kg^{-1} for humans, which is at least an order of magnitude more toxic than most pesticides. LD_{50}s for representative agricultural OPs and CBs are shown in **Table 4**.

Assay Techniques

An early assay for ChE activity was a manometric method in which the change in pH due to ACh hydrolysis released CO_2 from a reaction buffer. A common technique (that of Michel) directly determines ACh hydrolysis by changes in pH. Another assay, that of Hestrin, utilizes the reaction of ACh with hydroxyl amine and ferric chloride, producing a reddish-purple complex. A test developed by Okabe and

Table 4 Representative acute LD_{50}s of selected organophosphates and carbamates

Compound	LD_{50} (mg kg^{-1})	
	Oral	*Dermal*
Organophosphates		
Dimethoxy compounds		
Azinphosmethyl (*O*,*O*-dimethyl-*S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl]phosphorodithioate)	13	220
Malathion (*O*,*O*-dimethyl-*S*-(1,2-dicarbethoxyethyl)phosphorodithioate)	1375	>4000
Methyl parathion (*O*,*O*-dimethyl-*O*-(*p*-nitrophenyl)phosphorothioate)	14	67
Diethoxy compounds		
Parathion (*O*,*O*-diethy-*O*-(4-nitorphenyl)phosphorothioate)	13	21
Diazinon (*O*,*O*-diethyl-*O*-(2-isopropyl-6-methyl-4-pyrimidnyl)phosphorothioate)	108	200
Carbamates		
Aldicarb (2-methyl-2-(methylthio)propylideneamino-*N*-methylcarbamate)	0.8	3.0
Carbaryl (1-naphthyl-*N*-methylcarbamate)	850	>4000

Adapted from Gaines TB (1969) Acute toxicity of pesticides, *Toxicology and Applied Pharmacology* 14: 515–534.

co-workers, oxidizes the choline released from ACh hydrolysis and determines the H_2O_2 produced. Several assays use radioactive ACh; one method counts the acetate produced by the reaction by separating it into an organic phase, leaving the unhydrolyzed ACh behind in an aqueous phase. Another common approach utilizes thioanalogs of ACh and other esters. In the assay developed by Ellman and co-workers, hydrolysis of thiocholines such as acetylthiocholine (AcTh) is measured at 410 nm with the color reagent dithionitrobenzoate. Although assays that rely on pH or radioactivity of ACh have the advantage of using a natural substrate, assays utilizing thiocholine esters are inexpensive, readily automated, and do not require expensive disposal of radioactive wastes. Negative features are the possibility of interference of hemoglobin (Hb) in RBC samples and a nonlinear reaction of the reduced glutathione in some RBCs with the color reagent. Some of the methods have been adapted for field use. Whatever the assay, it is important that its conditions be validated for the species, tissues, and chemicals under study. Unfortunately, there are no national or international standards for ChE assays making it difficult to compare results from one clinical laboratory with another. Recently the state of California specified a version of the Ellman assay as its clinical standard and required all laboratories monitoring blood ChEs to comply or harmonize with this standard methodology.

Biochemistry of Cholinesterase Inhibition

The inhibition of the activity of ChEs by OPs and CBs proceeds in a manner similar to the action of the enzymes on ACh. However, instead of forming a rapidly hydrolyzed acetyl–enzyme complex, the OPs and CBs, respectively, phosphorylate and carbamylate the catalytic sites of the enzymes. The major biochemical features of the inhibition of ChEs by OPs and CBs involve (1) activation of the inhibitors; (2) detoxification; (3) reaction of the inhibitor with the serine-active site of the enzyme and loss of a 'leaving group'; (4) hydrolysis of the complex and spontaneous reactivation of the enzyme; (5) loss of a second group, known as aging; and (6) recovery by synthesis of new enzyme.

One way to visualize the biochemical mechanisms underlying the toxicity of OPs and CBs is to trace the fate of an OP such as parathion from its entry into the body. Mixed function oxidases (MFO) in the liver (or in other tissues) convert parathion, a thionophosphate, to its oxygen analog, paraoxon, increasing its anti-ChE potential by orders of magnitude. The paraoxon may exert its toxic action by inhibiting AChE or be inactivated by conjugation with glutathione, reaction with glutathione transferases, further oxidation by MFO, or hydrolysis by A-esterases, in this case paraoxonase. Such reactions may lead to a loss in toxicity of either parathion or paraoxon. Paraoxon may also be inactivated by binding and reacting with B-esterases other than AChE, such as BuChE and carboxylesterases.

The reaction of an OP with AChE, BuChE, or other B-esterases is similar to the reaction of AChE with ACh, except that the hydrolysis step is much slower or, in some cases, may not occur at all. Its basis is a phosphorylation of the enzyme via a nucleophilic attack. The electronegative serine hydroxyl at the catalytic site reacts with the electropositive phosphorus atom of the inhibitor to form an OP–ChE complex and loss of a side group on the phosphorus atom, known as the leaving group (X). The phosphorylated enzyme may, in time, reactivate by

rehydrolysis. A similar set of reactions leads to carbamylation, except that the spontaneous reactivation tends to be more rapid than that for an OP. Spontaneous reactivation of an OP may take hours to days, whereas CBs may reactivate as soon as 30 min. In addition, OPs undergo a further reaction known as 'aging' in which a second group (often an alkyl group) is lost from the phosphate, stabilizing the OP–ChE complex.

Structure/Activity

Some general rules for OPs based on their structures include the following:

1. The P = O group is more toxic than the P = S group because it is more reactive. It is more reactive due to its higher electronegativity, which causes a more electropositive P atom, facilitating its reaction with the serine hydroxyl at the active site.
2. The electron-withdrawing ability of the leaving group X is predicted by the strength of its acid. For example, fluoride is a more powerful leaving group than nitrophenol since HF is a strong acid.
3. Reactivity of the R groups is in the order methoxy ⩾ ethoxy ⩾ propoxy ⩾ isopropoxy ⩾ amino groups. The more difficult a compound is to hydrolyze, the weaker is likely to be its ChE inhibition.
4. Steric effects are also important. The longer and more branched a compound, the more reduced is its rate of inhibition, probably because of the conformation of the proteins around the catalytic site.

The terms 'reversible' and 'irreversible' are often misused in describing ChE inhibitions. For example, statements such as 'OPs are irreversible inhibitors and CBs are reversible inhibitors' are useful insofar as they refer to the stability of the aged OP–enzyme and to the more rapid hydrolysis of the CB–enzyme compared to that of the un-aged OP–enzyme. Technically one could argue that the term 'reversible' should be reserved for cases in which there is an equilibrium between the substrate and the enzyme–substrate complex.

Spontaneous Reactivation of Organophosphates

Table 5 lists the half-lives of recovery for some OP-inhibited AChEs. In general OP–AChE complexes from dimethoxy-substituted OPs (e.g., malathion) spontaneously dephosphorylate faster than diethoxy (e.g., parathion) or diisopropoxy (e.g., DFP) complexes. Eto pointed out in 1974 that the stability of a phosphorylated AChE may be predicted from the stability of the specific OP inhibitor itself. One possibility is that methyl groups have less steric hindrance and greater electronegativity than ethyl or isopropyl groups.

Chemical Reactivation of Organophosphates

It has been almost 40 years since B. Wilson *et al.* observed that nucleophiles, oximes like hydroxamic acid, reactivated OP-inhibited AChE above and beyond that occurring from spontaneous reactivation, opening the way to a treatment for OP poisoning. The oxime registered for use in the United States is 2-PAM Cl (Protopam); its methanesulfonate salt (P2S) is used in Europe. Oxime therapy should be recommended with caution for carbamate poisonings. Although beneficial in the case of aldicarb, there is evidence that 2-PAM treatment increases the toxicity of carbaryl.

The mechanism of action of oxime reactivation involves transfer of the substituted phosphate or phosphonate residue from the catalytic site of the

Table 5 Spontaneous reactivation of aging of selected organophosphates

Compound	*Tissue*	*Spontaneous* (h)	*Aging* (h)
Malathion	Human RBC	0.85	3.9
Methamidophos	Bovine RBC	0.13	0.54
Chlorpyrifos	Bovine RBC/mouse brain	58	36
Diazinon	Human RBC	58	41
Parathion	Rat brain/bovine RBC	103	58
Tabun	Human RBC	ND	13
Sarin	Human RBC	ND	3.0
DFP	Human RBC	ND	4.6
Soman	Human RBC	ND	0.02

Adapted from Wilson *et al.* (1992) In: Chambers JE and Levi PE (eds.) *Organophosphates, Chemistry, Fate, Effects*. New York: Academic Press.

enzyme to the oxime. In addition, 2-PAM may react directly with the free OP molecule itself. Other oximes such as TMB-4, obidoxime, and HI-6 are reported to be superior to 2-PAM as reactivators and antidotes to chemical warfare agents. Oxime therapy should not be used in the absence of ChE inhibition since 2-PAM itself is a weak ChE inhibitor. In addition to the reactions discussed previously, direct effects of these compounds on muscle contraction and nicotinic receptors led Albuquerque and colleagues to propose that oximes also act directly on cholinergic receptors.

Aging

Research on oximes revealed an important phenomenon: the extent of reactivation of an OP–AChE complex decreased with time and depended on the OP used. This 'aging' prevents both spontaneous and chemical reactivation. Evidence indicates aging is due to the loss of a second group from the phosphorus atom. Harris and colleagues in 1966 demonstrated the loss of an alkyl group from a soman–AChE complex and showed that the percentage of enzyme losing an alkyl group correlated with the percentage of enzyme resistant to oxime activation. In general, OP–ChE complexes that spontaneously reactivate slowly tend to age rapidly. Exceptions are dimethoxy-phosphorylated AChEs, which both rapidly age and spontaneously reactivate. In general, agricultural chemicals (e.g., malathion, parathion, and diazinon) have half-lives of aging of hours and longer, while chemical warfare agents age rapidly (e.g., 10 min for soman).

Treatment for Anticholinesterase Poisoning

The information included here is educational; it should not be construed as specific recommendations for treatment of patients.

Inhibition of AChE by OPs or CBs is one of the few types of toxicity for which there are antidotes. The usual treatment for OP poisoning is atropine and 2-PAM (**Table 6**). The presence of atropine reduces the effectiveness of the ACh receptors, counterbalancing the excess ACh present. The recommended doses for humans are 1 g 2-PAM Cl (intramuscular or intravenous) two or three times a day and 2 mg atropine (intravenous) at 15–30-min intervals as needed. Higher doses may be used depending on the extent of the OP intoxication. Environmental Health Criteria No. 63 describes the case of a patient who drank a large amount of dicrotophos while inebriated. Treatments were progressively increased up to 6 mg atropine intravenously every 15 min with continuous infusion of 2-PAM Cl at 0.5 g h^{-1}. All told, 92 g of 2-PAM and 3912 mg of atropine were given to the patient, who was discharged after 33 days.

Table 6 Treatments for anticholinesterase poisoning

Atropine
2 mg intravenously, at 15 to 30 min intervals as needed to suppress symptoms 2 Pralidoxime
1 g either intramuscularly or intravenously two or three times per day or to suppress symptoms
Diazepam
10 mg subcutaneously or intravenously, repeated as required

Adapted from Environmental Health Criteria 63, 1986.

Much of the research on treatments of ChE inhibitions has concerned chemical warfare agents, providing little direct information for the treatment of agricultural chemicals.

Considerable attention has been given to prophylactic treatments to protect military units and civilian populations in the event of either accidental or deliberate release of nerve gas agents. One kit contains a combination of atropine, 2-PAM, and the anxiolytic, diazepam. Another contains pyridostigmine, a carbamate with actions similar to physostigmine. Diazepam is included to lessen CNS symptoms. The use of pyridostigmine is based on the idea that a readily rehydrolyzable carbamate will compete for AChE catalytic sites with the high-affinity binding nerve gas agents, reducing the percentage of AChE that becomes 'irreversibly' inhibited. Using these agents is not without risk since they are themselves toxic. Issuance of atropine kits to the general population of Israel during the Persian Gulf crisis led to the accidental injection of more than 200 children; some had systemic effects but fortunately there were no fatal consequences.

The discovery of methods to isolate relatively large amounts of ChE enzymes in essentially pure form has led to a unique method of treating OP intoxication – that of adding ChEs to the blood. Several experiments indicate enough of the OPs bind to the ChEs to reduce their toxicity in experimental animals. One issue is that of possible immune responses to what might be recognized by the body as a foreign protein, but to date there is no evidence suggesting this to be a problem.

Treatments with Anticholinesterase Agents

Several anticholinesterase agents have been used to treat human disorders.

Alzheimer's Disease and Tacrine

The finding that senile dementia of the Alzheimer's type was accompanied by a loss of AChE activity (as well as other neurochemical markers for cholinergic neurons) in parts of the brain has stimulated study of cholinergic nerve activity, learning and memory, and the use of anti-ChE compounds in the treatment of Alzheimer's disease. The strategy is to increase the effective level of ACh by reducing the activity of the AChE present. Tacrine (tetrahydroaminoacridine) was the first drug to be evaluated for this purpose. Tacrine is a weakly binding anti-ChE agent approved for treatment by the US FDA. The dose of Tacrine recommended ($100\,mg\,day^{-1}$) was chosen on the basis of the side effects the drug has on liver function rather than on unequivocal demonstration of its effectiveness. (In some trials, up to a third of the patients were removed from the studies due to side effects of the drug.) A subsequent cholinesterase inhibitor approved for use was donepezil (E 2020; Aricept). This drug appears less capable of eliciting adverse side effects. Other cholinesterase inhibitors evaluated for use in Alzheimer's disease include physostigmine and trichlorfon.

Glaucoma

Glaucoma is a disorder of vision accompanied by an increase in ocular pressure. Although mostly replaced by other drugs (e.g., beta blockers and pilocarpine), anti-ChE drugs such as ecothiopate are still used in the treatment of these common disorders.

Myasthenia Gravis

Myasthenia gravis is a progressive disorder characterized by muscle weakness; eye muscles are often the first affected. Research has shown it to be an autoimmune disease in which the victim forms antibodies to his or her nicotinic acetylcholine receptors at motor endplates. It is characterized by fatigability and weakness of the skeletal muscles, especially those of the eyes. Approximately 90% of the patients have droopy eyelids and double vision. Treatments include corticosteroids and thymectomy to reduce the actions of the immune system and anti-ChE agents such as pyridostigmine to improve the effectiveness of the receptors that remain.

Wildlife and Domestic Animal Exposures

The recognition that chlorinated hydrocarbons are a persistent danger to wildlife led to a decrease in their use as agricultural chemicals and to an increase in the use of OPs and CBs. In general, OPs and CBs do not bioaccumulate as do chlorinated hydrocarbons and they are relatively biodegradable. However, they are more acutely toxic than chlorinated hydrocarbons to humans and wildlife. A thorough discussion of the comparative toxicology of OPs and CBs is outside the scope of this entry. ChE inhibitions are generally the same, regardless of the animal; differences between species are often in the overall pharmacokinetics and metabolism. For example, although birds have higher brain AChE activities than mammals, they also have less hepatic MFOs to activate OPs and less A-esterases to hydrolyze them. Much research has been done on the toxicology of OPs to wild birds from sparrows to hawks and eagles. For example, Hill *et al*. of the US Fish and Wildlife Service studied the toxicity of 19 OPs and eight CBs to 35 species of birds. In general, such studies showed that over 50% of OPs and 90% of CBs have LD_{50}s of $<40\,mg\,kg^{-1}$ for most birds.

Route of exposure may have much to do with the recovery from OPs. When pigeons were treated orally with an OP, inhibition of blood ChE was rapid, and recovery of activity occurred within a few days. However, when the treatment was conducted dermally, putting the OP on the feet, recovery of enzyme activity took several weeks, implying the presence of a depot for OPs and the possibility that birds can accumulate OPs by flying from site to site. The possibility of bioaccumulation of OPs in a food chain (usually considered to be a characteristic of chlorinated hydrocarbons) was demonstrated by the report of an eagle poisoned by an OP (Warbex) in magpies that, in turn, had obtained the OP by ingesting hair from a steer that had been treated with it for parasites.

Beef cattle, horses (more than sheep), goats, and swine are treated several times each year with OPs to control parasites and some are fed tetrachlorvinphos to prevent fly larvae hatching in their feces. Carbaryl is commonly used for flea and tick control. Oehme states that insecticides are a common cause of poisoning of domestic animals and that "the majority of insecticide problems in domestic animals result from ignorance or mismanagement." Indeed, there is some epidemiological evidence that animal technicians in pet grooming and veterinary hospitals are exposed to the OP and CB chemicals used to control fleas and ticks while washing the animals. Sheep 'dipping' methods have been changed to minimize exposure to the worker.

Exposures in the Workplace

Worldwide, estimates of the number of humans requiring treatment due to anti-ChE chemicals run into many thousands annually. Concern for those who

manufacture and use agricultural chemicals has resulted in studies of pesticide residues, protective clothing, urinary metabolites, and blood ChE levels of farmworkers, greenhouse workers, and spray applicators. In general, the rule has been to consider decreases of blood cholinesterases of 30% or more as meaningful, signifying the worker should be removed from contact with the agent. In the United States, California requires workers to be monitored; however, even there, until recently, there has been no single standard method to determine ChE activities.

Chemical Warfare and Terrorism

The use of chemical weapons, nerve gases, mustard gases, and blistering agents is banned by international treaty. Nerve agents were inadvertently released from storage sites during the Persian Gulf conflict. A decade later the role that nerve agents may have played, whether alone or in company with other chemicals, in a baffling set of symptoms known as the Gulf War syndrome is still under investigation.

Millions of pounds of chemical warfare agents are stored throughout the world. Their destruction by incineration at high temperatures, up to 2500°F (1480°C) is planned or under way in several countries. These include eight sites in the United States, such as the Tooele Army Depot in Utah and Johnston Atoll in the Pacific, which is 750 miles from Hawaii. Some of the ordinance has been stored since World Wars I and II. Complaints have been lodged by citizens groups concerned about possible risks to residents during the destruction of the chemicals.

Sadly, chemical warfare weapons are dangerous instruments of terror. Two recent episodes in which sarin was used by terrorists in Japan cast a cloud over attempts to control the use of these weapons. Sarin was released in a residential area of the city of Matsumoto on June 27, 1994, and in a crowded Tokyo subway less than a year later, on March 20, 1995. In Matsumoto, about 600 residents and rescuers were affected and seven died. More than 5500 people were poisoned and 12 died in the Tokyo incident. Many more might have perished if it were not for the quick action and bravery of firemen, police, and others and the availability of antidotes in Japanese hospitals. (Two subway attendants died removing containers of sarin from subway cars.)

Significance of Blood Cholinesterase Levels

There has been a continuing discussion of the significance of monitoring blood ChEs of humans and wildlife. The setting of no-observed-adverse-effect levels (NOAELs) is an example. (NOAELs are the highest dose levels at which no important effect of a toxicant is observed.) Determining NOAELS is an important step in assigning risks and safe levels for the use of a toxic chemical. Some propose that batteries of behavioral tests performed under controlled laboratory conditions provide the best data for setting safe levels of exposure. Under field conditions others propose that measurements of residues on skin and clothing, urinary metabolites of agricultural workers, and fecal metabolites of wild animals provide evidence of exposure to chemicals without invasive procedures. Proponents of the use of ChE levels point out that they represent standardized, relatively inexpensive measurements that directly demonstrate a biochemical effect of an exposure to a toxic chemical rather than merely providing evidence of the exposure itself. Recent technology permits determinations of enzyme activities on 100 µl or less of blood, obtainable by a finger prick.

Regardless, as long as millions of pounds of OPs and CBs are used annually, ChE measurements will be an important tool in the protection of humans, domestic animals, and wildlife from overexposure to these toxic agents.

See also: A-Esterases; Anticholinergics; Carbamate Pesticides; Carboxylesterases; Neurotoxicity; Nerve Agents; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates; Pesticides; Veterinary Toxicology.

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Cholinesterases *See* Cholinesterase Inhibition.

Chromated Copper Arsenate *See* CCA-Treated Wood.

Chromium

Abbi Heilig

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- Chemical Abstracts Service Registry Number: CAS 7440-47-3
- Synonyms: Chrome; Chromium
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Cr^{3+}; Cr^{5+}

Uses

Chromium is a transitional element with many industrial uses. It is mainly used in imparting a shiny appearance to metal surfaces. In the early 1800s, the mineral, now known as chromite, was widely used in the production of paint as well as in the production of chromium compounds. These compounds can be used in a variety of applications. For example, potassium dichromate is used in the dyeing industry and chromium salts are used in leather tanning and wood preservation. Today, perhaps its most important use is in the production, in combination with iron, of stainless steel.

Background Information

Chromium as a metallic element was first discovered two hundred years ago, in 1797. But the history of chromium really began several decades before this. In 1761, in the Beresof Mines of the Ural Mountains Johann Gottlob Lehmann obtained samples of an orange-red mineral, which he called 'Siberian red lead'. He analyzed this mineral in 1766 and discovered that it contained lead "mineralised with a selenitic spar and iron particles." The mineral he found was crocoite, a lead chromate ($PbCrO_4$).

In 1770, Peter Simon Pallas also visited the Beresof Mines and observed the same type of mineral. He described it as "a very remarkable red lead mineral which has never been found in any other mine. When pulverised, it gives a handsome yellow guhr which could be used in miniature painting...." Chromium from the Beresof Mines and Siberia was used as a paint pigment. Due to its rarity, this later became a collector's item and increased in popularity in the paint industry. A bright yellow made from crocoite fast became the fashionable color for the carriages of the nobility in both France and England.

In 1797, chromium received its name from a professor of chemistry and assaying at the School of Mines in Paris, Nicolas-Louis Vauquelin. He received some samples of crocoite ore and his subsequent analysis revealed a new metallic element, which he called chromium after the Greek word khrôma, meaning color. After further research he detected trace elements of chromium in precious gems – giving the characteristic red color of rubies and the distinctive green of emeralds, serpentine, and chrome mica.

In 1798, Lowitz and Klaproth independently discovered chromium in a sample of a heavy black rock found further north from the Beresof Mines and in 1799 Tassaert identified chromium in the same mineral from a small deposit in the Var region of South-Eastern France. The chromite ore deposits discovered in the Ural Mountains greatly increased the supplies of chromium to the growing paint industry and even resulted in a chromium chemicals factory being set up in Manchester, England around 1808. In 1827, Isaac Tyson identified deposits of chromite ore on the Maryland-Pennsylvania border and the United States became the monopoly supplier for a number of years.

But high-grade chromite deposits were found near Bursa in Turkey in 1848 and with the exhaustion of the Maryland deposits around 1860, it was Turkey that then became the main source of supply. This continued for many years until the mining of chromium ore started in India and Southern Africa around 1906. And although paint pigments remained the main application for many years, chromium was finding other uses: Kochlin introduced the use of

potassium dichromate as a mordant in the dyeing industry in 1820. The use of chromium salts in leather tanning was adopted commercially in 1884. While chromite was first used as a refractory in France in 1879, its real use started in Britain in 1886.

The first patent for the use of chromium in steel was granted in 1865 – but the large-scale use of chromium had to wait until chromium metal could be produced by the alumino-thermic route, developed in the early 1900s and when the electric arc furnace could smelt chromite into the master alloy, ferrochromium.

Among the products chromium is used in, shiny finishes on surfaces and stainless steel are the most popular.

Exposure Routes and Pathways

Most chromium exposure in the general population is through ingestion of the chemical in food containing chromium(III), although exposure is also possible as a result of drinking contaminated well water, or living near uncontrolled hazardous waste sites containing chromium or industries that use chromium. Inhalation of chromium dust and skin contact during use in the workplace are the main routes of occupational exposure.

Studies have shown that inhalation, oral, and dermal exposures can result in chromium deposits in liver, kidney, heart, and lungs. Chromium in the breast milk of mothers can be passed down to infants and fetuses can be exposed to chromium that passes through the placenta.

Toxicokinetics

The toxicokinetics of a given chromium compound depend on the valence state of the chromium atom and the nature of its ligands. In contrast to chromium(III), which is bound to plasma proteins such as transferrins, chromium(VI) entering the blood stream is taken up selectively by erythrocytes, reduced, and bound predominantly to hemoglobin.

The absorption of chromium(VI) into the blood system through the skin has been reported but not investigated extensively, mainly because the reported health effects are rare. Once absorbed into the blood system there are various antioxidants that act as reducing agents, such as glutathione and ascorbate, which rapidly reduce chromium(VI) to chromium(III). Chromium absorbed through the lungs into the blood system is excreted by the kidneys and the liver. The kidney appears to absorb chromium from the blood through the renal cortex and releases it into the urine. Thus, sampling of urine for chromium can be used for biological monitoring of certain types of welding fumes that contain water-soluble chromium(VI).

Mechanism of Toxicity

Chromium may cause adverse health effects following inhalation, ingestion, or dermal exposure. The toxicity of chromium is mainly caused by hexavalent compounds as a result of a higher cellular uptake of chromium(VI) compounds than chromium(III). This is explained by the fact that the chromate anion $(CrO_4)^{2-}$ can enter the cells via facilitated diffusion through nonspecific anion channels (similarly to phosphate and sulfate anions). Absorption of chromium(III) compounds is via passive diffusion and phagocytosis.

Hexavalent chromium is unstable in the body and is reduced intracellularly (by many substances including ascorbate and glutathione) providing very reactive pentavalent chromium and trivalent chromium. Both of these intermediates can alter DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chromium can cause irritation in both the eyes and skin. Hexavalent chromium is corrosive to the skin and eyes.

Human

Ingesting large amounts of chromium(VI) can cause stomach upsets and ulcers, convulsions, kidney and liver damage, and even death. Skin contact with certain chromium(VI) compounds can cause skin ulcers. Some people are extremely sensitive to chromium(VI) or chromium(III). Allergic reactions consisting of severe redness and swelling of the skin have been noted.

Chronic Toxicity (or Exposure)

Animal

The hexavalent form of chromium is a potent teratogen, primarily affecting bone formation. However, trivalent chromium was not found to be teratogenic. Animal studies also show an increase in the risk of cancer after exposure to chromium(VI) compounds.

Human

Chromium(III) is an essential nutrient that helps the body use sugar, protein, and fat. Chronic liver and

kidney damage due to long-term exposure of chromium(VI) has been reported. However, chronic low-level exposure to chromium does not appear to produce measurable renal damage. Dermal exposure to chromium compounds can cause irritant dermatitis and skin ulcerations (chrome holes). Breathing high levels of chromium(VI) can cause irritation to the nose, such as runny nose, nosebleeds, and ulcers and holes in the nasal septum. Inhalation of chromium(VI) compounds is also associated with lung cancer and these compounds are classified as human carcinogens.

Clinical Management

There are no specific antidotes for chromium poisoning. Since most human overexposure is by ingestion, gastric lavage is appropriate in some cases. However, emesis should not be induced. Maintaining the proper fluid balance is critical due to impact on the kidney's ability to reabsorb fluid. It is necessary to establish that there is no impairment with breathing due to fluid accumulation in the lungs. Another important step is to decrease the intake of dietary supplements that contain chromium.

Environmental Fate

Chromium enters the air, water, and soil mostly in the chromium(III) and chromium(VI) forms. In air, chromium compounds are present mostly as fine dust particles, which eventually settle over land and water. Chromium can strongly attach to sediment and soil and only a small amount is expected to dissolve in water and leach though the soil to groundwater. Fish do not accumulate much chromium in their bodies.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit time-weighted averages (TWAs) for chromium compounds are: for, chromium(0) and salts – 1.0 $mg\,m^{-3}$; for chromium(II) and chromium(III) – 0.5 $mg\,m^{-3}$. The American Conference of Governmental Industrial Hygienists threshold limit value – TWA for chromium(VI) is 0.01 $mg\,m^{-3}$. The US Environmental Protection Agency maximum contaminant level in drinking water is 0.1 $mg\,l^{-1}$.

See also: Cardiovascular System; Chromium Hexavalent Compounds; Metals; Respiratory Tract.

Further Reading

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chromium.

Chromium Hexavalent Compounds

Robert Kapp

Chromium occurs in three basic forms: metallic chromium (Cr(0)), trivalent chromium (Cr(III)), and hexavalent chromium (Cr(VI)). Hexavalent chromium can exist as chromium hexavalent ion and as part of a number of compounds including calcium chromate, chromic acid, chromium trioxide, lead chromate, strontium chromate, potassium dichromate, and zinc chromate.

- Chemical Abstracts Service Registry Numbers:

Chemical	*CAS nos.*	*EINECS nos.*
Chromium	7440-47-3	231-157-5
Hexavalent chromium ion	18540-09-9	Not Listed
Ammonium dichromate	7789-09-5	232-143-1
Calcium chromate	13765-19-0	237-366-8
Chromic acid	13530-68-2	236-881-5
Chromium trioxide	1333-82-0	215-6-7-8
Lead chromate	7758-97-6	231-846-0
Strontium chromate	7789-06-2	232-142-6
Potassium dichromate	7778-50-9	231-906-6
Zinc chromate	13530-65-9	236-878-9

- Synonyms:
  - Hexavalent chromium ion – Chromium hexavalent ion; Chromium(6+); Chromium(6+) ion; Chromium(VI); Cr(VI); Chromium ion (Cr^{6+})
  - Ammonium dichromate – Ammonium bichromate; Ammonium dichromate(VI); Ammonium chromate; Chromic acid, diammonium salt; Diammonium dichromate
  - Barium chromate – Barium chromate(VI); Barium chromium oxide; Baryta Yellow; C.I. Pigment Yellow 31; Chromic acid, barium salt; Lemon Yellow; Lemon chrome; Ultramarine yellow
  - Calcium chromate – Calcium chrome yellow; Calcium chromium oxide; Calcium monochromate; Yellow ultramarine
  - Chromium trioxide – Chromic(VI) acid; Chromic oxide; Chromium oxide; Chromium(6+) oxide, Monochromium trioxide
  - Chromic acid – Dichromic acid; Dichromic(VI) acid
  - Lead chromate – Lead chromate(VI); Phoenicochroite; Plumbous chromate
  - Strontium chromate – C.I. pigment yellow; Deep lemon yellow; Strontium yellow; Strontium chromate; Sutokuro T
  - Potassium dichromate – Bichromate of potash; Chromium potassium oxide; Dipotassium dichromate heptaoxide; Iopezite; Potassium dichromate(VI)
  - Zinc chromate – Basic zinc chromate; Buttercup yellow; C.I. 77955; Chromium zinc oxide; Pigment yellow 36; Zinc chromate hydroxide; Zinc hydroxychromate; Zinc tetraoxychromate; Zinc yellow; Zincro ZTO
- Chemical/Pharmaceutical/Other Class: Metals

Uses

Metallic chromium is used in the following applications: to harden steel, the manufacture of stainless steel, the manufacture of other alloys, in electroplating, and as a catalyst. Trivalent chromium is used in the following applications: to make metal alloys, to make high-temperature bricks for industrial furnaces, and in leather tanning. Hexavalent chromium is used in the following applications: the production of pigments, metal finishing, and in wood preservatives.

Ammonium chloride is used in the following applications: as a fluxing agent, as an electrolyte for plating baths and batteries, in personal care products, in pharmaceuticals, and as a general anticaking agent.

Barium chromate is used in the following applications: as an anti-corrosive agent, as a pigment in paint, in ignition control devices, in safety matches, as a constituent in pyrotechnic compositions, and as a coloring agent in ceramics.

Calcium chromate is used in the following applications: as a pigment, as a corrosion inhibitor, as an oxidizing agent, and as a coating for light metal alloys.

Chromium trioxide is used in the following applications: in chromium plating, in copper stripping, in aluminum anodizing, as an anticorrosive, in photography, in hardening microscopic preparations, and in purifying oil and acetylene.

Lead chromate is used in the following applciations: as a pigment, in printing fabric, in chemical analysis of organic material, and as a constituent in pyrotechnic compositions.

Potassium dichromate is used in the following applications: as an anticorrosive agent, in the manufacture of other potassium/chromium compounds, in the manufacture of glass and glazes, and as a constituent in pyrotechnic compositions.

Strontium chromate is used in the following applications: as a pigment, as an anticorrosive agent in aluminum and magnesium alloys, in vinyl sheeting, and in chemical-resistant coatings.

Zinc chromate is used in the following applications: in pigments in paints, varnishes and oil colors, as an anticorrosive in primer coatings, as metal conditioners prior to priming, and as a catalyst.

Background Information

Elemental chromium is an odorless, hard, steel gray, lustrous metal that is available in crystal or powder. The symbol is Cr and the atomic weight is 52. Johann Gottlob Lehmann originally found chromium as a metallic element in 1761 on a visit to the Beresof Mines in the Ural Mountains of Siberia. He obtained samples of an orange-red mineral, which he named Siberian red lead. He later analyzed the sample and discovered it contained lead "mineralized with a selenitic spar and iron particles." The mineral was later found to be crocoite – which is lead chromate ($PbCrO_4$). Peter Simon Pallas also visited the Beresof Mines and observed "a very remarkable red lead mineral which had never been found in any other mine. When pulverized, it gives a handsome yellow guhr which could be used in miniature painting." Subsequent to that finding, the red lead mineral was used as a paint pigment and the crocoite yellow became a fashionable color for the carriages of the French and English nobility. Professor Nicolas-Louis Vauquelin, of the School of Mines in Paris,

discovered trace amounts of chromium in some precious gems – giving the characteristic red color to rubies and the distinctive green color to emeralds, serpentine, and chrome mica. In 1797 Vauquelin subsequently analyzed a sample of crocoite ore and found a new metallic element that he termed 'chromium' after the Greek word khrôma, meaning color because of the many different colors of its compounds. This metal can be in several forms, the most common are the metals chromium(0), chromium(III), and chromium(VI) compounds. Chromium(III) occurs naturally in the environment and types (0) and (VI) are produced in industrial reactions. The divalent state (chromous) is readily oxidized to the more stable trivalent (chromic) state. The hexavalent (chromate) state is more stable than the divalent state, but it is not found in nature. Hexavalent chromium compounds are highly corrosive and are strong oxidizers. They are generally reduced to the trivalent state in nature.

Other hexavalent chromium compound descriptions include the following:

- ammonium chromate occurs as a yellow crystalline substance;
- barium chromate occurs as a yellow powder;
- calcium chromate occurs as yellow monoclinic prisms;
- chromic acid occurs as brownish-red flakes;
- chromium trioxide occurs as odorless purplish to red rhombus crystals;
- lead chromate occurs as yellow to orange monoclinic crystals;
- potassium dichromate occurs as orange-red crystals;
- strontium chromate occurs as monoclinic yellow crystals; and
- zinc chromate occurs as lemon yellow prisms.

Chromium metal does not react with air, oxygen, nitric acid, alkalis, or water at room temperature.

Exposure Routes and Pathways

Human exposure to chromium and hexavalent chromium compounds can occur by inhalation, ingestion and skin contact; however, ingestion is the main route of exposure for the general population. Chromium compounds are widely distributed in the air, water, soil, and food. The trivalent form in trace amounts is considered to be an essential dietary component. Chromium has been detected in vegetables, fruits, grains, cereals, eggs, meat and fish at concentrations between 20 and 520 $\mu g\,kg^{-1}$. The mean daily intake of chromium from the air varies from <0.2 to 0.6 μg; from water is calculated to be <4 μg while the intake from food is approximately 60 μg. Skin exposure can occur through contact with wood treated with chromated copper arsenate. The Agency for Toxic Substances and Disease Registry estimates that citizens that live near industrial facilities or waste sites that can release chromium to the environment would have a higher exposure level than the average citizen.

The highest exposures occur occupationally. The National Occupational Hazard Survey conducted by the National Institute for Occupational Safety and Health from 1972 through 1974 concluded that some 2.5 million workers could be exposed to chromium and its compounds in the workplace. The National Occupational Exposure Survey conducted a decade later from 1981 through 1983 estimated a total of almost 200 000 workers were exposed to hexavalent chromium compounds (barium chromate, calcium chromate, chromium trioxide, lead chromate, strontium chromate, and zinc chromate). Occupational exposure occurs primarily from stainless steel production and welding, chromate production, chrome plating, ferrochrome alloys, chrome pigment and tanning industries.

Toxicokinetics

Both hexavalent and trivalent chromium are poorly absorbed from the lung or gastrointestinal (GI) tract.

Gastric absorption of hexavalent chromium is more efficient than absorption of trivalent chromium; however, absorption of ingested hexavalent chromium is less than 5%. Hexavalent chromium is reduced to the trivalent form upon contact with gastric juices, which appears to significantly reduce its absorption by the oral route of exposure. The size, oxidation state, and solubility of the chromium particles and the activity of the alveolar macrophages can affect absorption by inhalation exposure. In most cases, hexavalent chromium is more readily absorbed from the lungs than the trivalent compounds due, in part, to differences in the capacity of biological membranes. A significant amount of chromium is absorbed into the bone. Chromium is also concentrated in tissues of the liver, kidney, and spleen. Once absorbed, hexavalent chromium readily enters red blood cells through the phosphate and sulfate anion-exchange carrier pathway and some residual may remain in the plasma for an extended time period Dermal absorption is dependent upon the state of the chromium, the vehicle and the integrity of the skin.

Mechanism of Toxicity

Hexavalent chromium is significantly reduced to the trivalent state by glutathione in all tissues. During this reduction process, it has been shown that chromium may interact with cellular macromolecules and DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hexavalent chromium compounds are severely irritating to skin, eyes, and respiratory tissues.

Human

Humans who ingest toxic amounts of hexavalent chromium present clinical features of vomiting, diarrhea, hemorrhagic diathesis, and GI bleeding.

Chronic Toxicity (or Exposure)

Animal

Hexavalent chromium is nephrotoxic and tumorigenic. It has been reported in experimental animals that the hexavalent form of chromium can affect bone formation in fetal development. The mechanisms for this effect have yet to be elucidated; however, it is suggested that the potent peroxidant properties of hexavalent chromium may be involved. Animal studies have revealed a deficiency in lactation and male sterility resulting from hexavalent chromium exposure.

Intrabronchial implantations of zinc chromate and strontium chromate produced bronchial carcinomas.

Human

It is generally accepted that chromium is an essential element for humans. World Health Organization has estimated that the minimum chromium requirement is $33\,\mu g\,day^{-1}$. Some investigators have proposed that chromium deficiency may cause postnatal growth retardation and impaired glucose tolerance. There are no reports documenting chromium deficiency in human reproduction.

Chronic exposure to excess hexavalent chromium results in irritation of the skin and mucous membranes. Exposure to low doses of any form of chromium can induce allergic reactions causing skin rashes and swelling of the skin in sensitive individuals. Ulcerations (or chrome holes) can occur among workers who are exposed to high concentrations of chromic acid, sodium or potassium dichromate or chromate or ammonium dichromate. The ulcers generally occur in nail root areas, the creases over the knuckles, finger webs and forearms. Primary irritation can be attributed to hexavalent chromium. Cross hypersensitization to other metals, such as cobalt and nickel are not uncommon upon exposure to chromium. Systemic toxicity has been noted in humans following dermal exposure to chromium compounds.

Chronic inhalation exposure to hexavalent chromium may give rise to nasal septum bleeding and perforation with an accompanying loss of the sense of smell and taste. Bronchial asthma may result from chronic exposure to chromate dust or chromium trioxide.

Hexavalent chromium compounds are classified as substances known to be carcinogenic to humans. This is based upon sufficient evidence of carcinogenicity in humans exposed in chrome production facilities, chromium-alloy facilities, in the chrome plating industry as well as in chrome pigment industries. This exposure results in an increased incidence of lung cancer among these workers. The incidence of cancers at other sites may be increased in these occupational workers There is not sufficient evidence to show that barium chromate, calcium chromate, chromium trioxide, lead chromate, sodium dichromate and strontium chromate are carcinogenic in humans.

There are no reports documenting excess chromium as a teratogen in the human fetus.

In Vitro Toxicity Data

Hexavalent chromium has been shown to be a strong clastogen in experimental animals producing chromosome aberrations, sister chromosome exchanges, DNA strand breaks, oxidized base damage, DNA – DNA and DNA – protein cross-links. Human genetic studies have shown mixed results and have been limited by insufficient numbers of subjects.

Clinical Management

Upon exposure to any chromium compounds, the initial approach includes an assessment of the clinical status with subsequent support of basic cardiopulmonary functions. Once the airway has been stabilized, further measures can be taken.

After ingestion, emesis should not be induced due to the corrosive effects of chromium compounds. Ascorbic acid should be administered orally or nasogastrically to help reduce the hexavalent compounds to the trivalent forms. Dilution of the GI tract contents is indicated if the dilution can be accomplished within a few minutes after the ingestion.

Dilution may be accomplished with water or with demulcent fluids such as milk. Gastric lavage is indicated in certain circumstances. Hemodialysis and charcoal hemoperfusion may be employed in the event of renal failure. Fluid balance should be monitored and supportive measures taken as indicated.

After dermal exposure, the skin should be irrigated copiously with water. Topical application of freshly made 10% ascorbic acid solution or of a barrier cream containing 2% glycine and 1% tartaric acid has been beneficial in reducing thermal and chemical burns.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value – time-weighted average for chromium(VI) is $0.01\,\mathrm{mg\,m^{-3}}$.

See also: Metals.

Further Reading

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chromium.

Chromosome Aberrations

Antone L Brooks

DNA is the genetic material responsible for passing genetic information from one cell to its daughter and in whole organisms passing the information from one generation to the next. In each cell, the DNA is packaged with proteins into units called chromosomes. The chromosomes in each cell have a characteristic number, size, and shape depending on its species. These characteristics provide what is called the karyotype for any cell from that species. For example, the human karyotype has 46 chromosomes, each of which can be identified and evaluated to determine if the cell or individual is normal. Such evaluation can predict many genetic diseases.

Chromosomes provide the units or trains that the genes ride on during their travels during cell replication and division. This genetic material (DNA) is organized into genes that are responsible for the production of proteins that regulate cell function. Each of the chromosomes has a unique location in the cell and the chromosomes maintain their respective locations during cell division. In mammals and other higher organisms, each chromosome is divided into two chromatids containing the same information. The chromosome also contains a centromere. This is the site where spindle fibers attach to the chromosome during cell division to ensure that each of the daughter cells has identical numbers and types of chromatids as the parent cell. This provides a way for genetic material to be carefully transmitted with very few errors during cell division, one cell to another, and during reproduction from one generation to the next.

Any physical or chemical agent that causes chromosome damage or aberrations is known as a clastogen or chromosome breaker. Chromosome aberrations can be induced by physiological changes as well as physical and chemical agents. The frequency of aberrations increases with age, cigarette smoking, or exposure to other environmental insults such as exposure to ionizing radiation. The number and type of aberrations can be used to determine if individuals have been exposed to specific environmental insults and to estimate the amount or magnitude of that exposure. The aberrations in this case can serve as a biomarker of radiation or chemical exposure or dose.

With the advances in molecular biology it has become possible to label or 'paint' each of the human chromosomes a different color. This was done by sorting the chromosomes according to their size, isolating the DNA from each chromosome, and making a probe using a different color to match the unique DNA on the sorted chromosome. This chromosome painting makes it possible to accurately determine which chromosomes are involved in each chromosome aberration or rearrangement. With these paints it has been possible to determine that many of the chromosome aberrations produced by ionizing radiation that in the past were thought to be between two chromosomes actually involve many chromosomes.

Such specific identification of aberrations has made it possible, in some cases, to determine which environmental insult is responsible for the chromosome alterations. The chromosome aberrations thus become biomarkers of exposure.

Chromosome damage is involved during the induction of cancer and birth defects. That is why it is important to be able to characterize the type of aberrations and the chromosomes that are involved. For example, if there is an exchange of one piece of a chromosome with a piece of another chromosome it is called a translocation. All the genes are still present after these translocations have occurred, but they are located next to different genes. This can cause a change in the way the genes produce messages and result in alterations in the message and proteins produced. These alterations change the physiology of the cells. For example, in many individuals who have leukemia there is a translocation between human chromosomes 9 and 22. This change results in a unique protein being produced from the altered karyotype. These changes in karyotype and protein provide useful markers of this disease. The understanding of the production of these proteins has been important in developing molecular medicines that can block the action of the new protein. Such medicines have resulted in useful cancer treatments.

The study of chromosome aberrations is important to provide useful biomarkers of exposure, dose, and disease. Understanding chromosome aberrations can result in a better understanding of genetics and the role that genetics plays in the induction of disease in exposed populations.

See also: Biomarkers, Environmental; Biomarkers, Human Health; Carcinogenesis; Radiation Toxicology, Ionizing and Nonionizing.

Further Reading

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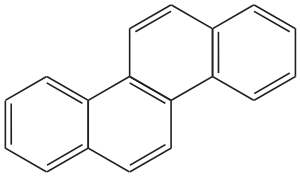
Chronic Toxicity *See* Toxicity, Chronic.

Chrysene

Linda A Malley

This article is a revision of the previous print edition article by Linda A Malley and David M Krentz, volume 1, pp. 344–346, © 1998, Elsevier Inc.

- Representative Chemicals: Polynuclear aromatic hydrocarbons
- Chemical Abstracts Service Registry Number: CAS 218-01-9
- Synonyms: 1,2,5,6-Dibenzonaphthalene; 1,2-Benzophenanthrene; 1,2-Benzphenanthrene; Benz(*a*)-phenanthrene; Benzo(*a*)phenanthrene
- Chemical/Pharmaceutical/Other Class: Polynuclear aromatic hydrocarbons
- Chemical Formula: $C_{18}H_{12}$
- Chemical Structure:



Uses

Chrysene is not produced commercially except for research purposes.

Background Information

Chrysene occurs as a product of combustion of fossil fuels and has been detected in automobile exhaust. Chrysene has also been detected in air samples collected from a variety of regions nationally and internationally. The concentrations were dependent on proximity to nearby sources of pollution such as traffic highways and industries, and was also dependent on seasons (generally higher concentrations were noted in winter months). Chrysene has also been detected in cigarette smoke and in other kinds of soot and smoke samples (carbon black soot, wood smoke, and soot from premixed acetylene oxygen flames). It has been detected as a component in petroleum products including clarified oil, solvents, waxes, tar oil, petrolatum, creosote, coal tar, cracked petroleum residue, extracts of bituminous coal, extracts from shale, petroleum asphalts, and coal tar pitch.

Exposure Routes and Pathways

Occupational exposure to chrysene may occur through inhalation of air contaminated with products of incomplete combustion and dermal contact with soot, motor oil, and coal tar. It has been detected in air samples from the following types of industrial operations and locations: coke ovens, furnaces for silicon carbide process plants, carbon anode plant, graphite plant, metal recycling plant, bitumen paving plant, aluminum refinery, smoking kilns for meat processing, and chimney sweeping. Chrysene has also been detected in surface water and soil samples and in a variety of cooked foods (particularly charcoal broiled/smoked); therefore, exposure to chrysene by ingestion is also possible. Dermal exposure to chrysene can also occur as a result of skin contact with soot or petroleum products.

Toxicokinetics

In general, polynuclear aromatic hydrocarbons (PAHs; the generic class name for chrysene) are highly soluble in lipids and adipose tissue and are expected to be readily absorbed by the dermal, oral, or inhalation routes of exposure.

Following oral administration in rats, peak concentration of chrysene occurred within 1 h in the blood and liver. Chrysene concentrated in the adipose and mammary tissue, and the majority of the dose was eliminated in the feces within 2 days.

In vitro rat liver and mouse skin preparations have been reported to metabolize chrysene to its 1,2-, 3,4-, and 5,6-dihydrodiols and to some monohydroxy derivatives including 1- and 3-phenols. In addition, the dihydrodiols have been reported to undergo further transformation to form 1,2-diol-3,4-epoxide and 3,4-diol-1,2-epoxide.

Mechanism of Toxicity

The primary toxic effect of concern for chrysene is carcinogenicity, which is most likely the result of the mutagenic activity of its metabolites, 1,2-dihydrodiol and 1,2-diol-3,4-epoxide. The 1,2-dihydrodiol and the 1,2-diol-3,4-epoxide have been shown to be mutagenic *in vitro* in bacterial and mammalian cells and have induced pulmonary adenomas when administered to newborn mice. In addition, the 1,2-dihydrodiol was active as a tumor initiating agent on mouse skin. DNA adducts in hamster cells resulting from a reaction of the DNA with 1,2-diol-3,4-expoxide have also been detected.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} of chrysene in mice, administered by intraperitoneal injection, is $320\,mg\,kg^{-1}$. Application of chrysene (0.1% in a petroleum hydrocarbon mixture known to have low embryotoxicity) to the eggshell of Mallard duck embryos resulted in embryotoxic and teratogenic effects in the ducklings. A single oral dose of chrysene administered to pregnant rats on day 19 of gestation induced hepatic P-450 enzymes in the fetal rat liver. In another study, chrysene induced benzo(*a*)pyrene hydroxylase activity in the placenta of pregnant female rats.

Human

As a general class of compounds, PAHs have low acute toxicity.

Chronic Toxicity (or Exposure)

Animal

The primary toxic effect elicited by chrysene is oncogenicity. Several studies have been conducted in mice in which chrysene (diluted in a variety of agents) was applied dermally either as a single dose (followed by a tumor promoting agent) or as multiple doses. Increased incidences of dermal tumors (papillomas and carcinomas) were observed in mice administered chrysene and a tumor promoting agent. Several studies were also conducted in which mice or rats received intramuscular or subcutaneous injections of chrysene

in various dilutants (single or multiple doses). Most of the treatment protocols resulted in an increased incidence of tumors at the injection site. In addition, male mice that were treated on days 1, 8, and 15 after birth with an intraperitoneal injection of chrysene had a higher incidence of pulmonary and liver tumors and lymphosarcomas compared to similarly treated controls.

Human

Chronic exposure to PAHs can produce a variety of effects. Exposure to the eyes can result in irritation and photosensitivity. Dermal exposure can result in erythema, burns, and 'coal tar warts' (precancerous lesions enhanced by ultraviolet light exposure). Inhalation exposure may cause irritation to the respiratory tract accompanied by cough and bronchitis. Oral exposure may produce a thickening and/or whitening of the oral mucous membranes. In addition to the local effects at the site of entry, systemic toxicity may occur, which could result in hepatic or renal effects. Some PAH compounds have been noted to cause hematological effects (anemia, leukopenia, and pancytopenia) in animals and suppression of selective components of the immune system.

Chrysene is classified as A2 (suspected human carcinogen) by the American Conference of Governmental Industrial Hygienists (ACGIH) and B2 (probable human carcinogen) by Environment Protection Agency (EPA) Integrated Risk Management System based on its carcinogenic effects in other species. Specific reports of toxicity or carcinogenicity in humans resulting from exposure to chrysene were not found. However, increased incidences of skin, bladder, and lung tumors and tumors of the gastrointestinal tract have been reported among workers exposed to PAHs.

In Vitro Toxicity Data

The potential for mutagenic effects was determined in bacterial, fungal, and mammalian cell systems *in vitro*. Although chrysene produced negative results in *Escherichia coli* and *Saccharomyces*, it produced positive results in *S. typhimurium* in TA100. In mammalian cells, chrysene produced positive effects in Syrian hamster embryo cells *in vitro*. Administration of chrysene to Chinese hamsters by intraperitoneal injection also produced increased sister chromatid exchanges in bone marrow cells. Increased aberrations were also noted in phase II oocytes collected from NMRI mice treated orally with chrysene. Chrysene induced aryl hydrocarbon hydroxylase in cultured human lymphocytes.

Clinical Management

Toxicity from PAH-containing compounds generally occurs following chronic exposure. Toxicity following acute ingestion is unlikely, and gastric decontamination is generally not necessary. For inhalation exposure, the patient is moved to a place with fresh air. If cough or difficulty breathing develops, oxygen is administrated and assisted ventilation provided as needed. Any broncho-spasm can be treated with inhaled beta-2-agonist and/or oral or parenteral corticosteroids or inhaled sympathomimetic agents. For eye exposure, eyes are irrigated with copious amounts of water for at least 15 min and then a follow-up evaluation should be made. For dermal exposure, the exposed area is washed with soap and water. If dermal hypersensitivity develops, reactions may require topical or systemic corticosteroids or antihistamines.

Since PAH compounds have been noted to cause hepatic, renal, and hematopoietic abnormalities, liver function tests, renal function tests, and a complete blood count are recommended for patients with chronic exposure. If tests indicate organ function abnormalities, supportive treatment should be undertaken. Treatment for cancer should follow standard therapeutic protocols for the type and location of the cancer.

Environmental Fate

Chrysene is expected to be immobile in soil and is not expected to volatilize from either moist or dry soil. Biodegradation rates in soil range from 77 to 387 days depending on soil type. In water, chrysene is expected to absorb to suspended solids and sediment. Bioconcentration in marine organisms is expected to range from low to high. Chrysene is not expected to undergo hydrolysis due to lack of hydrolyzable functional groups. In the atmosphere, chrysene is expected to exist in the particulate phase and may be physically removed by wet and dry depositions.

Ecotoxicology

The 96 h LC_{50} in fish was greater than 1 mg l^{-1}. The 24 h LC_{50} in amphibians was greater than 6.7 mg l^{-1}. The 24 h LC_{50} in insects was 1.7 mg l^{-1}. The 2 h LC_{50} in *Daphnia magna* was 1.9 mg l^{-1}.

Other Hazards

Chrysene emits acrid smoke and fumes when heated to decomposition.

Exposure Standards and Guidelines

Chrysene is classified as A2 (suspected human carcinogen) by the ACGIH and B2 (probable human carcinogen) by EPA Integrated Risk Management System based on its carcinogenic effects in other species.

Table 1 Summary of exposure criteria for chrysene

Agency	*Criteria*		*Averaging time*
ACGIH		A3 Confirmed animal carcinogen	NA
OSHA	PEL (TWA)	$0.2\,mg\,m^{-3}$	8 h/40 h week

OSHA, Occupational Safety and Health Administration; ACGIH, American Conference of Governmental Industrial Hygienists; PEL, permissible exposure limit; NA, not applicable.

Specific reports of toxicity or carcinogenicity in humans resulting from exposure to chrysene were not found. However, increased incidences of skin, bladder, and lung tumors and tumors of the gastrointestinal tract have been reported among workers exposed to PAHs (see **Table 1**).

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Polycyclic Aromatic Hydrocarbons.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Chrysene.

Ciguatoxin

David Elridge and Christopher P Holstege

This article is a revision of the previous print edition article by Gaylord P Lopez, volume 1, p. 346, © 1998, Elsevier Inc.

- Synonyms: Ichthyosarcotoxism; Ciguatera poisoning
- Chemical Formula: $C_{60}H_{86}O_{19}$
- Implicated Sources (Fish): Amberjack; Barracuda; Cinnamon; Coral trout; Dolphin; Eel; Emperor; Spanish mackerel; Surgeon fish; Grouper; Kingfish; Paddletail; Parrot fish; Red snapper; Reef cord; Sea bass; Swordfish; Yankee whiting

Background Information

Ciguatoxin is a lipophilic, heat-stable compound composed of multiple ether rings. Though overall a global problem that can be imported anywhere, this toxin is largely found in coral reef waters between 35° south to 35° north latitude.

Exposure Routes and Pathways

Ciguatoxin is produced by the dinoflagellate, *Gambierdiscus toxicus*, and related dinoflagellates. These microorganisms live attached to microalgae that are subsequently eaten by fish found in the area described above. This toxin is then stored in the viscera and flesh of the fish where it can remain for years. Ciguatoxin becomes progressively concentrated as one follows up the food chain (i.e., typically more toxin is found in larger, carnivorous fish). Human exposure may occur upon ingestion of these fish. Ciguatoxin is tasteless and odorless and does not degrade with cooking or freezing.

Toxicokinetics

After ingestion, the onset of signs and symptoms is highly variable and can range from 30 min to 30 h (average time is 6 h). The toxin may be found in the muscle, mucous, skin, and internal organs of fish. The highest concentration of toxin is typically found in the liver, gonads, and intestines.

Mechanism of Toxicity

Ciguatoxin binds tightly to voltage-sensitive sodium channels. This binding leads to increased opening of sodium channels and subsequent increased cell membrane permeability to sodium. As a result, the electrical potential of involved cells is altered.

Acute and Short-Term Toxicity (or Exposure)

Human

A multitude of signs and symptoms, predominantly neurological and gastrointestinal, can be seen following ingestion of ciguatoxin. In the early stages, nausea, vomiting, diarrhea, and abdominal pain are

common. Neurological abnormalities include tremors, tingling, and numbness of the lips and extremities, disturbance of temperature sensation (classically described as hot and cold reversal), and pruritus. Pain is common and is seen as myalgias, arthralgias, and toothaches. Bradycardia, hypotension, and respiratory failure may occur. Death is rare. Typically, the gastrointestinal symptoms resolve in 24–48 h while the neurological effects may last up to 2 months.

Chronic Toxicity (or Exposure)

Human

Humans exposed repeatedly to ciguatoxins have greater sensitivity compared to those with single exposures. Symptoms may be more pronounced and of greater duration in those with multiple exposures.

In Vitro Toxicity Data

Studies on human embryotic cells demonstrated that ciguatoxins target and bind to site 5 voltage gated sodium channels in both cardiac and muscle cells.

Clinical Management

Treatment is primarily symptomatic and supportive. Basic and advanced life support should be used as necessary. Bradycardia may be treated with IV atropine. Intravenous mannitol infusion has been advocated to alleviate neurological effects in the acute phase. Its utility, however, is controversial and has been questioned in recent studies. Exercise and the ingestion of nuts, grains, alcohol, seafood, opiates, and barbiturates have been reported to aggravate neurological signs and symptoms. Relief of chronic neurological effects has been described with the use of oral amitriptyline. Primary prevention consists of avoiding ingestion of characteristic fish.

See also: Atropine; Fish Consumption Advisory.

Further Reading

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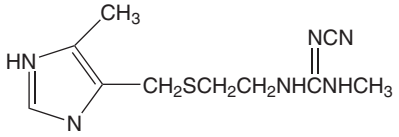
Geller RJ, Olson KR, and Senecal PE (1991) Ciguatera fish poisoning in San Francisco, California, caused by imported barracuda. *The Western Journal of Medicine* 156: 639–642.

Cimetidine

Michael D Reed

- Chemical Abstracts Service Registry Number: CAS 51481-61-9
- Synonyms: Numerous salts and brand names available; Tagamet
- Chemical/Pharmaceutical/Other Class: Histamine-2(H-2) receptor antagonist
- Chemical Formula: $C_{10}H_{16}N_6S$
- Chemical Structure:



Uses

Cimetidine is indicated for the treatment of disorders associated with hypersecretion of gastric acid, for example, gastric and duodenal ulcer disease, gastroesophageal reflux. The drug competitively antagonizes the H-2 receptor of the parietal cells.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to cimetidine. The drug is available as a parenteral formulation for intravenous administration and with improper dosing may result in acute toxicities.

Toxicokinetics

Cimetidine is rapidly and well absorbed (~70% bioavailability) after oral administration. The drug is

extensively distributed throughout the body with a V_d of $\sim 2\,l\,kg^{-1}$ and minimal protein binding, 13–25%. The vast majority of administered cimetidine is excreted unchanged via the kidney (70%) with ~15% undergoing hepatic metabolism and the remainder excreted via the bile. The drug's elimination half-life in patients with normal renal function is ~2 h. Cimetidine inhibits the activity of a number of cytochrome (CYP) P450 drug metabolizing enzymes, including CYP 1A2, CYP 2C9, and CYP 3A4. This interaction with these important human drug-metabolizing enzymes is responsible for a large number of clinically important metabolic-based drug–drug interactions.

Mechanism of Toxicity

Blockade of the cardiac H-2 receptors is the postulated mechanism for the cardiovascular toxicity associated with cimetidine overdosage. Cimetidine penetrates the blood–brain barrier and is associated with central nervous system effects in predisposed individuals, including elderly, and patients with poor renal function.

Acute and Short-Term Toxicity (or Exposure)

Human

Adverse effects associated with cimetidine overdose are rare. Patients are usually asymptomatic or experience minor to moderate gastrointestinal side effects/discomfort. Serious effects are rare with only case report descriptions of possible cimetidine-associated tachycardia. Rapid intravenous cimetidine administration in seriously ill patients has resulted in hypotension, bradycardia, and cardiac arrest. Large therapeutic or an overdose in patients with poor renal function, particularly the elderly, have resulted in central nervous system effects including confusion, delirium, hallucinations, and slurred speech.

Chronic Toxicity (or Exposure)

Animal

Rats given cimetidine at doses up to 4–48 times the recommended human dose over 2 years developed a slight increase in the incidence of benign Leydig cell tumors compared with the control group. There is no evidence of impaired mating or fertility in rabbits, rats, or mice at doses up to 40 times the human dose.

Human

Chronic exposure to cimetidine may be associated with gynecomastia in males and galactorrhea in females, possibly resulting from the drug's affinity for the androgen receptors combined with CYP inhibition of estradiol hydroxylation. Reductions in sperm count and impotence have been described in men chronically receiving the drug.

In Vitro Toxicity Data

Studies of radioprotective effects of cimetidine have demonstrated reduced frequency of radiation-induced micronuclei and chromosomal aberrations at various doses in rat bone marrow cells.

Clinical Management

Though the clinical need for such measures would be expected to be rare, basic and advanced life-support measures as well as aggressive decontamination may be instituted as clinically necessary. Gastric decontamination with a single dose of activated charcoal will effectively adsorb ingested cimetidine.

See also: Charcoal; Cytochrome P-450; Gastrointestinal System.

Further Reading

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MacMahon B, Bakshi M, and Walash MJ (1981) Cardiac arrhythmias after intravenous cimetidine. *New England Journal of Medicine* 305: 832–833.

Price W, Coli L, and Brandstetter RD (1985) Ranitidine-associated hallucinations. *European Journal of Clinical Pharmacology* 29: 375–376.

Ciprofloxacin

Teresa Dodd-Butera and Molly Broderick

- Chemical Name: 1,4-Dihydro-1-cyclopropyl-1,6-fluoro-4-dihydro-oxo-7-(1-piperazinyl)-3-quinolinehydroxycarboxylic acid
- Chemical Abstracts Service Registry Number: CAS 85721-33-1
- Chemical/Pharmaceutical/Other Class: Synthetic broad spectrum antibiotic
- Chemical Structure: $C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$. Ciprofloxacin has a fluorine atom at the 6-position, a piperazine moiety at the 7-position, and a cyclopropyl ring at the 1-position

Uses

Ciprofloxacin hydrochloride is a fluoroquinolone antimicrobial used in the treatment of infections from a wide range of aerobic Gram-positive and aerobic Gram-negative microorganisms. It has been shown to be effective against the following: inhalational anthrax, some types of respiratory infections, urinary tract infections, typhoid fever, gonorrhea, and septicemia. It is used as a secondary agent in the treatment of tuberculosis and has been used, occasionally, for conditions associated with cystic fibrosis.

Exposure Routes and Pathways

Ciprofloxacin is available in oral dosage forms, both pills and suspension. It may also be administered intravenously.

Toxicokinetics

Ciprofloxacin is well absorbed in the gastrointestinal tract. Serum concentration peaks in 1.5–2 h postingestion. The elimination half-life with normal renal function is 4 h. Ciprofloxacin is 20–40% bound to serum proteins and distributed widely throughout the body. Four metabolites in human urine have been identified, which account for ~15% of an oral dose. The metabolites have antimicrobial activity, which are less active than the parent compound of ciprofloxacin. Ciprofloxacin inhibits CYP3A4 enzyme system.

Mechanism of Toxicity

Ciprofloxacin acts by inhibiting the bacterial enzymes DNA gyrase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ciprofloxacin has produced tonic and clonic seizures in high doses in animal studies. In addition, areas of retinitis were noted on investigation of the effects of the drug to the eyes of rabbits; however, this was not a consistent finding in all studies. Hypotensive episodes were found with rapid administration.

Human

Nausea, vomiting, and diarrhea are most common from the oral dosage form. Central nervous system effects have been reported with intravenous administration, including increased intracranial pressure, dizziness, and convulsions. Local skin irritation at the intravenous site may also occur with rapid infusion of the drug. Rarely, cardiovascular symptoms have also been reported. All routes of exposure should be avoided in persons who are sensitive to the hypersensitive effects of ciprofloxacin or any other quinolones, as allergic reactions are possible. Interstitial nephritis has been attributed to hypersensitivity. Ciprofloxacin decreases the clearance of the drug, theophylline. Fatal reactions have been reported when these two drugs are concurrently administered. Theophylline levels must be monitored if the drugs are given together.

Chronic Toxicity (or Exposure)

Animal

Crystalluria and secondary nephropathy were noted, especially under conditions of alkaline urine. No impairment of fertility was noted and no congenital defects could be directly related to the administration of the drug.

Human

Abnormalities of liver, renal, and hematological parameters have been reported. As with other antibiotic regimens, pseudomembranous colitis may occur during or after treatment. Fluoroquinolones have the potential to cause adverse effects on developing cartilage and bone; thus, ciprofloxacin should be used with caution in pregnant women and young children.

Clinical Management

Symptomatic and supportive treatment is recommended, as is monitoring liver, renal, and hematological

parameters and drug levels especially with concurrent administration of other agents.

See also: Gastrointestinal System.

Further Reading

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Zimpfer A, Propst A, Mikuz G, *et al.* (2004) Ciprofloxacin-induced acute liver injury: Case report and review of literature. *Virchows Archiv* 444(1): 87–89.

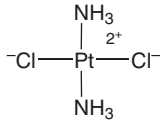
Relevant Website

http://www.fda.gov – US Food and Drug Administration.

Cisplatin

Linda A Malley

- Representative Chemicals: Platinum compounds
- Chemical Abstracts Service Registry Number: CAS 15663-27-1
- Synonyms: Biocisplatinum; CDDP; *cis*-DDP; *cis*-Diaminodichloroplatinum(II); *cis*-Diaminedichloroplatinum; *cis*-Platin; *cis*-Platinous diaminodichloride; *cis*-Platinous diamine dichloride; *cis*-Platinum; *cis*-Platinum diaminodichloride; *cis*-Platinum(II) diaminedichloride; Cisplatyl; Diaminedichloroplatinum; Neoplatin; NSC-119875; Platiblastin; Platinol
- Chemical Formula: $C_{12}H_6N_2Pt$
- Chemical Structure:



Uses

Cisplatin is primarily used for the treatment of a variety of malignancies.

Background Information

Cisplatin is a solid, ranging in color from white to yellow; and is soluble in water.

Exposure Routes and Pathways

Cisplatin is only available for intravenous use. It is generally supplied in vials as a solution or as a lyophilized powder. The possibility exists for dermal, oral, or inhalation exposure during production, and during preparation of dosing formulations.

Toxicokinetics

Following an intravenous injection, several mammalian species demonstrate a similar general organ distribution. Cisplatin is rapidly distributed to all tissues, followed within the first hour by an accumulation in kidneys, liver, skin, bone, ovaries, and uterus. Approximately 60–80% is excreted in the urine within 24 h. However, up to 4 weeks after a single dose, platinum is still detectable in kidneys, liver, skin, and lung. Following a single oral dose of cationic platinum, little absorption occurred and almost the entire dose was excreted in the feces, indicating that it would be unlikely for significant absorption to occur following oral administration of cisplatin.

The chloride atoms of the molecule may be displaced directly by reaction with neucleophiles such as thiol groups. However, hydrolysis of the chloride ion may also occur and may be responsible for formation of an active metabolite, which then reacts with nucleic acids and proteins. Investigation of metabolism of cisplatin in rat liver and kidneys following an intraperitoneal dose was conducted over a 24 h period. Maximum platinum concentrations in kidney cortex and medulla were reached within 1 h after dosing. In addition, the parent compound and five platinum-containing metabolites were present at 1 h postdosing, with cisplatin being the primary species detected. Similarly, there were five platinum-containing metabolites

detected in the liver at 1 h postdosing; however, in contrast to the kidneys, cisplatin was not the primary species detected in the liver.

Mechanism of Toxicity

Cisplatin reacts with nucleosides and nucleic acids and can cross-link cellular DNA. The effects on cross-linking with DNA appear to differ among cell type; however, the effects on cross-linking are most pronounced during the S-phase of the cell cycle. In addition, cisplatin inhibits a number of enzymes that contain a catalytically active sulfydryl group. Ribonucleotide reductase is extremely sensitive to the effects of cisplatin, with greater than 90% inhibition observed *in vitro* in the presence of a two-molar excess of cisplatin. The inhibition was nearly instantaneous and was irreversible.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the oral LD_{50} was $20\,mg\,kg^{-1}$, the intravenous LD_{50} was $8\,mg\,kg^{-1}$, and the intramuscular LD_{50} was $9200\,\mu g\,kg^{-1}$. The toxic effects observed in rats included leukopenia, decreased numbers of circulating platelets, lymphoid depletion, intestinal epithelial injury, bone marrow depression, and sloughing of the renal tubular epithelium. The LD_{50} in mice following a single intraperitoneal injection was $13.0\,mg\,kg^{-1}$, and following a single intravenous injection the LD_{50} was $13.36\,mg\,kg^{-1}$ for males and $12.32\,mg\,kg^{-1}$ for females. The minimum lethal dose for dogs was a single intravenous injection of $2.5\,mg\,kg^{-1}$ or five daily consecutive injections of $0.75\,mg\,kg^{-1}$. Toxic symptoms in dogs included severe hemorrhagic enterocolitis, severe damage to the bone marrow and lymphoid tissue, and marked renal necrosis. Occasionally, pancreatitis was also observed in dogs. In monkeys, the minimum lethal dose was five daily intravenous injections of $2.5\,mg\,kg^{-1}$. Toxic effects observed in monkeys included nephrosis, myocarditis, and some degeneration of spermatogenic cells.

Cisplatin ($13\,mg\,kg^{-1}$) administered to pregnant mice by intraperitoneal injection on gestation day 8 was lethal to all the fetuses. A dose of $8\,mg\,kg^{-1}$ cisplatin was lethal to 98% of the fetuses, and a dose of $3\,mg\,kg^{-1}$ was lethal to 31% of the fetuses. Surviving fetuses exhibited growth retardation and minor skeletal anomalies.

Female rats were administered twice weekly intraperitoneal injections of cisplatin for a cumulative dose of 15 or $34\,mg\,kg^{-1}$. Following the last dose, sensory and motor nerve conduction velocities were determined. Both doses of cisplatin significantly decreased sensory nerve conduction velocity. In addition, the level of cisplatin DNA binding in dorsal root spinal ganglion satellite cells equaled that in liver cells; however, the level of cisplatin DNA binding in spinal cord and brain was very low.

Rats were administered a single intravenous dose of $6\,mg\,kg^{-1}$ cisplatin, which was either preceded (30 min prior) or was followed by (30 or 60 min) $500\,mg\,kg^{-1}$ of reduced glutathione. The reduced glutathione, administered 30 min before or 30 min after the cisplatin, offered significant protection from toxicity and did not interfere with the antitumor effectiveness of cisplatin in a tumor model. However, in mice, the protective effect of reduced glutathione was only partial for some strains.

Human

Cisplatin is corrosive to the skin, and dusts cause eye and respiratory irritation. Renal dysfunction is the major toxic effect of this drug. It reduces the single-nephron glomerular filtration rate and causes a back leak of inulin across the renal tubule. In addition, myelosuppression, peripheral neuropathy (paraesthesias), central extrapyramidal disorders, loss of deep tendon reflexes, metabolic acidosis, headaches, taste disturbance, retrobulbar neuritis, seizures, ototoxicity, nausea, vomiting, diarrhea, thirst, metallic taste, leukopenia, allergic reactions, azotemia, hypokalemia, hypophosphatemia, hypocalcemia, and hypomagnesemia are commonly reported side affects of treatment with cisplatin. Some patients have also experienced anaphylactic reactions to treatment with platinum-containing compounds.

Chronic Toxicity (or Exposure)

Animal

Studies in animals indicate that cisplatin increases the occurrence of tumors. Cisplatin was administered once weekly by intraperitoneal injection to mice for 10 weeks for a total dose of $108\,mol\,kg^{-1}$. Cisplatin-treated mice had a significantly higher incidence (100%) of pulmonary adenomas compared to similarly treated control mice (26%). In another study, mice received weekly intraperitoneal injections of cisplatin 1.62 mg in $5\,ml\,kg^{-1}$ saline for 16 weeks, followed by dermal application of croton oil, dermal application of croton oil alone (control), or saline alone (control). Mice treated with croton oil and cisplatin had a higher incidence of skin papillomas compared to cisplatin alone or the control groups.

Human

Cisplatin caused azoospermia in humans within 2 months after initiation of treatment. Recovery of sperm counts occurred in most patients within 1–2 years after cessation of treatment. Cisplatin administered in combination with etopsoside is carcinogenic in humans.

In Vitro Toxicity Data

Cisplatin was mutagenic in *Salmonella typhimurium* strains G46/pkM101, TA100, and TA98, without metabolic activation. Cisplatin also induced increased mutations in Chinese hamster ovary cells and V79 Chinese hamster cells. Postreplication repair was induced in V79 cells and in HeLa cells; and sister chromatid exchanges were induced in V79 cells. Chromosomal damage and sister chromatid exchanges were also induced by cisplatin in human lymphocyte cultures. Similar to the *in vitro* data, intraperitoneal injection of mice with 13.85 mg kg^{-1} cisplatin induced a significant increase in sister chromatid exchanges and in chromosome aberrations.

Clinical Management

Nephrotoxicity may be prevented or diminished by prehydration with 2 l of normal saline administered over a 6–8 h period, followed by continued hydration during and after the cisplatin infusion. Nausea and vomiting may be managed with antiemetics. Electrolyte concentration should be monitored and supplemented as needed. Treatment for an anaphylactic reaction would include antihistamines, administered with or without epinephrine. If accidental exposure to the eyes or skin occurs, the affected skin area should be washed thoroughly with soap and water, and eyes should be flushed with copious amounts of tepid water for at least 15 min. Seizures should be treated with diazepam, lorazepan, phenobarbital, or phenytoin.

Environmental Fate

During production, *cis*-diaminedichloroplatinum is released, which could leach through soil. Abiotic or biotic processes can convert this to an ionic species, which could enhance its adsorption to soil. *cis*-Diaminedichloroplatinum will slowly convert to *trans*-diaminedichloroplatinum in water. This form will remain dissolved unless it precipitates to the sediment or is adsorbed to suspended particulates.

Ecotoxicology

Toxicity of cisplatin to aquatic organisms was not reported.

Other Hazards

Cisplatin is incompatible in solutions having low chloride content. Cisplatin interacts with aluminum, and only administration equipment that does not contain aluminum should be used for this medication. When heated to decomposition, toxic fumes of hydrogen chloride and nitrogen oxide are emitted.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists recommends an 8 h time-weighted average of 0.002 mg m^{-3}.

See also: Platinum.

Further Reading

Barnes KR and Lippard SJ (2004) Cisplatin and related anticancer drugs: Recent advances and insights. *Metal Ions in Biological Systems* 42: 142–177.

US Department of Health & Human Services/National Toxicology Program; Tenth Report on Carcinogens. *cis*-Diaminedichloroplatinum (15663-27-1).

Clean Air Act (CAA), US

Robert Kapp

- TITLE: CAA
- AGENCY: US Environmental Protection Agency (EPA)
- YEAR ENACTED: 1970; amended 1977, 1990, and 1997

Background

The first US federal legislation dealing with controlling air pollution at its source was Public Law 84-159, the Air Pollution Control Act of 1955. The legislation granted $5 million annually for five years for research

by the Public Health Service. While the act did not prevent any air pollution, it did provide research and technical assistance to air pollution control efforts. In 1960, the Act was amended to extend research for four additional years. In 1962, the Act was further amended to add research by the US Surgeon General to determine the health effects of various motor vehicle exhaust substances.

The Clean Air Act of 1963 (Public Law 88-206) was passed to improve, strengthen, and accelerate programs for the prevention and abatement of air pollution. This legislation granted $95 million over a 3 year period to state and local governments and air pollution control agencies to conduct research and create control programs. This Act encouraged the development of emissions standards for motor vehicles and from stationary sources, and it led to research on the removal of sulfur from high sulfur coal and oil fuels. The Act was amended in 1965 to establish standards for automobile emissions. It was further amended in 1966 and 1967 to expand local air pollutions control programs. The 1967 Amendment established national emissions standards for stationary sources and created Air Quality Control Regions as a means of monitoring ambient air. The states were given fixed timetables in which to implement State Implementation Plans to meet emission standards.

Overview of Clean Air Act

The Clean Air Act (CAA) is the federal law designed to assure that the air is safe to breathe. While public health is the primary goal, the Act also seeks to prevent environmental damage caused by air pollution. The fundamentals of the CAA were set up in the Clean Air Act of 1970 and then were amended several times. The basic framework of the Act and the objective of public health have remained intact.

The Clean Air Act of 1970 (Public Law 81-604) essentially rewrote the original Clean Air Act of 1963, by making it a more effective program to improve the quality of the ambient air. The legislation set ambitious National Ambient Air Standards to protect the public health with six 'criteria' pollutants, which included:

1. carbon monoxide,
2. nitrogen dioxide,
3. ozone,
4. sulfur dioxide,
5. particulate matter with aerodynamic size less than or equal to 10 μm (PM_{10}), and
6. lead.

The CAA also set New Source Performance Standards that strictly regulated emissions of any new sources of air pollution entering an area. It also established two categories of air-quality standards: Primary Standards set limits to protect public health, and Secondary Standards set limits to protect against public welfare effects, such as damage to farm crops and vegetation. The CAA further required leaded gasoline be phased out by the mid-1980s. In addition, the Act allowed citizens the right to take legal action against anyone or any organization, including government itself, who was in violation of the emissions standards.

In 1977, this Act was amended to extend the deadline of meeting the motor vehicle emissions standards. These amendments also made a first attempt to control stratospheric ozone and created the New Source Review, which required older 'grandfathered' facilities to install pollution control technologies as they modernized.

In 1990, the Clean Air Act was again amended and rewritten (Public Law 101-549). These amendments extended the prohibition of leaded gasoline to 1995. However, additional changes drastically strengthened the measures for attaining air quality standards provided in the CAA including:

- Provisions relating to mobile pollution sources
- Expanding the regulation of hazardous air pollutants
- Requiring substantial reductions in power plant emissions for control of acid rain (SO_2 and NO_x abatement). Utilities had the choice of using any of the following ways to meet the standard annual emissions allowance limit:
  - using cleaner fuel or choosing lower sulfur coal or fuel blending;
  - obtaining additional allowances;
  - installing flue gas desulfurization equipment (scrubbers);
  - using previously implemented controls;
  - retiring units;
  - boiler repowering;
  - establish operating permits for all major sources of air pollution;
  - establish provisions for stratospheric ozone protection; and
  - expand enforcement powers and penalties.

Section 112 of the CAA provides a list of 189 hazardous air pollutants for which the Environmental Protection Agency (EPA) must establish emissions standards for sources that emit any listed pollutant. There was considerable debate concerning the costs of emissions control, which came to a head in 1986

when CAA issued a standard for vinyl chloride. This standard was set aside by the courts; however, the court case (Natural Resources Defense Council, Inc. vs. United States Environmental Protection Agency, 1987) set a precedent by recognizing that the EPA could, in fact, consider costs in deciding if any additional margin of safety was necessary.

The 1990 amendments replaced the health-based standard with a two-tiered system of regulation. With this system, EPA must first issue standards that are technology based, designed to require the maximum achievable control technology (MACT) available. If the MACT values are insufficient to protect human health with an 'ample margin of safety', EPA must issue residual risk standards as well. These amendments define a sufficient margin of safety for carcinogens by requiring EPA to establish residual risk standards for any pollutant that poses a lifetime excess cancer risk of greater than 1:1 000 000.

In 1997, the Act was again amended to tighten the permissible ozone levels from 0.12 ppm per 1 h to 0.08 ppm per 8 h. In addition, the amendment revised the 24 h particulate matter up to 10 μmin diameter (PM_{10}) to simplify data handling requirements. Finally, new particulate matter up to 2.5 μm ($PM_{2.5}$) standards with an annual limit of 15 $\mu g\,m^{-3}$ were also added to the Act.

See also: Environmental Protection Agency, US; Environmental Toxicology; Pollution, Air.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency (EPA), Clean Air Act information.

Clean Water Act (CWA), US

Robert Kapp

- Agency: US Environmental Protection Agency (EPA)
- Year of Enactment: 1948, reauthorized 1972; amended 1977–83, 1987, 1988, 1990–92; 1994, 1995, and 1996; reauthorized in 1997

Background

Federal legislation on water began when Congress enacted the River and Harbor Act of 1886 that was recodified in the Rivers and Harbors Act of 1899. The Federal Water Pollution Control Act is a comprehensive statute aimed at restoring and maintaining the chemical, physical, and biological integrity of the US water supply. It was originally enacted in 1948. The Water Pollution Control Act Amendments of 1956 strengthened the enforcement by no longer requiring the federal government to receive consent from the States. The Water Quality Act of 1965 (Public Law 89-234) provided for the setting of enforceable water quality standards and the basis for interstate water quality standards. The Clean Water Restoration Act of 1966 (Public Law 89-753) imposed fines ($100 per day) on polluters who failed to submit a required report. The Water Quality Improvement Act of 1970 (Public Law 91-224) further expanded federal authority to certify water quality. These various amendments created cumbersome legislation that was, at best, difficult to implement. Growing public concern for controlling water pollution in the environment led the enactment of the Federal Water Pollution Control Act Amendments of 1972 (Public Law 92-500). Subsequently, this law was amended in 1977 and became known as the Clean Water Act.

Overview of Clean Water Act (CWA)

In a step toward resolving numerous administrative and implementation problems with the previous water pollution legislation, the 1972 amendments to the Federal Water Pollution Control Act restructured water pollution control under the authority of the Administrator of the US EPA. The objective of the 1972 reauthorization was to restore and maintain the chemical, physical, and biological integrity of the nation's waters. Initially there were two critical goals:

1. The elimination of the discharge of all pollutants into the navigable waters by 1985.
2. The creation of an interim level of water quality to provide for the protection of fish, shellfish, and wildlife and recreation by July 1, 1983.

The 1972 legislation also required federal effluent limitations and state water quality standards,

required permits for the discharge of pollutants into navigable water, provided enforcement mechanisms, authorized funding for wastewater treatment works, and provided construction grants to states and tribes for their water quality programs. The 1972 legislation changed the enforcement from regulating the quality of existing water to regulating the amount of effluents being discharged from particular point sources. (Point source is defined as "any discernible, confined, and discrete conveyance from which pollutants are or may be discharged.")

The EPA Administrator originally published guidelines for 63 chemicals and many other materials – sewage, garbage, dirt, discarded equipment, even heat – that could not be indiscriminately dumped in the water. The Act was amended in 1977 (Public Law 95-217) to include 126 materials, which EPA had identified as toxic under newly-developed health-based 'water quality criteria'. The Act also addressed previously unrecognized but widespread sources of water pollution such as municipal storm water, and new sources such as land application of manure from Confined Animal Feeding Operations. To meet this challenge, the most recent water act, the 1998 National Pollutant Discharge Elimination System focused not just on waterways but on watersheds. The Act authorized numerous research programs to study the prevention, reduction and elimination of water pollution. The act authorized the development of plans for the control of pollution within all or any part of the watersheds of the Great Lakes. In 1992, it authorized EPA to conduct a comprehensive survey of data on aquatic sediment quality and report the findings to Congress. The Act prohibited the discharge of pollutants except those in compliance with the effluent limitations with the best practicable control technology.

The Act provided for construction grants and loans to publicly owned treatment works (POTWs) to implement improved water pollution control measures. Another significant feature of the Act was the creation of a national pollutant discharge elimination system (NPDES). The NPDES required POTWs as well as industrial sources to acquire a permit that mandated certain effluent limitations had to be met before any discharges could occur in navigable waters. Other water pollution issues covered by the Act included the Clean Lakes Program, thermal discharges, non-point source pollution, estuaries, marine sanitation devices, oil and hazardous substance liability, and sewage sludge.

The pollutants regulated under the CWA include biochemical oxygen demand; fecal coliform; total suspended solids, oil and grease; and pH ('conventional pollutants'). Also included in the CWA are 'priority pollutants', that is, toxic pollutants as well as 'nonconventional pollutants' not identified as either conventional or priority.

The critical requirements of the CWA include the following:

1. Direct discharges from 'point source' limitations. Point sources include sewers, pipes, drainage ditches, etc. Any facility that intends to discharge into a lake or stream or river must obtain a permit prior to initiating the discharge. The discharge must meet conditions and effluent limitations set by the state and/or EPA.
2. Pretreatment requirements for indirect discharges into POTWs must meet pretreatment requirements as set forth in 40 CFR 403.6 National Pretreatment Standards: Categorical Standards. Effluent guidelines for direct discharges and pretreatment standards for specific chemical industry manufacturers and users are listed in the Code of Federal Regulations as follows:
   - 40 CFR 414 Organic Chemicals, Plastics, and Synthetic Fibers;
   - 40 CFR 415 Inorganic Chemicals Manufacturing;
   - 40 CFR 417 Soap and Detergent Manufacturing;
   - 40 CFR 418 Fertilizer Manufacturing;
   - 40 CFR 422 Phosphate Manufacturing;
   - 40 CFR 428 Rubber Manufacturing;
   - 40 CFR 446 Paint Formulation;
   - 40 CFR 447 Ink Formulation;
   - 40 CFR 454 Gum and Wood Chemicals Manufacturing;
   - 40 CFR 455 Pesticide Chemicals;
   - 40 CFR 457 Explosives Manufacturing; and
   - 40 CFR 458 Carbon Black Manufacturing.
3. Storm water runoffs were addressed in the 1987 CWA Amendments. These regulations required that manufacturers with any sort of storm sewer connected with any aspect of the chemical process apply for a permit under these conditions: (1) a discharge is associated with industrial activity; (2) a discharge from a large or medium municipal storm sewer system; or (3) a discharge, which has been determined to contribute to a violation of any water standard or is a significant contributor of pollutants to waters of the nation. These specific regulations are located in 40 CFR 122.26.
4. Oil and hazardous substance spill prevention and responses are generally incorporated into the Comprehensive Environmental Response, Comprehension, and Liability Act and EPCRA regulations. However, the Spill, Prevention, Control and

Countermeasure Plan applies to any facility that has oil or hazardous materials that has the potential to reach the nation's waters. These regulations are located in 40 CFR 112.

5. Wetlands modifications and/or the placement of dredge and fill materials into surface waters is covered by a permit program administered by the US Army Corp of Engineers. The CWA defines surface waters to include wetlands, hence, activities that involve any modification of wetlands are covered by the US Army Corps of Engineers. The regulations governing this permit program are located in 40 CFR 404.

See also: Comprehensive Environmental Response, Compensation, and Liability; Effluent Biomonitoring; Environmental Toxicology; Pollution, Water; Safe Drinking Water Act, US; Toxicity Testing, Aquatic.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency, Clean Water Act.

Clinical Chemistry

Shayne C Gad

The function of clinical chemistry in toxicology (as well as in human and veterinary medicine) is to provide, via laboratory analysis, evaluations of the qualitative and quantitative characteristics of specific endogenous chemical components present in samples of blood, urine, feces, spinal fluid, and tissues. The purpose is to help identify abnormal or pathological changes in organ system functions. The most common specimens used in clinical chemistry are blood and urine, and many different tests exist to test for almost any type of chemical component in blood or urine; for example, blood glucose, electrolytes, enzymes, hormones, lipids (fats), other metabolic substances, and proteins. The tests used were all initially applied to human clinical medicine, and may not possess the same utility when performed as part of nonclinical toxicity studies in a wide variety of other species.

Clinical chemistry evaluations are commonly recommended in animal toxicology studies. Regulatory agencies such as the US Food and Drug Administration and the US Environmental Protection Agency have set guidelines for clinical pathology testing in nonclinical toxicity and safety studies. Measurement of chemical components of biological fluids allows the toxicologist to do serial sampling, detect metabolic injury or organ-specific effects, and perhaps gain additional information helpful in establishing the no effect level and determining the mechanism of toxicity. When using serum enzymes as markers of tissue or organ damage, the enzyme of interest must reasonably reflect pathological change in a specific tissue, organ, or group of organs and must be easily measured.

The tests that are routinely performed provide information concerning hepatocellular and biliary integrity and function, renal function, carbohydrate, protein and lipid metabolism, and mineral and electrolyte balance. Modern analytical techniques require only small sample volumes to make accurate determinations, allowing in-life evaluations of effects in rats and larger species at multiple times during the course of a study without compromising animal health.

Table 1 summarizes the commonly measured endpoints and the probable causes behind findings.

See also: Toxicity, Chronic; Toxicity, Subchronic.

Further Reading

Burtis CA and Ashwood ER (1999) *Tietz Textbook of Clinical Chemistry*, 3rd edn. Philadelphia: Saunders.

Relevant Websites

http://www.aacc.org – American Association for Clinical Chemistry (AACC) website. Also considering accessing the AACC Listservs

http://www.clinchem.org – Clinical Chemistry: International and Journal of Molecular Diagonistics and Laboratory Medicine.

http://www.e-c4.org – European Communities Confederation of Clinical Chemistry and Laboratory Medicine.

Table 1 Association of changes in biochemical parameters with actions at particular target organs

Parameter	*Blood*	*Heart*	*Lung*	*Kidney*	*Liver*	*Bone*	*Intestine*	*Pancreas*	*Notes*
Albumin				↓	↓				Produced by the liver. Very significant reductions indicate extensive liver damage
ALP					↑	↑	↑		Elevations usually associated with cholestasis. Bone alkaline phosphatase tends to be higher in young animals
Bilirubin (total)	↑				↑				Usually elevated due to cholestasis, either due to obstruction or hepatopathy
BUN				↑	↓				Estimates blood filtering capacity of the kidneys. Does not become significantly elevated until the kidney function is reduced 60–75%
Calcium				↑					Can be life threatening and result in acute death
Cholinesterase				↑	↓				Found in plasma, brain, and RBC
CPK		↑							Most often elevated due to skeletal muscle damage but can also be produced by cardiac muscle damage. Can be more sensitive than histopathology
Creatinine				↑					Also estimates blood-filtering capacity of kidney as BUN does
Glucose								↑	Alterations other than those associated with stress are uncommon and reflect an effect on the pancreatic islets or anorexia
GGT					↑				Elevated in cholestasis. This is a microsomal enzyme and levels often increase in response to microsomal enzyme induction
HBDH		↑			↑				
LDH		↑	↑	↑	↑				Increase usually due to skeletal muscle, cardiac muscle, or liver damage. Not very specific
Protein (total)				↓	↓				Absolute alterations are usually associated with decreased production (liver) or increased loss (kidney). Can see increase in case of muscle 'wasting' (catabolism)
SGOT		↑		↑	↑			↑	Present in skeletal muscle and heart and most commonly associated with damage to these
SGPT					↑				Elevations usually associated with hepatic damage or disease
SDH					↑↓				Liver enzyme that can be quite sensitive but is fairly unstable. Samples should be processed as soon as possible

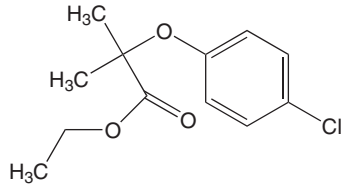
↑: increase in chemistry values; ↓: decrease in chemistry values.

ALP, alkaline phosphatase; BUN, blood urea nitrogen; CPK, creatinine phosphokinase; GGT, gamma glutamyl transferase; HBDH, hydroxybutyric dehydrogenase; LDH, lactic dehydrogenase; RBC, red blood cells; SDH, sorbitol dehydrogenase; SGOT, serum glutamic oxaloacetic transaminase (also called AST (aspertate amino transferase)); SGPT, Serum glutamic pyruvic transaminase (also called ALT (atanine amino transferase)).

Clofibrate

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 637-07-0
- Synonyms: 2-(*p*-Chlorphenoxy)-2-methylpropionic acid ethyl ester; α-*p*-Chlorophenoxyisobutyryl ethyl ester; Amotril; Angiokapsul; Anparton; Antilipid; Ateculon; Ateriosan; Atheropront; Atromid; Atromidin; Hyclorate; Lipavil; Liponorm; Liporil; Lipofaction; Neo-Atromid; Normet; Regelan; Serotinex
- Chemical/Pharmaceutical/Other Class: Antihyperlipoproteinemic agent
- Chemical Structure:



Uses

Clofibrate is a drug used to lower plasma concentration of very low density lipoprotein and also to lower plasma triglyceride concentration.

Background Information

Clofibrate, a well-known hypolipidemic agent, has drawn attention over a past few years with regard to its efficacy in coronary artery disease. It has been shown to exert protective influence against the gross morbid effects in experimental myocardial infarction, development of new infarction, and sudden death in clinical cases. Furthermore, it has recently been reported to reduce incidence and severity of arteriosclerosis, an important risk factor in precipitating myocardial infarction. However, the enthusiasm for prophylactic use of clofibrate has received a major setback following a 5 year US multicenter study report regarding failure of clofibrate in reducing or preventing mortality in cardiovascular disorders.

Exposure Routes and Pathways

Accidental overdose or ingestion is the most common exposure pathway.

Toxicokinetics

Clofibrate is rapidly and completely absorbed after oral administration. It is hydrolyzed to clofibric acid during absorption and in its passage through the liver. The acid binds strongly to plasma proteins. Sixty percent of it is excreted as glucuronide conjugate in the urine. Some is secreted into the bile and reabsorbed. The plasma elimination half-life is 25 h. Clofibrate appears in plasma as *p*-chlorophenoxyisobutyric acid. An acyl-linked metabolite of clofibrate has been identified in human urine.

Mechanism of Toxicity

Clofibrate acyl glucuronide is an electrophilic metabolite that reacts with sulfhydryl groups and causes hepatotoxicity.

Acute and Short-Term Toxicity (or Toxicity)

Animal

Rats given food containing 1% clofibrate or subcutaneous injections of 0.3–0.9 g kg^{-1} daily for 2 days showed spontaneous electromyographic responses in hind limbs. Clofibrate has induced relative enlargement of the liver in proportion to the body of newborn rats as well as an abnormal postnatal fetal thrombosis syndrome. The postnatal thrombosis consisted of an extension of the normal thrombosis in the umbilical arteries, and this caused necrosis of the tail or parts of the hindlimbs. Reproduction studies in both dogs and monkeys using clofibrate dosages approximately four to six times the usual human dosage have demonstrated arrest of spermatogenesis.

Human

Nausea, diarrhea, skin rash, alopecia, weakness, flu-like syndrome, and severe muscle cramps are symptoms of acute toxicity.

Chronic Toxicity (or Exposure)

Animal

Clofibrate causes hepatic tumors in rodents. Fifteen male Fischer 344 rats, weighing 84–100 g, were fed clofibrate at dietary concentration of about 250 mg kg^{-1} body weight per day in ground rat chow for up to 28 months. One or more hepatocellular

carcinomas developed in 10/11 rats, compared with 0/14 controls. Five of the animals showed metastasis. In addition, pancreatic exocrine acinar carcinomas were found in 2/11 rats, a dermatofibrosarcoma in one rat, and a leiomyoma of the intestine in one rat in the clofibrate-fed group. No such tumors were seen in controls.

Human

Chronic administration increases the incidence of cholesterolic gallstones twofold. It also causes a small increase in thromboembolic phenomenon, pulmonary embolism, intermittent claudication, and angina pectoris. The drug may increase the incidence of bowel cancer. Overall, clofibrate cannot be classified as carcinogenic in humans.

In Vitro Toxicity Data

Clofibrate at a concentration of 0.5 mmol in culture medium maintained the cytochrome P-450 content of rat hepatocytes for up to 96 h. This effect was associated with a marked induction of lauric acid hydroxylation whereas little effect was observed on the metabolism of three other cytochrome p450 dependent mixed function oxidase substrates.

Clinical Management

Exposure should be terminated as soon as possible. If toxic amount in overdose is unknown, then gastric decontamination is required. Gastric decontamination is probably usually not necessary and should only be considered if several times the daily therapeutic dose were ingested. Activated charcoal as a slurry (240 ml water/30 g charcoal) should be given. The usual dose is 25–100 g in adults/adolescents, 25–50 g in children (1–12 years), and 1 g kg^{-1} in infants less than 1 year.

Exposure Standards and Guidelines

Manufacturers, packers, and distributors of drug and drug products for human use are responsible for complying with the labeling, certification, and usage requirements as prescribed by the Federal Food, Drug, and Cosmetic Act.

See also: Charcoal; Cytochrome P-450.

Further Reading

Clofibrate (1996) IARC Monographs. *Evaluation of Carcinogenic Risks to Humans* 66: 391–426.

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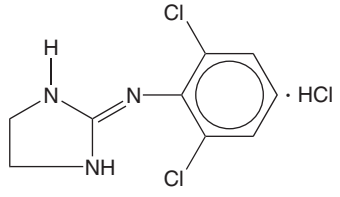
Clonidine

Elizabeth J Scharman

- Chemical Abstracts Service Registry Numbers: CAS 4205-90-7; CAS 4205-91-8 (hydrochloride)
- Synonyms: Clonidine hydrochloride; 2-(2,6-Dichloroanilino)-2-imidazoline hydrochloride; Catapres®
- Chemical/Pharmaceutical/Other Class: Imidazoline-derivative hypotensive agent; A selective α-2 adrenergic receptor agonist
- Chemical Formula: $C_9H_9Cl_2N_3$
- Chemical Structure:



Uses

Clonidine is used in the management of hypertension, attention-deficit hyperactivity disorder, opiate or nicotine withdrawal, vascular headache prophylaxis, and as an aid in the diagnosis of pheochromocytoma. It is also used as an epidural infusion for pain management.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to clonidine. Toxicity may also occur via dermal exposure from transdermal patches.

Toxicokinetics

Clonidine is well absorbed (75–90%) orally with peak plasma concentrations occurring in 2–4 h. Following transdermal application, therapeutic plasma concentrations are reached within 2–3 days and last for 8 h after patch removal. Peak analgesia occurs within 30–60 min when clonidine is given epidurally. Clonidine is metabolized by hydroxylation of the phenol ring and cleavage of the imidazole ring to six inactive metabolites. The volume of distribution is 2.1–4 l kg^{-1}. Clonidine is distributed into the cerebral spinal fluid and into breast milk; it also crosses the placenta. Approximately 65% is excreted by the kidneys; 32% as unchanged drug. The half-life is 6–20 h; 18–41 h in patients with renal insufficiency.

Mechanism of Toxicity

Clonidine acts at postsynaptic α-2 adrenergic receptors in the lower brainstem and medulla oblongata, resulting in inhibition of sympathetic discharge. This results in a decrease in cardiac output and heart rate. Following overdose, peripheral α-2 receptors may be stimulated, resulting in transient hypertension followed by hypotension.

Acute and Short-Term Toxicity (or Exposure)

Animal

Clonidine produces decreases in blood pressure and heart rate in animals as those seen in humans. In a rat model of seizure activity secondary to soman administration, clonidine pretreatment provides some seizure protection compared with controls.

Human

As little as 0.1 mg of clonidine has produced toxicity in children; determination of adult toxicity is based on observation as there is no milligram per kilogram toxic dose established. Clonidine levels are not clinically useful. Toxicity can result from ingestion of used clonidine transdermal patches as residual clonidine remains after full therapeutic use. Symptoms generally begin within 30–90 min and include hypotension, central nervous system depression, bradycardia, and miosis; cardiac dysrhythmias and hypothermia may be noted. Hypotension may be preceded by transient hypertension. Toxic symptoms may persist for up to 24–48 h; longer durations of toxicity have been reported.

Chronic Toxicity (or Exposure)

Animal

Albino rats on therapy with clonidine for 6 months or greater have developed retinal degeneration.

Human

Side effects of clonidine therapy include dry mouth, drowsiness, sedation, and constipation. Abrupt discontinuation of therapy may result in a withdrawal syndrome manifested as restless and headache in addition to significant rebound hypertension. Withdrawal can be avoided by tapering therapy over 2–4 days. The incidence of a local dermatitis or an extended dermal reaction with use of the transdermal patch is $\sim$15–20%.

Clinical Management

Induction of emesis with syrup of ipecac is not recommended due to the potential for rapid onset of lethargy and coma. Clonidine is adsorbed by activated charcoal. Whole bowel irrigation may be useful if a transdermal patch is ingested. Respiratory depression, hypotension, and coma may respond to naloxone (initial dose, 0.4–2 mg intravenously repeated as necessary) although this therapy is not routinely effective. Standard supportive therapies, such as vasopressors, should be utilized as clinically indicated. Paradoxical hypertension following an acute overdose is transient and normally does not require treatment. Therapy for rebound hypertension, which follows abrupt discontinuation of clonidine therapy, includes reinstitution of clonidine or the combined administration of an α- and β-adrenergic blocking agent (e.g., phentolamine or prazosin with a beta blocker such as propranolol).

See also: Charcoal; Nicotine; Opium.

Further Reading

Anderson RJ, Hart GR, and Crumpler CP (1981) Clonidine overdose: Report of six cases and review of the literature. *Annals of Emergency Medicine* 10: 107–112.

Wiley JF, Wiley CC, and Torrey SB (1990) Clonidine poisoning in young children. *Journal of Pediatrics* 116: 654–658.

Clostridium perfringens

Lee R Shugart

Description

Clostridium perfringens is a gram-positive, obligate anaerobic spore-forming rod. It is widely distributed in the environment and frequently occurs in low numbers in the intestines of humans and domestic animals. Spores of the organism persist in soil, sediments, and areas subject to human or animal fecal pollution. Any raw food may contain spores or the bacteria.

Mechanism of Toxicity

In all clostridial diseases, pathogenesis is attributable to potent exotoxins released by the organism. *C. perfringens* strains produce numerous toxins; over 20 have been described scientifically. For toxicological identification, *C. perfringens* strains have been divided into five types (A–E) based on the production of four main toxins (alpha, beta, epsilon, and iota), which combine with other toxic substances created by the bacteria to produce ~25 different diseases.

Nature of Disease

Perfringens food poisoning is the term used to describe the common acute food-borne illness caused by *C. perfringens*. In most instances, poor temperature control is the cause of perfringens food poisoning especially where large quantities of food such as meats, meat products, gravy, and poultry are prepared several hours before serving. Under these conditions, *C. perfringens* (usually type A) proliferates with little or no toxin production. Upon ingestion, the organism starts to sporulate after encountering the acidic conditions found in the stomach and produces toxins, which are released in the gastrointestinal tract. Food poisoning by *C. perfringens* is characterized by intense abdominal cramps and diarrhea, which begin 8–22 h after consumption of tainted food. The illness is usually over within 24 h but less severe symptoms may persist in some individuals (elderly or infirm) for 1–2 weeks. Ingesting food contaminated with the type C strain of the organism may causes a more serious but rare illness in humans that involves invasion of the intestine. In animals, especially domesticated ones, enteritis is almost always fatal. *C. perfringens*-related livestock infections have been reported in every state in the United States and in most parts of the world.

C. perfringens is the most important of the histotoxic clostridia that cause tissue infections in humans, especially of the muscle tissue (clostridial myonecrosis or gas gangrene). The organism is more aerotolerant than most other anerobes. In addition to toxins and enzymes, many of which have lethal, cell-destroying and hemolytic properties, a number of nonlethal enzymes are also produced and apparently contribute to the invasiveness of the organism in the tissue. These include collagenase, deoxyribonuclease, and hyaluronidase.

Control

In most instances, poor temperature control of prepared food is the cause of acute *C. perfringens*-related food poisoning. Therefore, it is important to keep hot foods hot (above 140°F) and cold foods cold (below 40°F) before serving. Perfringens food poisoning is a mild, self-limiting disease. Symptomatic and supportive therapy, including fluid and electrolyte replacement, is normally adequate intervention. The toxicity is produced by a toxin and is not an invasive infectious process; therefore, antibiotics play no role in its management. In some instances where a particular strain of *C. perfringens* has been isolated from domesticated animals, a vaccine can be produce to target the disease.

See also: Gastrointestinal System.

Further Reading

Brynestad S and Granum PE (2002) *Clostridium perfringens* and foodborne infections. *International Journal of Food Microbiology* 74(3): 195–202.

Salyers AA and Whitt DD (1994) *Bacterial Pathogenesis: A Molecular Approach*. Washington, DC: American Society for Microbiology.

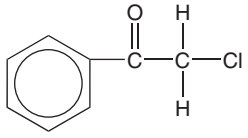
Sarker MR, Singh U, and McClane BA (2000) An update on *Clostridium perfringens* enterotoxin. *Journal of Natural Toxins* 9(3): 251–266.

CN Gas

Harry Salem, Bryan Ballantyne, and Sidney A Katz*

Published by Elsevier Inc.

- Military Designation: CN (Mace)
- Chemical Name: Chloroacetophenone
- Chemical Abstracts Service Registry Number: CAS 532-27-4
- Synonyms: Tear gas; Less-than-lethal; Nonlethal; Lacrimator, Harassing agent; Incapacitant; 2-Chloro-1-phenylethanone; 2-Chloroacetophenone, chloroacetophenone, phenacyl chloride; Chloromethyl phenyl ketone
- Chemical Formula: C_8H_7ClO
- Chemical Structure:



Pharmacological Action

Riot control agents such as CN are those that cause disabling physiological effects when they come into contact with the eyes or skin, or when inhaled. They have the capacity to cause intense sensory irritation of the skin and mucous membranes of the eye and respiratory tract. They are peripheral sensory irritants that pharmacologically interact with sensory nerve receptors in skin and mucosal surfaces at the site of contamination resulting in local pain and discomfort sensations with associated reflexes. The reflex associated with the inhalation exposure of irritants is the Kratschmer reflex. This reflex causes apnea, bradycardia, and a biphasic fall and rise in aortic blood pressure.

Pharmacological Class

CN is a peripheral sensory irritant, lacrimator, and incapacitant.

Uses

CN is used as a nonlethal or less-than-lethal chemical in riot control situations, to distract, deter, incapacitate, disorient, or disable disorderly people, to clear facilities, areas, deny areas, or for hostage rescue. It can also be used in peacekeeping operations. It is also used in military training as a confidence builder for the protective mask.

Exposure Routes and Pathways

CN is a white solid with low vapor pressure that can be dispensed as a fine powder or as a jet or stream of solution from small or large spray tanks, as well as aerosols or smokes by pyrotechnic generation. Its solubility in water is limited, but it is soluble in organic and chlorinated organics. Exposure of eyes, nose, mouth, skin, and respiratory tract produces irritation and pain. If swallowed, CN may produce vomiting.

Toxicokinetics

Evaporation of organic solvent may concentrate CN in the eyes and intensify damage. Hydrolysis of CN is very slow in water and is difficult to decompose. Environmental contamination may be persistent and difficult to remove.

Mechanism of Toxicity

CN is considered less than lethal or nonlethal because it has a large safety ratio. That is, its effective dose or concentration ECt_{50} is low compared to its lethal dose or concentration (LCt_{50}). In the body, CN is converted to an electrophilic metabolite. It is an SN2 alkylating agent that reacts with SH groups and other nucleophilic sites of biomolecules. Alkylation of SH-containing enzymes leads to enzyme inhibition with disruption of cellular processes. CN was found to inhibit human plasma cholinesterase via a non-SH interaction, and some of the toxic effects may be due to alkylation of SH-containing enzymes.

CN as well as CS is an SN2-alkylating agent with activated halogen groups that react readily at nucleophilic sites. The prime targets include sulfhydryl-containing enzymes such as lactic dehydrogenase. Alkylation of SH-containing enzymes leads to enzyme inhibition with disruption of cellular processes. It has been suggested that tissue injury may be related to inactivation of certain of these enzyme systems. The initial response to the inhalation of CN or other sensory irritants is consistent with the Kratschmer reflex and the Sherrington pseudoaffective response. These aerosols stimulate the pulmonary irritant receptors to produce bronchoconstriction and

*The views of authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

increased pulmonary blood volume by augmenting sympathetic tone. The chlorine atoms released from CN on contact with skin and mucous membranes are reduced to hydrochloride acid that can cause local irritation and burns. CN was also found to inhibit human plasma cholinesterase via a non-SH interaction.

Human Toxicology

The incapacitant effects of CN in human volunteers during exposure included lacrimation, some blurring of vision, and conjunctivitis. On the nose and throat, CN causes a tingling sensation, irritation, pain, and some increase in secretions; while on the respiratory track it causes irritation, burning, and pain. CN on the skin causes burning in the periorbital area, and other areas of tender skin, especially where sweating is present. Occasionally, nausea and gagging occur during and soon after exposure. Most of these effects disappear within 20 min after exposure, but conjunctivitis and blepharospasm usually disappear after a few days leaving no aftereffect. Incapacitating dosages (ICt_{50}) of CN have ranged from 20 to 50 mg min m^{-3}. The estimates of human LCt_{50} values, extrapolated from animals exposed to CN dispersed from a solvent, is 7000 mg min m^{-3}, and 14 000 mg min m^{-3} when dispersed from commercially available grenades. Other estimates range from 8500 to 25 000 mg min m^{-3}. The maximum safe inhalation dosage of CN for humans is estimated to be 500 mg min m^{-3}. Acute injuries to the eyes, primarily from effects of blast and missiles, may occur from tear-gas weapons such as pen guns. The immediate effects of these injuries include swelling and edema of the lids with penetration of skin, conjunctivitis, cornea, sclera, or globe by gunpowder and CN. Conjunctival ischemia and chemosis, corneal edema, erosion, inflammation or ulceration, and focal hemorrhage have been reported. A few hand injuries resulting from accidental discharges of tear gas guns at close range have been reported. Surgery was required in all to relieve pain and to remove the foreign material. All of these few victims suffered continuing pain and some loss of sensation, apparently from the toxic action of CN on nerves.

Clinical Management

Effects of exposure in open air are generally self-limiting and require no specific therapy. Most effects disappear in 15–30 min following exposure, although erythema may persist for an hour or longer.

CN can produce intense blepharospasm, pain, lacrimation, conjunctival erythema, periorbital edema, and a rise in intraocular pressure. These generally diminish within 30 min postexposure. CN also produces rhinorrhoea, nasal irritation and congestion, bronchorrhoea, sore throat, cough, sneezing, and unpleasant taste and burning of the mouth immediately after exposure. These effects rapidly resolve within minutes postexposure. Symptomatic treatment of ocular irritation consists of use of a topical solution to relieve the irritation with topical antibiotics. The eyes should be examined for corneal abrasions. Treatment with oral analgesics, topical antibiotics, and mydriatics should be considered. Since CN is a solid, it is possible for a particle or clump to become embedded in the cornea or conjunctiva and cause tissue damage. Medical care for eye pain after exposure should include thorough decontamination of the eyes and a thorough ophthalmological examination. The injured eye should be carefully irrigated with isotonic saline and the remaining powder removed with a cotton swab. Any remaining stromal particles should be removed with a needle tip under slit lamp illumination. Airway problems may occur in individuals with lung disease, especially if exposed to higher than average field use concentrations. If these occur, the immediate priority is the removal from the exposure and to ensure a patent airway.

Severe and prolonged erythema or severe dermatitis may occur several hours after exposure followed by vesiculation. These are generally second-degree burns and should be treated as such.

If the release of irritant incapacitants is in a confined, unventilated space, exposure may be to very high concentrations. Some individuals may be more susceptible to high concentrations, possibly because of an existing medical condition such as asthma, and will require intensive supportive medical treatment post exposure.

Animal Toxicology

Acute and sublethal effects following aerosol exposure from commercially available thermal grenades or from acetone solutions in experimental animals were lacrimation, conjunctivitis, copious nasal secretions salivation, hyperactivity, lethargy, and dyspnea, which occurred in all animals. Effects on the skin of exposed animals were primarily erythema. The estimated LCt_{50} values calculated for CN in the various animal species were 8878 mg min m^{-3} in the rat, 7984 mg min m^{-3} in the guinea pig, and 7033 mg min m^{-3} in the dog. The pathological findings in the animals that died from inhalation of CN consisted of congestion of the alveolar capillaries, alveolar hemorrhage, and excessive secretions in the bronchi and

bronchioles, as well as areas of acute inflammatory cell infiltration of the trachea, bronchi, and bronchioles. The early deaths exhibited lesions of the upper respiratory tract, with marked pseudomembrane formation, excessive salivation, and nasal secretion. The animals that died later exhibited edema, and hemorrhage of the lungs. In repeated exposures for 10 consecutive days in guinea pigs, dogs, and monkeys, the toxicity of CN was found to be considerably less when administered in divided doses. Overall, studies demonstrated a lack of cumulative toxicity. Changes in biochemical endpoints measured following multiple exposures of CN in mice were a decrease in hepatic glutathione and increased lipid peroxidation. Hepatic acid phosphatase increased after the 5 day exposure to CN, and the glutathione levels decreased after 10 day CN exposures. CN-induced elevation in acid phosphatase levels reflected the release of lysosomal enzymes from the liver, indicative of tissue injury. Additionally, hyperglycemia was observed after exposure to CN. Stress-mediated release of epinephrine is known to elevate glucose levels and thus may be responsible for the hyperglycemia. Significant decreases in body weight gain were also noted on exposure to CN. Histopathologic changes following CN exposures included hemorrhage, perivascular edema, congestion of the alveolar capillaries, occluded bronchioles, and alveolitis. Renal histopathology demonstrated congestion and coagulative necrosis in the cortical renal tubules in CN-exposed mice. Hepatic histopathology consisted of cloudy swelling and lobular and centrolobular necrosis of hepatocytes following CN exposures.

At high concentrations, CN may result in chemical injury to the eye with corneal and conjunctival edema, erosion or ulceration, chemosis, and focal hemorrhages. CN-induced ocular effects on the rabbit eye following exposure to various formulations included lacrimation, chemosis, iritis, blepharitis, and keratitis, with severity dependent on the formulation.

CN is also a potent skin irritant that may cause serious injury to the skin that includes severe generalized itching, diffuse and intense erythema, severe edema, and vesication. CN is considered a potent skin irritant, and sensitizer.

In 2 year carcinogenicity inhalation bioassays in rats and mice, there were no indications of carcinogenicity in male rats, while equivocal evidence was found in female rats. These findings were evidenced by increased fibroadenomas of the mammary gland. In these 2 year studies in mice, there was no evidence of carcinogenic activity in males and females.

Decontamination

Contaminated clothing should be removed and sealed in a plastic bag. Disposable rubber gloves should be used when handling contaminated clothes. The eyes should be irrigated copiously with saline for 15–20 min. Contaminated skin should be washed thoroughly with copious amounts of water, alkaline soap and water, a mildly alkaline solution (sodium bicarbonate or sodium carbonate), or mild liquid soap and water. The use of sodium hypochlorite solution will exacerbate the skin lesions and should not be employed. Only a saline irrigation should be used over vesiculated skin.

See also: CS Gas; Non-Lethal Weapons, Chemical; Riot Control Agents.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Coal Tar

Richard D Phillips

- Chemical Abstracts Service Registry Number: CAS 8007-45-2
- Synonyms: Coal tar; Creosote; Coal tar pitch

Uses

Coal tar is a viscous liquid mixture of hydrocarbon compounds, derived along with coke, from the destructive distillation of coal in coking ovens. Coal tar itself may be subjected to distillation, a process that separates groups of the components of coal tar from groups of others. The substances derived from this process are often called ‘coal tar distillates’.

Coal tar products are ingredients in medicines used to treat skin diseases such as psoriasis. Coal tar, along with coal tar pitch and car tar pitch volatiles, are used or produced in several industries including roofing, aluminum, smelting, road paving, rubber production, and coking.

Exposure Routes and Pathways

Most people are exposed to very low levels of coal tar. In the general population, exposure is most likely through products that contain coal tar or similar materials to improve a health problem such as eczema or psoriasis. Occupational exposure to coal tar could occur through contact with the skin or by inhalation exposure to volatile fractions when coal tar is heated.

Toxicokinetics

Coal tar is a complex mixture of hydrocarbons including polyaromatic hydrocarbons (PAHs). Coal tar constituent can enter the body through the lungs, skin, and by ingestion. There is no information that describes how fast or how much of coal tar might enter the body after one or several exposures.

Generally, the PAH components of coal tar are metabolized by oxidative enzymes in the liver and lungs to generate active metabolites that can bind to macromolecules. Principal metabolic products include phenols, dihydrodiols, quinones, anhydrates, and conjugates (e.g., glutathione) of these products.

Mechanism of Toxicity

Defining a general mechanism of toxicity for coal tar is difficult because of the diversity and variability in biological effect and composition. However, PAHs are a major constituent, and their carcinogenic activity is related to their metabolism to reactive intermediates that bind to macromolecules, thus initiating a carcinogenic response. The degree and magnitude will vary based on the composition of the coal tar.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute and prolonged periods of dermal exposure to coal tar in animals are associated with dermal irritation and more severe damage.

Dermal exposure to coal tar products is also toxic to animals but at fairly high doses. For example, the dermal LD_{50} in rabbits is greater than $7950\,mg\,kg^{-1}$.

Inhalation exposure of rats and mice to high doses of coal tar has produced some toxic effects in the form of weight and morphologic changes to the lymphoreticular tissues.

Coal tar products do not appear to be potent reproductive toxins based on experimental findings. Treatment of pregnant rats with coal tar by all routes of exposure produced reduced growth of offspring and an increase with incidence of cleft palate and small lungs.

Human

Coal tar exerts its acute toxic effects in humans primarily via dermal contact, causing structural damage to the tissues that it comes in contact with, such as the skin and eyes. In addition, respiratory effects are likely to occur after inhalation exposure to coal tar products. Over exposure to coal tar is likely to produce a number of systemic effects in the central nervous system, liver, and kidney.

Cutaneous photosensitivity from coal tar pitch has been described.

Chronic Toxicity (or Exposure)

Animal

Coal tar applied to the skin of 259 mice every third or fourth day resulted in skin papillomas in half of the mice that survived 100 days.

Light and heavy coal tar oil were applied dermally for the lifespan of mice or until persistent papillomas developed at the application site. Test solutions were applied three times weekly in male and female mice. Both produced skin tumors: the light coal tar oil contained benzene, toluene, xylene, solvent naphtha, and was the residual oil drained from a naphthalene recovery operation; the heavy coal tar oil was a mixture of creosote, anthracene oils, and the oil drained from the naphthalene recovery operation. The heavy oil was less potent compared to the effects observed in the BaP group of the study.

Five different coal tars were tested for carcinogenic potency in mice. Four samples were crude tars from the coking of bituminous coal, and one sample was produced by the coking of lignite coal. Lengths of exposure were not reported for any of the groups studied. Tumors developed in all five groups. Onset of tumors was delayed when animals were washed with aqueous detergent 5–60 min after application.

In a study with hard coal tar pitch (50% in benzene) and soft coal tar pitch (50% in benzene), 30 mice per group plus controls were skin-painted with one drop of test material twice weekly for 5 months. The hard-pitch solution produced an average of one papilloma/mouse in 21 surviving mice. The soft-pitch solution produced an average of 2.9 papillomas/mouse in 28 surviving mice, plus 14 mice with malignant tumors. Soft pitch was more carcinogenic than hard pitch, and pitches were more carcinogenic than coal tars tested in the same study.

Human

Various case reports and the results of cross-sectional occupational surveys associated chronic occupational exposure to coal tar products with the development of skin cancer. Disease etiology included the development of dermatoses, such as squamous papillomas, that progressed to carcinoma, usually squamous-cell carcinoma. Cancer of the scrotum in chimney sweeps has also been associated with prolonged exposure to coal tar creosote. The latency period for the development of dermatoses, such as squamous papillomas, was usually 20–25 years. Worker exposure in the past was much greater than it now is because of less sophisticated industrial practices used in the past, the lack of knowledge concerning occupational hygiene, and the current recognition of the dangers of excessive exposure to the health of workers.

However, no association between exposure to coal tar products and cancer in humans has been found.

In Vitro Toxicity Data

Coal tar is mutagenic in the *Salmonella typhimurium* assay and the mouse lymphoma assay in the presence of an exogenous mammalian metabolic system.

Clinical Management

Emergency management of direct cutaneous exposure to creosote is prompt and comprehensive decontamination. Treatment commonly includes removal of all contaminated clothing and washing of the skin, hair, and nails with large volumes of soapy water. In order to reduce dermal irritation from creosote-contaminated soil and water, wearing protective clothing, and immediate washing of exposed skin will limit exposure.

The treatment to manage exposure from ingestion also focuses on the acute effects of the phenolic and PAH components. Phenolic compounds cause corrosive esophageal burns, and there may also be a risk of causing pneumonitis in the patient by aspiration of PAHs in coal tar derivatives, so emesis is contraindicated as a means of elimination.

The treatment for contaminated eyes commonly includes irrigation with copious amounts of room temperature water, or saline if available, for at least 15 min.

Should an inhalation exposure occur, treatment commonly includes moving the exposed individual to fresh air and monitoring for respiratory distress.

Environmental Fate

As with other chemical mixtures, the fate and transport processes affecting coal tar can be extremely complex. Coal tar components may partition to the air, water, soil, or biota depending on their physical and chemical properties. Compounds initially released into the atmosphere may undergo atmospheric deposition and reach surface water directly or through runoff carrying soil-bound compounds.

Coal tar constituents released into surface waters will differentially partition to the water column or to sediments depending on their water solubility and sorptive properties. For example, PAHs, the major constituents of coal tar, generally tend to sorb strongly to soil and sediment particulates, and often have low aqueous solubilities and mobility. Many components in the PAH fraction, particularly the higher molecular weight PAHs, will remain in a virtually stationary tar-like mass at the place where they were deposited.

Many of the same bacteria and fungi capable of biodegrading coal tar components in aqueous systems can be found in soils. Especially where the coal tar is close to the surface and under aerobic conditions, the vast majority of the phenolics can be consumed in less than a year. The majority of the lighter fractions of the PAH components (from 53 to 75% by weight) can be biodegraded within 2 months.

Ecotoxicology

For fish and wildlife, the aromatics of coal tar pose the most danger. The aquatic toxicity in the water-soluble fraction is mostly from the aromatics.

See also: Charcoal; Coke Oven Emissions; Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

IARC (1985) *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, vol. 35, pp. 83–159.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Creosote (including Wood Creosote, Coal Tar Cresote, and Coal Tar).

Cobalt

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-48-4
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Co^{2+}

Uses

Cobalt is a relatively rare metal produced primarily as a by-product of the mining of other metals, chiefly copper. It is the essential trace element found in cyanocobaltamine (vitamin B_{12}). This vitamin protects against pernicious anemia and is required in the production of red blood cells. Medicinally, cobalt salts have been used to stimulate the formation of red blood cells in individuals suffering from anemia.

Commercially, cobalt is used primarily in high-temperature alloys, in tungsten carbide tools, and (with iron and nickel) in permanent magnets. Cobalt salts are used in pigments, in paint dryers, and as catalysts in the petroleum industry.

Background Information

Cobalt exists in valence states from 0 to 5, with the most stable ($+2$ and $+3$) being most common. While there is only one stable isotope of cobalt, there are a number of unstable isotopes. Two of these, cobalt-60 and cobalt-57, are in use commercially. Cobalt-60 is used for cancer treatment and for food irradiation. Cobalt-57 has research applications.

Exposure Routes and Pathways

For the general population, ingestion is the primary exposure pathway for cobalt. For persons working in industrial settings, inhalation is a significant pathway (e.g., carbide industry emissions and airborne particulate from grinding processes) as is dermal exposure. There can also be internal exposure from implanted medical devices.

Toxicokinetics

Oral ingestion of cobalt salts results in ready absorption, probably in the jejunum. Although cobalt is readily absorbed, increased levels do not tend to cause significant accumulation. The majority (80%) of cobalt is excreted in the feces of rats and cattle. In contrast, in humans, ~80% of absorbed cobalt is excreted via the urine and 15% is excreted in the feces by an enterohepatic pathway. Breast milk and sweat are secondary routes of excretion. The total body burden for the average person is estimated as 1.1 mg. Muscle contains the greatest mass of cobalt but the highest concentrations are found in fat. Cobalt present in the blood is associated with the red blood cells.

Mechanism of Toxicity

Cobalt most often depresses the activity of enzyme including catalase, amino levulinic acid synthetase, and P-450, enzymes involved in cellular respiration. The Krebs citric acid cycle can be blocked by cobalt resulting in the inhibition of cellular energy production. Cobalt can replace zinc in a number of zinc-required enzymes like alcohol dehydrogenase. Cobalt can also enhance the kinetics of some enzymes such as heme oxidase in the liver. Cobalt interferes with and depresses iodine metabolism resulting in reduced thyroid activity. Reduced thyroid activity can lead to goiter.

Acute and Short-Term Toxicity (or Exposure)

Human

Ingestion of cobalt may result in the production of an unusually high number of red blood cells (similar to a cancer of red blood cells (polycythemiavera)). Ingestion of cobalt salts (once added to beer as a defoaming agent) has resulted in cardiomyopathy. The signs and symptoms of cardiomyopathy due to beer consumption are similar to those of congestive heart failure. Autopsy results indicated a 10-fold increase in cobalt concentrations in heart tissue. The alcohol may have potentiated cobalt absorption or toxic effects.

Chronic Toxicity (or Exposure)

Animal

When implanted intramuscularly in rats, cobalt metal produced fibrosarcomas at the site but no other routes of exposure have elicited a carcinogenic response.

Human

Cobalt is an essential nutrient at low levels ($\sim 40\,\mathrm{mg\,day^{-1}}$). In industrial settings, inhalation of high concentrations of cobalt compounds has led to hard-metal pneumoconiosis, which may result in interstitial fibrosis. Workers with this condition typically develop hypersensitivity to cobalt compounds (symptoms include coughing and wheezing). A few workers have developed skin hypersensitivity after dermal contact with cobalt and its compounds. Cobalt can cause cardiomyopathy and (if inhaled as a dust) interstitial lung disease.

Clinical Management

The oil-soluble BAL (British Antilewisite; 2,3-dimercaptopropanol) appears to be the antidote of choice for cobalt poisoning.

Environmental Fate

The sources of cobalt in the environment are both natural and man-made (anthropogenic). Natural sources include soil, seawater spray, volcanic eruptions, and forest fires. Anthropogenic sources include combustion of fossil fuels, metal smelting, sewage sludge, and processing of cobalt alloys. Cobalt is found in the atmosphere in particulate form and returns to the Earth's surface through dry deposition and with rain or snow. Once in surface water, cobalt generally moves into sediment. Cobalt does not appear to biomagnify significantly in the aquatic food chain. The cobalt that does accumulate in fish is largely found in the nonedible parts of the fish.

Under normal environmental conditions, cobalt is expected to bind strongly to soil and thus migration through soil would be very limited. Cobalt in soil can be taken up by plant roots and root vegetables but is not translocated to the aboveground parts of plants.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value – time-weighted average for cobalt (elemental and inorganic compounds) is $0.02\,\mathrm{mg\,m^{-3}}$. ACGIH classifies cobalt as an animal carcinogen.

See also: Metals.

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Cobalt.

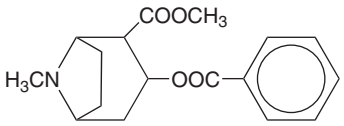
Cocaine

Michael Wahl

This article is a revision of the previous print edition article by William A Watson, volume 1, pp. 355–356, © 1998, Elsevier Inc.

- SYNONYMS: Ecgonine methyl ester benzoate; Benzoylmethylecgonine; [1*R*-(*exo*–*exo*)]-3-(benzoyloxy)-8-methyl-8-azabicyclo[3,21]octane-2-carboxylic acid methyl ester; Crack; Rock; Toot; Blow; Snow; Dama blanca; Coke; Lady
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: A naturally occurring alkaloid with local anesthetic and vasoconstrictor properties and central nervous system stimulant and euphoric effects
- CHEMICAL FORMULA: $C_{17}H_{21}NO_4$

- Chemical Structure:



Uses

Cocaine is used for topical local anesthesia of mucous membranes. It is also a drug of abuse.

Exposure Routes and Pathways

Smoking the base alkaloid (as crack or freebase) and nasal insufflation of the hydrochloride salt are the most common routes of exposure in abuse. Intravenous injection and application of the hydrochloride salt to mucous membranes are also methods of abuse. In therapeutic use, a solution of hydrochloride salt is applied to mucous membranes.

Toxicokinetics

Smoked cocaine is absorbed in seconds from the lungs, which results from volatilization of the alkaloid. Peak plasma concentrations occur within a few minutes. Absorption through mucous membranes is initially rapid, then slowed secondary to the vasoconstrictive effects of cocaine. Peak plasma concentrations occur within 1 h after oral ingestion and nasal application. After oral administration, bioavailability is decreased secondary to presystemic hydrolysis in the gastrointestinal tract.

Cocaine is metabolized by hydrolysis to benzoylecgonine, by cholinesterase to ecgonine methyl ester, and hepatically to norcocaine and ecgonine. In the presence of ethanol, cocaine is also metabolized to cocaethylene. Cocaine metabolism is probably dose dependent at the high doses that are abused, especially with binge use. Cocaine is widely distributed in the body with an apparent volume of distribution of 1.2–1.9 l kg^{-1}. It rapidly appears in the central nervous system (CNS) and crosses the placenta. The elimination half-life of cocaine is ~1 h at doses of less than 2 mg kg^{-1}. The measurement of low concentrations of cocaine in chronic users suggests half-lives as long as 3 days. Low doses of cocaine are excreted in the urine primarily as metabolites, with less than 10% of the dose found as unchanged cocaine.

Mechanism of Toxicity

Cocaine toxicity is primarily secondary to its ability to prevent the reuptake of neurotransmitters including serotonin, dopamine, and norepinephrine. Direct cardic toxicity may be due to inhibition of sodium influx across the nerve cell membrane (type 1C antidysrhythmias). The role of cocaine metabolites in producing clinical toxicity is unclear. Cocaethylene, ecgonine methyl ester, and benzoylecgonine may produce some toxicity. Additional effects of cocaine, which are important in clinical toxicity, include direct vasoconstriction, increased cellular oxygen consumption, increased platelet aggregation, and direct organ toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal models have demonstrated acute toxicity similar to that present in humans. Dogs develop toxicity at lower doses than rats, and death appears to be associated with the development of hyperthermia. The functional status of organ enervation and the presence of anesthetics may alter cocaine toxicity in animal models.

Human

Cocaine toxicity can present after a wide range of doses, with reports of toxicity at doses of less than 1 mg kg^{-1}. Toxicity includes most organ systems, and cocaine use and culture increases the risk of trauma and infections. Acute tolerance to many CNS and cardiovascular effects of cocaine develops. Kindling, the lowering of the seizure threshold with repeated subtoxic doses, can also occur. The CNS toxicity of cocaine includes stimulation, euphoria, agitation, seizures, intracranial hemorrhage, and, with larger doses, coma. Cardiovascular toxicity can include tachycardia, hypertension, coronary artery spasm, myocardial ischemia and infarction, bradycardia, hypotension, cardiovascular collapse, dysrhythmias, and sudden death. Pulmonary toxicity after smoking the alkaloid form of cocaine includes hemorrhage, barotrauma including pneumomediastinum, pulmonary edema, and 'crack lung', a hypersensitivity reaction that includes fever, productive cough, pulmonary infiltrates, and bronchospasm. Other toxicities seen with cocaine use include hyperpyrexia, rhabdomyolysis, metabolic acidosis, and respiratory alkalosis. Cocaine use during pregnancy can result in an increased risk of abruptio placentae, spontaneous abortion, and low-birth-weight infants with congenital malformations and potentially neurobehavioral impairment. Nasal insufflation of single doses of cocaine results in plasma concentrations of 100–500 ng ml^{-1}. Blood cocaine concentrations in fatalities are described as averaging ~6 mg l^{-1}, with a very wide range of reported concentrations of cocaine and metabolites.

Chronic Toxicity (or Exposure)

Animal

Animal models of chronic, self-administered cocaine use show regular patterns of use and abstinence. Administration of cocaine during a period of self-induced abstinence from cocaine restarts this cycle. Animals with free access 24 h a day to cocaine showed weight loss, self-mutilation, and death within 2 weeks.

Human

Toxicity associated with chronic use is not as well described as acute toxicity, but it appears to include cerebral atrophy, cardiomyopathy, and chronic pulmonary disease. Cocaine and its metabolites are most commonly identified in patient urine. An immunoassay directed toward identification of benzoylecgonine will frequently indicate the presence of cocaine and its metabolites for many days after use. The duration of qualitatively detected cocaine and metabolites in urine is probably dose dependent and may be up to 3 weeks in length. Chronic use of cocaine may lead to dependence.

In Vitro Toxicity Data

Cocaine has been demonstrated to covalently modify proteins *in vitro*. This finding has been seen in animals and humans chronically exposed to cocaine. Modified proteins are immunogenic and may explain why some people develop autoimmune effects after chronic cocaine exposure.

Clinical Management

The initial management of acute cocaine intoxication should include assessment and management of the patient's airway, breathing, and circulation. Supplemental oxygen and a benzodiazepine are frequently indicated for agitation and CNS stimulation. Many findings, such as hyperpyrexia, seizures, and rhabdomyolysis, should be managed using the basic treatment approaches for the complication. Concurrent use of alcohol and other drugs is frequent and should be considered during initial assessment. Treatment of cardiac toxicity should also include supportive care, oxygen, and a benzodiazepine. Beta-blockers can theoretically cause unopposed alpha stimulation; this may lead to paradoxical worsening of hypertension and vasoconstriction. If a beta-blocker is to be used, a short-acting beta-blocker (e.g., esmolol) is preferred. Nitrates, opiates, thrombolytics, and/or cardiac catherterization may be employed when appropriate based on the clinical and laboratory findings.

See also: Neurotoxicity.

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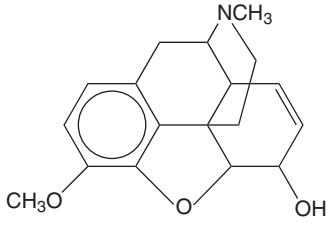
Codeine

F Lee Cantrell

- Chemical Abstracts Service Registry Number: CAS 76-53-3
- Synonyms: Methylmorphine; Morphine monomethyl ether
- Chemical/Pharmaceutical/Other Class: Opioid analgesic/antitussive
- Chemical Formula: $C_{18}H_{21}NO_3$
- Chemical Structure:



Uses

Codeine is used as an analgesic and an antitussive.

Exposure Routes and Pathways

Codeine is customarily ingested in the form of tablets and liquid preparations.

Toxicokinetics

Codeine is well absorbed via oral and intramuscular routes of administration; it is two-thirds as effective orally as parenterally. Peak serum levels are attained in 30–60 min. Codeine is metabolized in the liver by *O*-demethylation and *N*-demethylation and partial conjugation with glucuronic acid. The volume of distribution is $3.5\,l\,kg^{-1}$. Greater than 95% of a single dose is eliminated in 48 h by the kidneys. The elimination half-life is 1.9–4 h. Codeine metabolites are conjugated codeine, norcodeine, conjugated norcodeine, conjugated morphine, and hydrocodone.

Mechanism of Toxicity

Codeine stimulates opiate receptors in the central nervous system (CNS), producing sedation and respiratory depression.

Acute and Short-Term Toxicity (or Exposure)

Animal

Opioids have an excitatory effect on the CNS of cats and horses. Dogs experience similar CNS depressant effects as seen in humans. Death can occur within 12 h. Naloxone has been effective in treating animals.

Human

Depressant effects on the CNS are the most profound. Nausea, vomiting, and miosis may develop within 1 h. Infants and children may demonstrate unusual sensitivity while habituated adults may have extreme tolerance to opioids. In children, greater than $1\,mg\,kg^{-1}$ may produce serious symptoms and $5\,mg\,kg^{-1}$ may cause significant respiratory depression. The estimated lethal adult dose of codeine is $7–14\,mg\,kg^{-1}$.

Chronic Toxicity (or Exposure)

Animal

There is no evidence of carcinogenicity in 2 year feeding studies of mice and rats at doses up to 3000 ppm.

Human

Codeine is often subject to abuse and can cause a withdrawal syndrome after abrupt discontinuation of use.

In Vitro Toxicity Data

In vitro clearance of opiates is altered in hepatic cells obtained from mice with human alpha-globin and sickle beta-globin transgenes than in control mice.

Clinical Management

Basic and advanced life-support measures should be performed as necessary. Gastrointestinal decontamination procedures should be considered for substantial recent ingestions. Activated charcoal will adsorb codeine. Patients with respiratory or CNS depression can be treated with intravenous boluses of naloxone. A continuous naloxone infusion may be necessary if the toxic effects of codeine persist longer than the duration of action of naloxone.

See also: Charcoal; Hydrocodone; Morphine.

Further Reading

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Coke Oven Emissions

Shashi K Ramaiah and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 8007-45-2
- Synonyms: Primary synonym is coal tar pitch volatiles such as benzene soluble organics. However, synonyms vary depending on the specific constituents in the emissions; RTECS No. GH0346000

Uses

The primary use of coke is a fuel reductant and support for other raw materials in iron-making blast furnaces. Coke is also used to synthesize calcium carbide and to manufacture graphite and electrodes, and coke-oven gas is used as a fuel. Coal tar, a by-product of the production of coke from coal, is used in the clinical treatment of skin disorders such as eczema, dermatitis, and psoriasis.

Background Information

Petroleum coke is a chunky powdered carbon product derived from petroleum. If petroleum coke is heated to a high temperature, it may emit volatiles such as polynuclear aromatic hydrocarbons, which could be suspect carcinogens. Such exposures can occur in coke oven workers.

The production of coke by the carbonization of bituminous coal leads to the release of chemically complex emissions from coke ovens that include both gases and particulate matter of varying chemical composition. The chemical and physical properties of coke oven emissions vary depending on the constituents. The emissions include coal tar pitch volatiles (e.g., particulate polycyclic organic matter, polycyclic aromatic hydrocarbons, and polynuclear aromatic hydrocarbons), aromatic compounds (e.g., benzene and β-naphthyl amine), trace metals (e.g., arsenic, beryllium, cadmium, chromium, lead, and nickel), and gases (e.g., nitric oxides and sulfur dioxide).

Exposure Routes and Pathways

The primary routes of potential human exposure to coke oven emissions are inhalation and dermal contact. Occupational exposure to coke oven emissions may occur for those workers in the aluminum, steel, graphite, electrical, and construction industries. Coke oven emissions can have a deleterious effect on human health. Coke oven emissions contain literally several thousand compounds, several of which are known carcinogens and/or cocarcinogens including polycyclic organic matter from coal tar pitch volatiles, β-naphthylamine, benzene, arsenic, beryllium, cadmium, chromate, lead, nickel subsulfide, nitric oxide, and sulfur dioxide. Most regulatory attention has been paid to coal tar pitch volatiles.

Toxicokinetics

Since coke oven emissions are complex mixtures of coal and coke particles, specific information is not available. In general, coke oven emissions are well absorbed from the respiratory tract, skin, and the conjunctiva.

Mechanism of Toxicity

Complex chemical mixtures in coke oven emissions is known to result in DNA adduct formation. Free oxygen radicals and CYP450 are implicated in the pathogenesis. Polycyclic aromatic hydrocarbons, which are primary compounds in coke oven emissions generated by the coking process, cause cancer and mutagenesis by a multitude of mechanisms including DNA adduct formation and metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal studies have reported weakness, depression, dyspnea, general edema, and effects on the liver from acute oral exposure to coke oven emissions.

Human

Acute exposure to coke oven emissions produces irritation of the eyes, respiratory symptoms like cough, dyspnea, and wheezing.

Chronic Toxicity (or Exposure)

Animal

In animals, extracts and condensates of coke oven emissions were found to be carcinogenic in both inhalation studies and skin painting bioassays. The mutagenicity of whole extracts and condensates, as well as their individual components, provides supportive evidence for carcinogenicity. In addition, several inhalation exposure studies in laboratory animals have provided evidence of the carcinogenic effect of aerosols of coal tar and its fractions. The extract was found to produce papillomas and skin carcinomas in the mice and acted as an initiating agent, although the extent to which this extract is representative of coke oven emissions is uncertain since the sample was contaminated with particulate matter from ambient air. Numerous carcinogenicity studies have shown that coal tar samples applied topically to the skin of laboratory animals produce local tumors.

Human

The emissions are investigated as a carcinogen, tumorigen, and mutagen. The cancer sites include

the skin, respiratory system, kidneys, and urinary bladder. Studies of coke oven workers have shown increased risk of mortality from cancer of the lung, trachea, and bronchus; cancer of the kidneys; cancer of the prostrate; and cancer at all sites combined.

Chronic occupation-related exposure is associated with significant excess mortality from cancer of the respiratory system and of the prostate. Depending on the segment of the population considered, the respiratory cancer risk for coke oven workers was as high as 4.5 times the risk for nonoven workers. To evaluate a biologically effective exposure dose in human biomonitoring studies, DNA carcinogen adduct analysis is frequently used.

Occupational Safety and Health Administration (OSHA) has not identified thresholds for carcinogens that will protect 100% of the population. It usually recommends that occupational exposures to carcinogens be limited to the lowest detectable concentration. To ensure maximum protection from carcinogens through the use of respiratory protection, only the most reliable and protective respirators are recommended. The OSHA permissible exposure limit (PEL) for benzene-soluble fraction of coke oven emissions is $0.150\,\mathrm{mg\,m^{-3}}$.

In Vitro Toxicity Data

In vitro toxicity data are not available for coke oven emissions.

Clinical Management

The exposed person should be moved to fresh air at once. If breathing has stopped, mouth-to-mouth resuscitation should be performed. The affected person should be kept warm and at rest. Exposed eyes should be washed immediately with large amounts of water; the lower and upper lids should be lifted occasionally. Medical attention should be obtained immediately.

Ecotoxicology

There is no aquatic toxicity information available for coke oven emissions.

Exposure Standards and Guidelines

The OSHA standard for coke oven emissions is a PEL of $0.15\,\mathrm{mg\,m^{-3}}$ as an 8 h time-weighted average (TWA). Under this standard, specific engineering and work practice control requirements became effective. OSHA has also promulgated a PEL of $<0.2\,\mathrm{mg\,m^{-3}}$ as an 8 h TWA for coal tar pitch volatiles. National Institute for Occupational Safety and Health (NIOSH) and OSHA have recommended work practices to minimize the harmful effects of exposure to coke oven emissions.

Miscellaneous

Coke production in the United States steadily increased between 1880s and 1950s, peaking at 72 million tons in 1951. In 1976, the United States ranked number two in the world with 52.9 million tons of coke or $\sim$14.4% of the world production. Although, the by-product process is designed to collect the volatile materials given off during the coke process, emissions escape because of structural defects around the doors or changing lids, improper use of engineering controls, improper work practices, and insufficient engineering controls.

See also: Combustion Toxicology; Pollution, Air; Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

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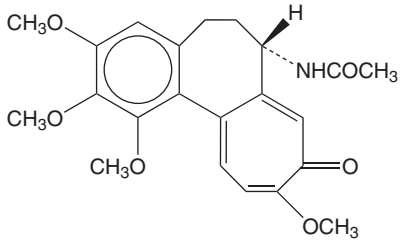
Colchicine

Henry A Spiller

- Chemical Abstracts Service Registry Number: CAS 64-86-8
- Chemical/Pharmaceutical/Other Class: Naturally occurring alkaloid
- Chemical Formula: $C_{22}H_{25}NO_6$
- Chemical Structure:



Uses

Colchicine is used in the treatment of acute gouty arthritis. Unlabeled uses include treatment of familial Mediterranean fever, neoplasms of the skin, and cirrhosis of the liver. It is available as a pesticide for moles and gofers.

Background Information

Colchicine is obtained from the autumn crocus, *Colchicum autumnale*, or the glory lily, *Gloriosa superba*. *C. autumnale* was first introduced for the treatment of gouty arthritis in 1763 by Von Storck.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to colchicine. It is available as an oral tablet and solution for injection.

Toxicokinetics

Colchicine is readily absorbed from the gastrointestinal tract. In therapeutic dosing, peak serum levels occur in 30–120 min. Colchicine undergoes deacetylation and hydrolysis in the liver. It has a rapid initial distribution phase, with a plasma half-life of 19 or 20 mm, suggesting swift uptake by the tissues. The volume of distribution is $2.2\,l\,kg^{-1}$. Up to 40% of colchicine is excreted in the urine, with 20–30% of this as unchanged drug. The majority of the drug undergoes enterohepatic recirculation and is excreted via bile and feces. The average elimination half-life is 20 h.

Mechanism of Toxicity

Colchicine binds to tubulin and prevents its polymerization into microtubules, subsequently disrupting microtubule function. Consequently, it alters nuclear structure, intracellular transport, and cytoplasmic motility, ultimately causing cell death. Colchicine is a potent inhibitor of cellular mitosis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal toxicity is primarily related to ingestion of the plant *C. autumnale*. Colchicine is available as a pesticide for burrowing animals. The estimated toxic dose for cows is $10\,g\,kg^{-1}$ with fresh leaves or 2 or $3\,g\,kg^{-1}$ with dried leaves. Symptoms may include gait disorders, hypersalivation, bloody vomitus, and diarrhea. Death within 72 h has occurred secondary to shock. It is only slightly toxic to cold-blooded and hibernating animals.

Human

Colchicine toxicity has been divided into three stages. The first stage, from 2 to 24 h, is the gastrointestinal phase, notable for abdominal pain, vomiting, diarrhea, and a prominent leukocytosis. The gastrointestinal symptoms may be relieved by atropine, but this does not prevent or alter the onset of the second stage. The second stage is marked by multisystem failure. Most life-threatening symptoms occur 24–72 h postexposure. Confusion, delirium, coma, seizures, and cerebral edema may occur. Progressive respiratory distress and pulmonary edema can occur. After an initial leukocytosis, bone marrow depression is seen with a nadir between the fourth and seventh days. Bone marrow depression, coupled with potential gastrointestinal hemorrhages and a hemolytic anemia, may produce profound anemia. Consumptive coagulopathy may also be seen. Renal function may be affected by direct organ damage as well as by decreased perfusion from profound and persistent hypotension. Cardiovascular instability along with metabolic acidosis may develop due to volume depletion, cardiac failure, and arrhythmias. Most deaths result from shock in the 24–72 h period. Stage three is the recovery phase. If patients survive to this convalescent phase, the main complication is sepsis.

Chronic Toxicity (or Exposure)

Animal

Colchicine has been shown to be teratogenic in mice and hamsters.

Human

Recently, a case report of development of colchicine induced cardiomyopathy was described. The patient also reported respiratory difficulty. Three weeks after discontinuation of colchicines, the patient returned to baseline status.

In Vitro Toxicity Data

Genotoxicity studies (micronucleus tests for chromosome aberrations) in mammalian polychromatic erythrocytes and nonhuman mammalian cell culture were positive. Ames *Salmonella* tests for mutagenicity have been negative.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Treatment of colchicine toxicity is largely supportive. Activated charcoal effectively adsorbs colchicines and should be administered for substantial recent ingestions. Aggressive early gastrointestinal decontamination may be life saving. Severe anemia may require packed red blood cell replacement. Coagulopathies may respond to vitamin K and fresh frozen plasma. Hypotension may be unresponsive to fluid replacement and pressor support. Due to rapid tissue distribution and the large volume of distribution, hemoperfusion and hemodialysis are ineffective. Colchicine Fab fragments have effectively reversed hypotension and increased survival in animals and humans in the research setting but are not commercially available. Colony stimulating factors have also been used for patients with profound bone marrow suppression from colchicine overdose.

Environmental Fate

No information is currently available on breakdown in soil groundwater or surface water. Colchicine alkaloids withstand storage, drying, and boiling.

See also: Atropine; Charcoal; Genomics, Toxicogenomics; Pesticides.

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Combustion Toxicology*

Barbara C Levin and Erica D Kuligowski

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Combustion toxicity research is the study of the adverse health effects caused by exposure to fire atmospheres. A fire atmosphere is defined as all of the effluents generated by the thermal decomposition of materials or products regardless of whether that effluent is produced under smoldering, nonflaming, or flaming conditions. The objectives of combustion toxicity research are to identify potentially harmful products from the thermal degradation of materials, to distinguish those materials that produce unusual or greater quantities of toxic combustion products, to determine the best measurement methods for the identification of the toxic products as well as the degree of toxicity, to determine the effect of different fire exposures on the composition of the toxic combustion products, and to establish the physiological effects of such products on living organisms. The ultimate goals of this field of research are to reduce human fire fatalities due to smoke inhalation, to determine effective treatments for survivors, and to prevent unnecessary suffering of fire casualties caused by smoke inhalation.

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Fire Death Statistics

The latest statistics from the (US) National Fire Protection Association (NFPA) report that the fire death rate in the United States was 1.3 times higher (2.1

times more if the New York City deaths from the September 11, 2001, terrorist attacks are included) than in the United Kingdom, 1.16 times more than in Sweden, 1.3 times less than Japan (or 1.3 times more if the deaths from the September 11, 2001 terrorist attacks are included), and about the same as that in Canada. Although the reasons are still being debated, the number of fire deaths per capita since 1977 have been higher in the United States and Canada than in most of the other industrialized countries outside of the former Soviet Union.

Fire statistics collected by NFPA indicated that 1 687 500 fires were reported in the United States in 2002, the latest year for which complete statistics are available at the time of this writing. Calculated another way, these statistics translate into a reported fire occurring in the United States every 19 s, in an outside property every 38 s, in a structure every 61 s, in a residence every 67 s, and in a motor vehicle every 96 s. These fires caused ~3380 civilian deaths and 18 425 reported injuries in 2002. Excluding New York City's World Trade Center deaths from the September 11, 2001, terrorist attacks in which 2326 civilian deaths occurred, the number of deaths in 2002 decreased by almost 10% from the previous year. However, there still was one civilian fire death every 156 min and one fire injury every 28 min. The number for injuries is believed to be less than the actual number, since many injuries are not reported. The property loss due to fires in 2002 is estimated at 10.3 billion dollars and indicates a decrease of 2.2% from the previous year, if one excludes the World Trade Center deaths from the 2001 numbers.

In 2002, residential fires accounted for only 24% of the total fires, but were responsible for 79% of all fire deaths and 76% of the reported injuries. Although in the years 1977–2002, the number of civilian fire fatalities in homes dropped from 5865 to 2670, fires in homes still cause the greatest concern to the fire community. Statistics show that children under five and adults over 65 years of age are the most frequent casualties of residential fires. This is attributed to their inherent difficulties in trying to escape. Statistics also show that males are more likely to die in fires than females. More fires and higher fire death rates occur in the South than any of the other geographical areas of the United States; the geographical region with the next highest fire death rate and number of fires is the Northeast.

One must distinguish between the causes of fires and the causes of fire deaths. The primary causes of residential fires have been shown to be heating and cooking. Lack of central heat and the incorrect use of portable space heaters are two of the reasons given for the high fire and death rate in the South. Heating fires result in the highest property losses, primarily because cooking fires are usually noticed and extinguished before getting out of control. Fire deaths, however, usually result from fires ignited by cigarettes. The most common fire scenario leading to fire deaths is one in which a person (usually intoxicated) falls asleep in an upholstered chair while smoking. The cigarette falls into a crevice and starts the upholstered chair smoldering. The individual awakes and goes to bed. The chair can smolder for an extended period of time (in laboratory tests, an hour was not unusual) before bursting into flames. It is after the flaming starts that the smoke fills the room and escapes to the other rooms. It is common to find people who have died from smoke inhalation (not burns) in or near their beds, indicating that the little or no effort to escape was probably due to lack of awareness of the danger. Smoke detectors in this type of scenario would save many lives. Statistics have shown that working smoke detectors double one's probability of escaping alive. Recent statistics have also shown that many homes have nonfunctioning smoke detectors due to being disconnected after a false alarm (usually from smoke from cooking or a wood stove) or when the beeping indicating the need for a new battery became annoying.

Since most of the deaths from fires occur in residences, the NFPA proposes the following safety initiatives to improve fire safety: (1) increase fire safety education on fire prevention and what to do if a fire occurs; (2) install smoke detectors in all homes and check them periodically to ensure they are working properly; (3) practice escape plans with the family; (4) install residential home sprinklers to prevent fires from spreading once they start; (5) develop products for the home that are more fire safe and produce less toxic combustion products (the latter is proposed by the authors); and (6) study the needs of the populations most at risk (the young, the elderly, and the poor) and implement preventive measures.

The US Fire Administration has issued the following 'Home Fire Safety Checklist':

- *Smoke detectors*, are they: (1) placed near bedrooms, (2) on every floor, (3) placed away from air vents, and (4) checked regularly for working batteries.
- *Electrical wiring*, is it: (1) replaced if frayed or cracked, (2) not placed under rugs, over nails or in high traffic areas. Are the outlets: (1) not overloaded, (2) cool to the touch, not hot, and (3) not exposed, that is, have cover plates.
- *Electric space heaters*, are they: (1) plugged directly into wall socket with no extension cords and (2) unplugged when not in use.

- *Kerosene heaters*, are they: (1) used only in permitted geographic areas, (2) filled only with K-1 kerosene, never gasoline or camp stove fuel, (3) only refueled outdoors, and (4) only refueled when cool.
- *Woodstoves and fireplaces*, are they: (1) used only with seasoned wood, never with green wood, Wolmanized chromated copper arsenate (CCA)-treated wood, pressure-treated wood, artificial logs or trash, (2) protected by screens, and (3) cleaned regularly along with the flues, interiors, hearths and chimneys. After December 30, 2003, Wolmanized CCA-treated wood (also called Wolmanized pressure-treated wood) will no longer be produced for nonindustrial applications. This wood has been preserved by pressure-treatment with a US EPA-registered pesticide containing CCA to protect it from termite attack and decay. Wood treated with CCA should be used only where such protection is important. Exposure to CCA or its combustion products presents certain hazards. Therefore, the following precautions should be taken both when handling the treated wood and in determining where to use and how to dispose of the treated wood. Treated wood should not be burned in open fires or in stoves, fireplaces or residential boilers because toxic chemicals are produced as part of the smoke and ashes (see section 'Relevant Websites'). The *Journal of the American Medical Association* (in 1984) reported that a Wisconsin family who had burned CCA-containing wood scraps in their home furnace for heating purposes experienced their hair falling out, rashes, severe reoccurring nosebleeds, extreme fatigue, and debilitating headaches. The parents spoke of black out periods that would last for several hours with long periods of extreme disorientation following it. The two children also reported experiencing frequent seizures. Since arsenic affects not just humans, but any pets and wildlife, the family noticed their houseplants and fish had died as well. Later, all the serious health effects were attributed to the family breathing in minute amounts of arsenic dust that had occurred from the ashes.
- *All alternate heaters*, are they: (1) used only in well-ventilated rooms, (2) stable such that they cannot be easily knocked over, (3) never used to dry clothing or other items, and (4) kept at a safe distance from curtains or furniture.
- *Home escape plan*: (1) is it practiced every 6 months, (2) are the emergency numbers, a whistle, and a flashlight kept near the telephone, and (3) is the outside meeting place identified.

Generation of Toxic Gases in Fires: Adverse Effects of Particulates

Eighty percent of the residential fire deaths are attributed to smoke inhalation, not to burns. Smoke is defined by ASTM International (ASTM, formerly the American Society for Testing and Materials) as "the airborne solid and liquid particulates and gases evolved when a material undergoes pyrolysis or combustion." The adverse effects from smoke inhalation are believed to be due mainly to the exposure to toxic gases, although the role of the particulates alone and in combination with fire gases needs further investigation. The importance, therefore, of determining the identities and concentrations of toxic gases produced from materials thermally decomposed under various fire conditions is evident. In addition, the increased variety of plastics in buildings and homes has raised the issue of whether synthetic materials may produce unusually or extremely toxic[1] combustion products. In 1975, the journal *Science* documented a case in which an experimental rigid polyurethane foam containing a fire retardant produced a very unusual toxic combustion product identified as 4-ethyl-1-phospha-2,6,7-trioxabicyclo [2.2.2]octane-1-oxide (commonly referred to as a bicyclic phosphate ester). Bicyclic phosphate compounds have been shown to cause seizures at very low concentrations. Based on these test results, this product never became commercially available. To a large extent, however, it was this case that generated the burgeoning interest in the field of 'combustion toxicology' and the widespread concern about the potential formation of 'supertoxicants'. Although research since the 1970s has shown that this concern is largely unfounded, the bicyclophosphate ester case and at least one other product that generated extremely toxic combustion products have indicated the need to test new formulations or materials containing new combinations of compounds to ensure that extremely or unusually toxic products are not generated.

The gas composition of smoke depends on the chemical composition, the molecular structure and polymer formulation of the burning material, which may include a variety of additives, plasticizers, stabilizers, flame retardants, cross-linking agents, fillers, and blowing agents. In addition, the conditions of

[1] Here, the phrase 'extremely toxic' is a relative term indicating that the effluent from the thermal decomposition of very small quantities of a material has been noted to cause death of experimental animals (usually rats or mice) under controlled laboratory conditions. 'Unusually toxic' indicates that the toxic effect cannot be totally attributable to the combustion gases (either singly or in combination) that are normally considered the main toxicants.

thermal degradation, for example, temperature, oxygen availability and ventilation, will affect the nature of the combustion atmosphere. In a series of reviews of the combustion products and toxicity of seven plastics (acrylonitrile-butadiene-styrenes (ABS), nylons, polyesters, polyethylenes, polystyrenes, poly(vinyl chlorides) (PVC), and rigid polyurethane foams) commonly found in residences, and decomposed under various thermal and atmospheric conditions, over 400 different decomposition products were noted. Many of these products were common to more than one plastic. In addition, there are probably many other combustion products that were not detected. The toxicity of most of these individual compounds is currently unknown and little has been done to tackle the enormous problem of determining the toxicity of combinations of these compounds. It is important to note that lack of detection of a specific combustion product from a material may only mean that the particular analytical techniques used were not suitable to detect that compound or that the investigator did not specifically analyze for that combustion product. Toxicity testing, for example, on animals, becomes important to ensure that an unsuspected and therefore, undetected toxic by-product has not been formed.

Since the number of compounds one can reasonably analyze in any one test is limited, knowledge of the chemical composition, molecular structure, and formulation of the polymer can be used to provide some indication of the main gaseous products which may or may not be generated under specified experimental conditions. However, one needs to be cautious when predicting the combustion products from generic materials of unknown formulations. For example, one would expect nitrogen-containing materials (e.g., ABS, nylons, rigid and flexible polyurethanes) to produce hydrogen cyanide (HCN) and not expect HCN from a material like PVC. However, a PVC containing zinc ferrocyanide[2] (an additive designed to suppress smoke) as well as a vinyl chloride–vinylidene chloride copolymer were found to generate HCN. In a similar fashion, based on the chemical composition, PVC is the only one of the seven plastics mentioned above that would be expected to generate chlorinated combustion products. However, widespread usage of halogenated fire retardants in plastic formulations makes predicting which materials will produce halogenated products extremely difficult.

[2]This material was never made commercially available after toxicity testing indicated that its combustion products produced very rapid deaths of experimental animals (rats).

Temperature also plays an important role in influencing the production of decomposition products. In general, as the temperature and thus the rate of decomposition increases, the quantity of the more complex compounds and heavier hydrocarbons decreases and the concentrations of carbon monoxide (CO), carbon dioxide (CO_2), and nitrogen dioxide (NO_2) increase. The generation of HCN has also been shown to increase as a function of temperature. Another example is hydrogen chloride (HCl), the detection of which begins when stabilized PVC is heated to $\sim 200^{\circ}C$; rapid dehydrochlorination then occurs at $\sim 300^{\circ}C$. On the contrary, more acrolein was generated from polyethylene under lower temperature, nonflaming conditions than under higher temperature flaming conditions.

As mentioned earlier, more work is needed to examine the adverse effects of the particulate matter produced when these materials are thermally decomposed. Examination of the smoke particulate and condensable matter is important for a number of reasons. First, many of the thermal degradation products may condense or be adsorbed by the soot particles and be transported along with the smoke into the body. Hydrogen chloride is one example of a compound that may be transported in such a fashion and can form a corrosive acid mist in moist air, such as that found in the lung. One study of the particulate matter that formed during the smoldering decomposition of rigid polyurethane foam showed that many of the compounds detected in the soot fraction were not found in the volatile fraction. Free radicals, which form in fires and are of toxicological concern due to their high reactivity, are usually considered to have very short life spans; however, if adsorbed onto soot particles, their lifetimes can be considerably longer and, if the soot particles are the correct size, they can be inhaled into the individual's deep lungs. In addition, the particulate matter may interfere with the escape and rescue of individuals by causing the obscuration of vision, eye irritation (the eyes clamp shut and the victim is unable to see), and upper respiratory distress. An extreme case indicating the adverse effect of particulates was noted in experiments conducted at the (US) National Institute of Standards and Technology (NIST). Rats exposed for 30 min to the smoke from polystyrene died during the exposures and the level of CO in the blood, even in combination with CO_2, was too low to be fatally toxic. Pathological examination of these rats showed that their respiratory passages were completely blocked by soot and that suffocation was the likely cause of death.

Toxic Potency versus Fire Hazard versus Fire Risk

Death in a fire may be caused by:

1. Carbon monoxide (CO)
2. Toxic gases in addition to CO
3. Oxygen (O_2) at levels too low to sustain life
4. Incapacitation – either physical (inability to escape) or mental (incorrect decision-making)
5. Bodily burns from flame contact
6. Very high air temperatures
7. Smoke density or irritants in smoke that affect vision and interfere with ability to escape
8. Psychological effects (e.g., fear, shock, and panic)
9. Physical insults (e.g., building or ceiling collapses, broken bones from jumping from upper floors)

Research in the field of combustion toxicology is primarily concerned with items 1–4, all of which are related to the toxic potency of the fire gas effluent. Toxic potency is defined by ASTM as "a quantitative expression relating concentration (of smoke or combustion gases) and exposure time to a particular degree of adverse physiological response, for example, death on exposure of humans or animals." This definition is followed by a discussion, which states, "The toxic potency of smoke from any material or product or assembly is related to the composition of that smoke which, in turn, is dependent upon the conditions under which the smoke is generated." One should add that the LC_{50}[3] is a common end point used in laboratories to assess toxic potency. In the comparison of the toxic potencies of different compounds or materials, the lower the LC_{50} (i.e., the smaller the amount of material necessary to reach the toxic end point), the more toxic the material is.

It is important to note that a toxicity assessment based on lethality due to toxic gases is only part of the total fire hazard that needs to be evaluated especially when one is making choices as to the best material for a specific end use. ASTM defines 'fire hazard' as the potential for harm associated with fire. The discussion that follows this definition states, "a fire may pose one or more types of hazard to people, animals or property. These hazards are associated with the environment and with a number of fire-test-response characteristics of materials, products or assemblies including but not limited to ease of ignition, flame spread, rate of heat release, smoke generation and obscuration, toxicity of combustion products and ease of extinguishment." Other factors that need to be evaluated when considering a material for use in a given situation include the quantity of material needed, its configuration, the proximity of other combustibles, the volume of the compartments to which the combustion products may spread, the ventilation conditions, the ignition and combustion properties of the material and other materials present, the presence of ignition sources, the presence of fire protection systems, the number and type of occupants, and the time necessary to escape.

'Fire risk' is defined by ASTM as "an estimation of expected fire loss that combines the potential for harm in various fire scenarios that can occur with the probabilities of occurrence of those scenarios." The discussion following the definition of fire risk states, "risk may be defined as the probability of having a certain type of fire, where the type of fire may be defined in whole or in part by the degree of potential harm associated with it, or as potential for harm weighted by associated probabilities. Risk scales do not imply a single value of acceptable risk. Different individuals presented with the same risk situation may have different opinions on its acceptability." A simple way to explain the difference between fire hazard and fire risk is to compare the fire to sky diving, a very hazardous sport; however, if one never goes sky diving, no risk is incurred.

Toxicity Assessment: Animal Exposures

In most combustion toxicology experiments, the biological end point has been lethality or incapacitation of experimental animals, usually rats or mice. Incapacitation in a fire can be as perilous as lethality if an individual becomes incapable of correct decision-making or physically unable to move. Under these circumstances, the ability to escape will be lost and death will occur unless the individual is rescued. Therefore, many fire scientists are concerned with the levels of combustion products or amounts of materials which when combusted will cause incapacitation. However, an incapacitation model for use in laboratory testing has been especially difficult to develop. Most of the tests for incapacitation that have been designed are based on the physical-motor capability of an experimental animal to perform some task (e.g., running in a motorized wheel, jumping onto a pole or lifting a paw to escape a shock, running in a maze, or pushing the correct lever to open a door to escape an irritating atmosphere). The concentration of toxic combustion products that cause the loss of these types of

[3]The LC_{50} value is the result of a statistical calculation based on multiple experiments, each with multiple animals, and indicates the concentration at which 50% of the experimental animals exposed for a specific length of time would be expected to die either during the exposure time or the post-exposure observation period.

physical-motor capabilities is usually close to the concentration that is lethal and does not usually add much additional information. More recently, however, there have been attempts at examining neurological end points such as measuring the increased number of errors by humans doing mathematical problems while exposed to low levels of CO or exposing rats and pigeons to a complete neurobehavioral battery of 25 tests following nonlethal toxic exposures.

Whether one needs to examine incapacitation or lethality depends upon the problem one is trying to solve. To determine the best material for a particular end use application, the lethality end point has proved to be more definitive and will flag the materials that produce extremely toxic combustion products better than an incapacitation end point. There are at least two reasons for this: (1) Incapacitation is only measured during the exposure, which is usually 30 min or less, but lethality can also occur during the postexposure observation period, which can be 2 weeks or longer. A material that only causes delayed effects during the postexposure period (e.g., a material that generates HCl) can thus have an LC_{50} value that is lower than the incapacitation EC_{50}[4] value. The amount needed to kill can be less than the amount needed to incapacitate because the amount of thermally decomposed material necessary to cause postexposure deaths can be less than the amount needed to cause incapacitation during the exposure. (2) In many cases in which the combustion products contain high concentrations of irritant gases, the animals would only appear to be incapacitated (i.e., they would stop responding to the test indicator due to the high irritant quality of the smoke), but when removed from the combustion atmosphere, would immediately start responding normally.

Other delayed effects from exposures to combustion atmospheres, such as tissue or organ injury, mutagenicity, carcinogenicity, and teratogenicity also need to be studied since they may ultimately lead to permanent disability or death. The current advances in the field of genetics provide investigators with new opportunities to examine the effects of combustion products at the molecular level. One objective could be to determine whether these toxic products cause DNA damage and/or mutations. Specific problems of interest include: does the damage occur in nuclear DNA and/or mitochondrial DNA, are certain areas of the DNA more prone to these mutations (i.e., are there hot spots?), can we categorize the types of mutations (e.g., transitions, transversions, deletions, insertions), and how efficient are the repair mechanisms? Are these mutagens also known to be carcinogens?

Toxicity Assessment: Predictive Models

In the 1970s, there were essentially two experimental strategies to examine the issues raised by the field of combustion toxicology: (1) an analytical chemical method and (2) an animal exposure approach. In the analytical chemical method, investigators thermally decomposed materials under different experimental conditions and tried to determine every combustion product that was generated. This approach generated long lists of compounds. The toxicity of most of these individual compounds was unknown and the concept of examining the toxicity of all the various combinations of compounds was and still is considered a formidable task. An additional problem with the analytical approach was that, as mentioned earlier, the detection and identification of the toxic combustion products depended on the analytical method used. Therefore, one could not be certain that every toxic product was detected and identified. This approach enabled one to identify many of the multiple products that were generated, without knowing the toxic potency of all the identified compounds, either singly or combined.

In the animal exposure approach, the animals (usually rats or mice) serve as indicators of the degree of toxicity of the combustion atmospheres. The materials of concern are thermally decomposed under different combustion conditions and the animals are exposed to the combined particulate and gaseous effluent. Multiple animal experiments (each with multiple animals) with different concentrations of material are conducted to determine an EC_{50} (incapacitation) or an LC_{50} (lethality) for a specific set of combustion conditions. Each material would then have a particular EC_{50} or an LC_{50} value that can be used to compare the toxicities of different materials decomposed under the same conditions. The lower the EC_{50} or LC_{50} value, the more toxic the combustion products from that material are considered to be. In this approach, one knows the relative toxicity of a material as compared to another material, but does not know which of the toxic gases are responsible for the adverse effects.

In the 1980s, investigators at NIST began examining the possibility of combining the analytical chemical method with the animal exposure approach to develop empirical mathematical models to predict the toxicity. These predictions were based

[4] The definition of the EC_{50} is essentially the same as that of the LC_{50} except incapacitation rather than lethality is the end point and incapacitation is monitored only during the exposure and not during the post-exposure period.

on actual experiments with animals and their response to each of the main toxic combustion gases; CO, CO_2, low O_2, HCN, NO_2, HCl, hydrogen bromide (HBr), and various combinations of these gases. The advantages of these predictive approaches are: (1) the number of necessary test animals can be reduced by first predicting the toxic potency from a limited chemical analysis of the smoke; (2) smoke may be produced under conditions that simulate any fire scenario of concern; (3) fewer tests are needed, thereby reducing the overall cost of the testing; and (4) information is obtained on both the toxic potency of the smoke (based on the mass of material burned) and the responsible gases (based on the primary toxic gases in the mixture). The prediction is checked with one or two animal tests to assure that an unexpected gas or toxic combination has not been generated. The results of using these empirical mathematical models indicated that, in most cases, one could predict the toxic potency of a combustion atmosphere based on the concentrations of the main toxic gases and did not need to worry about the effects of minor or more obscure gases.

Primary Toxic Combustion Gases

Complete combustion of a polymer containing carbon, hydrogen, and oxygen in an atmosphere with sufficient O_2 yields CO_2 and H_2O. It is during incomplete combustion under various atmospheric conditions in either flaming or nonflaming modes that compounds of greater toxicological concern are generated. When O_2 is limited, the primary gases formed during the combustion of most materials are CO, CO_2, and H_2O. If the materials contain nitrogen, HCN and NO_2, two principal thermo-oxidative products of toxicological concern, are also likely to be generated. Halogenated or flame-retarded materials generally produce HCl or HBr. Other commonly found fire gases include nitrogen oxides (NO_x), ammonia (NH_3), hydrogen sulfide (H_2S), sulfur dioxide (SO_2), and fluorine compounds. One also needs to consider that in fire situations, O_2 levels drop and exposure to low O_2 atmospheres will have additional adverse physiological effects. Some of these toxic combustion gases (e.g., CO, HCN, low O_2) produce immediate asphyxiant symptoms, while others (e.g., HCl, HBr, NO_2) fall into an irritant category and produce symptoms following the exposures.

The *N*-Gas Models

The *N*-gas models for predicting smoke toxicity were founded on the hypothesis that a small number (*N*) of gases in the smoke will account for a large percentage of the observed toxic potency. These predictive models were based on an extensive series of experiments conducted at NIST on the toxicological interactions of the primary gases found in fires. Both the individual gases and complex mixtures of these gases were examined. To use these models, materials are thermally decomposed using a bench-scale method that simulates realistic fire conditions, the concentrations of the primary fire gases – CO, CO_2, low O_2, HCN, HCl, HBr, and NO_2 – are measured, and the toxicity of the smoke using the appropriate *N*-gas model is predicted. The predicted toxic potency is checked with a small number of animal (Fischer 344 male rats) tests to assure that an unanticipated toxic gas was not generated or an unexpected toxicological effect (e.g., synergism or antagonism) did not occur. The results indicate whether the smoke from a material or product is extremely toxic (based on mass consumed at the predicted toxic level) or unusually toxic (the toxicity cannot be explained by the combined measured gases). These models have been shown to correctly predict the toxicity in both bench-scale laboratory tests and full-scale room burns of a variety of materials of widely differing characteristics chosen to challenge the system. The six-gas model (without NO_2) is now included in two national toxicity test method standards (ASTM E1678), approved by ASTM, and NFPA 269, approved by the NFPA. It is also included in an international standard (ISO 13344) that was approved by 16 member countries of the International Organization of Standardization (ISO), Technical Committee 92 (TC92). All three of these standards were published in 1996.

The objectives for developing the *N*-gas models were:

- To establish the extent to which the toxicity of a material's combustion products could be explained and predicted by the interaction of the major toxic gases generated from that material in the laboratory or whether minor and more obscure combustion gases needed to be considered.
- To develop a bioanalytical screening test and a mathematical model which would predict whether a material would produce extremely toxic or unusually toxic combustion products.
- To predict the occupant response from the concentrations of primary toxic gases present in the environment and the time of exposure.
- To provide data for use in computer models designed to predict the hazard that people will experience under various fire scenarios.

The Six-Gas *N*-Gas Model The six-gas model (see eqn (1)) was based on studies at NIST on the toxicological interactions of six gases: CO, CO_2, HCN, low O_2 concentrations, HCl, and HBr. First, individual gases in air were tested to determine the concentrations necessary to cause 50% of the laboratory test animals (Fischer 344 male rats) to die either during the exposure (within exposure LC_{50}) or during the exposure plus a 14 day postexposure observation period (within plus postexposure LC_{50}). The studies on HCl and HBr were conducted at Southwest Research Institute (SwRI) under a grant from NIST. Similar measurements for various combinations of these gases indicated whether the toxicity of the mixtures of gases was additive, synergistic, or antagonistic.

Based on these empirical results, the following six-gas model was developed:

$$\frac{m[\mathrm{CO}]}{[\mathrm{CO_2}]-b}+\frac{[\mathrm{HCN}]}{\mathrm{LC_{50}HCN}}+\frac{21-[\mathrm{O_2}]}{21-\mathrm{LC_{50}O_2}}+\frac{[\mathrm{HCl}]}{\mathrm{LC_{50}HCl}}+\frac{[\mathrm{HBr}]}{\mathrm{LC_{50}HBr}}=N\text{-gas value} \quad (1)$$

where the terms in brackets indicate the time-integrated average atmospheric concentrations during a 30 min exposure period ((ppm × min)/min or for O_2 (% × min)/min). The other terms are defined in the following paragraphs.

Under the experimental conditions used at NIST and with Fischer 344 male rats, the 30 min LC_{50} value of CO_2 is 47% (470 000 ppm) with 95% confidence limits of 43–51%. No deaths occurred in rats exposed to 26% CO_2 for 30 min. In a real fire, the highest theoretically possible concentration of CO_2 is 21%, a concentration that could only occur if all the atmospheric O_2 were converted to CO_2, a highly improbable event. Therefore, CO_2 concentrations generated in fires are not lethal. However, CO_2 is a respiratory stimulant causing an increase in both respiratory rate and tidal volume. It also increases the acidosis of the blood. When combined with any of the other tested gases, CO_2 has a synergistic toxicological effect, that is, the toxicity of the other gases is increased in the presence of CO_2 (**Table 1**). Empirically, however, it was found that the effect of CO_2 can only be added into the *N*-gas equation once. Therefore, the CO_2 effect was included with the CO factor since there was more data on the effect of different concentrations of CO_2 on the toxicity of CO, and CO is the toxicant most likely to be present in all fires. The results on the synergistic effect of CO_2 on CO indicated that as the concentration of CO_2 increases (up to 5%), the toxicity of CO increases. Above 5% CO_2, the toxicity of CO starts to revert back toward the toxicity of the CO by itself. The terms m and b in eqn (1) define this synergistic interaction and equal −18 and 122 000, respectively, if the CO_2 concentrations are 5% or less. For studies in which the CO_2 concentrations are above 5%, m and b equal 23 and −38 600, respectively.

Table 1 Synergistic effects of CO_2

Gas[a]	*LC_{50} values*	
	Single gas	*With 5% CO_2*
CO_2	470 000 ppm[b]	
CO	6600 ppm[b]	3900 ppm[c]
NO_2	200 ppm[d]	90 ppm[d]
O_2	5.4%[b]	6.4%[c]

[a] All gases were mixed in air; 30 min exposures of Fischer 344 rats.
[b] Deaths occurred during the exposure.
[c] Deaths occurred during and following the exposures.
[d] Deaths during the postexposure period.

In rats, the 30 min LC_{50} for CO is 6600 ppm and with 5% CO_2, this value drops to 3900 ppm. Exposure to CO in air only produced deaths during the actual exposures and not in the postexposure observation period; however, exposures to CO plus CO_2 also caused deaths in the postexposure period. Carbon monoxide is a colorless, odorless, tasteless and nonirritating poisonous gas. The toxicity of CO comes from its binding to the hemoglobin in red blood cells and the formation of carboxyhemoglobin (COHb). The presence of CO on the hemoglobin molecule prevents the binding of O_2 to hemoglobin (O_2Hb) and results in hypoxia in the exposed individual. Since the binding affinity of hemoglobin for CO is 210 times greater than its affinity for O_2, only 0.1% CO (1000 ppm) is needed to compete equally with O_2 which is normally present at 20.9% in air (20.9%/210 ~ 0.1%). Thus, only 1000 ppm of CO in the atmosphere is enough to generate 50% COHb, a value commonly quoted (but not necessarily proved) as the concentration which is lethal to humans. The time to get to 50% COHb at 1000 ppm CO would be longer than 30 min.

The LC_{50} value of HCN is 200 ppm for 30 min exposures or 150 ppm for 30 min exposures plus the postexposure observation period. HCN caused deaths both during and following the exposures.

The 30 min LC_{50} of O_2 is 5.4%, a value that is included in the model by subtracting the combustion atmospheric O_2 concentration from the normal concentration of O_2 in air, that is, 21%. The LC_{50} values of HCl or HBr for 30 min exposures plus postexposures times are 3700 and 3000 ppm, respectively.

HCl and HBr at levels found in fires only cause postexposure effects.

The pure and mixed gas studies showed that if the value of eqn (1) is 1.1 ± 0.2, then some fraction of the test animals would die. Below 0.9, no deaths would be expected and above 1.3, all the animals would be expected to die. Since the concentration–response curves for animal lethalities from smoke are very steep, it is assumed that if some percentage (not 0% or 100%) of the animals die, the experimental loading is close to the predicted LC_{50} value. Results using this method show good agreement (deaths of some of the animals when the *N*-gas values were above 0.9) and the good predictability of this approach.

This model can be used to predict deaths that will occur only during the fire exposure or deaths during and following the fire. To predict the deaths that would occur both during and following the exposures, eqn (1) is used as presented. To predict deaths only during the exposures, HCl and HBr, which only have postexposure effects, should not be included in eqn (1). In small-scale laboratory tests and full-scale room burns, eqn (1) was used successfully to predict the deaths during and following exposures to the combustion products from numerous materials. In the case of PVC, the model correctly predicted the results as long as the HCl was greater than 1000 ppm; therefore, it is possible that HCl concentrations under 1000 ppm may not have any observable effect on the model even in the postexposure period. More experiments are necessary to show whether a true toxic threshold for HCl does exist.

Although most of the work at NIST concentrated on deaths during or following 30 min exposures, the LC_{50} values of many of these gases, both singly and mixed, were determined at other times ranging from 1 to 60 min and in all the cases examined, the predictive capability of eqn (1) holds if the LC_{50} values for the other times are substituted into the equation.

The Seven-Gas Model; Addition of NO_2 to the *N*-Gas Model Nitrogen dioxide is an irritant gas that will cause lachrymation, coughing, respiratory distress, increases in methemoglobin (MetHb) levels, and lung edema. Single brief exposures to less than lethal concentrations can cause lung damage, emphysema, or interstitial fibrosis. Low levels have been alleged to increase one's susceptibility to respiratory infections, and aggravate one's reactions to allergens. Impairment of dark adaptation has also been noted. Delayed serious effects can be observed as late as 2–3 weeks following exposures. In the lungs, NO_2 forms both nitric (HNO_3) and nitrous (HNO_2) acids, which are most likely responsible for the observed damage to the lung cells and connective tissue.

In fires, NO_2 may arise from atmospheric nitrogen fixation, a reaction that is material independent, or from the oxidation of nitrogen from nitrogen-containing materials. To examine the generation of NO_2 from nitrogen fixation, a small study was undertaken at NIST. In two full-scale fires of rooms in which the main source of fuel was polystyrene-covered walls, only low levels of NO_x (10 and 25 ppm) were found indicating little nitrogen fixation under these conditions. A real example of burning nitrogen-containing materials was the 1929 Cleveland Clinic (Ohio, USA) fire in which 50 000 nitrocellulose X-ray films were consumed. The deaths of 97 people in this fire were attributed mainly to NO_x. An additional 26 people died between 2 h and 1 month following the fire, and 92 people were treated for nonfatal injuries. In laboratory tests of nitrogen-containing materials under controlled conditions, 1–1000 ppm of NO_x were measured. In military tests of armored vehicles penetrated by high temperature ammunition, NO_2 levels above 2000 ppm were found.

Individual and binary mixtures. In small-scale laboratory tests of NO_2 in air, deaths of Fischer 344 male rats occurred only in the postexposure period and the LC_{50} value following a 30 min exposure is 200 ppm. Carbon dioxide plus NO_2 show synergistic toxicological effects. The LC_{50} for NO_2 following a 30 min exposure to NO_2 plus 5% CO_2 is 90 ppm (postexposure deaths) (i.e., the toxicity of NO_2 doubled) (**Table 1**).

As mentioned above, CO produces only within-exposure deaths and its 30 min LC_{50} is 6600 ppm. In the presence of 200 ppm of NO_2, the within-exposure toxicity of CO doubled (i.e., its 30 min LC_{50} became 3300 ppm). An exposure of $\sim$3400 ppm CO plus various concentrations of NO_2 showed that the presence of CO would also increase the postexposure toxicity of NO_2. The 30 min LC_{50} value of NO_2 went from 200 to 150 ppm in the presence of 3400 ppm of CO. A concentration of 3400 ppm of CO was used as that concentration would not be lethal during the exposure and any postexposure effects of CO on NO_2 would become evident; the LC_{50} of CO (6600 ppm) would have caused deaths of the animals during the 30 min exposure.

The 30 min LC_{50} of O_2 is 5.4% and the deaths occurred primarily during the exposures. In the presence of 200 ppm of NO_2, the within-exposure LC_{50} of O_2 and its toxicity increased to 6.7%. In the case of O_2, increased toxicity is indicated by an increase in the value of the LC_{50} since it is more toxic to be adversely affected by a concentration of O_2 ordinarily capable of sustaining life. Exposure of the animals

to 6.7% O_2 plus various concentrations of NO_2 showed that the NO_2 toxicity doubled (i.e., its LC_{50} value decreased from 200 to 90 ppm).

One of the most interesting findings was the antagonistic toxicological effect noted during the experiments on combinations of HCN and NO_2. As mentioned above, the 30 min LC_{50} for NO_2 alone is 200 ppm (postexposure) and the 30 min within-exposure LC_{50} for HCN alone is also 200 ppm. This concentration of either gas alone is sufficient to cause death of the animals (i.e., 200 ppm HCN or 200 ppm NO_2 would cause 50% of the animals to die either during the 30 min exposure or following the 30 min exposure, respectively). However, in the presence of 200 ppm of NO_2, the within-exposure HCN LC_{50} concentration increases to 480 ppm or stated in other words, the toxicity of HCN decreases by 2.4 times.

The mechanism for this antagonistic effect is believed to be as follows. In the presence of H_2O, NO_2 forms nitric acid (HNO_3) and nitrous acid (HNO_2). These two acids are the most likely suspects responsible for the lung damage leading to the massive pulmonary edema and subsequent deaths noted following exposure to high concentrations of NO_2. Nitrite ion (NO_2^-) formation occurs in the blood when the nitrous acid dissociates. The nitrite ion oxidizes the ferrous ion in oxyhemoglobin to ferric ion to produce MetHb (eqn (2)). MetHb is a well-known antidote for CN^- poisoning. MetHb binds cyanide, forming cyanmethemoglobin, which keeps the cyanide in the blood and prevents it from entering the cells. In the absence of MetHb, free cyanide will enter the cells, react with cytochrome oxidase, prevent the utilization of O_2, and cause cytotoxic hypoxia. If, on the other hand, cyanide is bound to MetHb in the blood, it will not be exerting its cytotoxic effect. Therefore, the mechanism of the antagonistic effect of NO_2 on the toxicity of cyanide is believed to be due to the conversion of oxyhemoglobin [$O_2Hb(Fe^{2+})$] to methemoglobin [$MetHb(Fe^{3+})$] in the presence of nitrite (see eqn (2)).

$$2H^+ + 3NO_2^- + 2O_2Hb(Fe^{2+}) = 2MetHb(Fe^{3+}) + 3NO_3^- + H_2O \qquad (2)$$

Tertiary mixtures of NO_2, CO_2, and HCN. Earlier work indicated that the presence of 5% CO_2 with either HCN or NO_2 produced a more toxic environment than would occur with either gas alone. The antagonistic effects of NO_2 on HCN indicate that the presence of one LC_{50} concentration of NO_2 ($\sim$200 ppm) will protect the animals from the toxic effects of HCN during the 30 min exposures, but not from the postexposure effects of the combined HCN and NO_2. Thus, it was of interest to examine combinations of NO_2, CO_2, and HCN. In this series of experiments, the concentrations of HCN were varied from almost 2 to 2.7 times its LC_{50} value (200 ppm). The concentrations of NO_2 were approximately equal to one LC_{50} value (200 ppm) if the animals were exposed to NO_2 alone and approximately half the LC_{50} (90 ppm) if the animals were exposed to NO_2 plus CO_2; the concentrations of CO_2 were maintained at $\sim$5%; and the O_2 levels were kept above 18.9%. The results indicated that CO_2 does not make the situation worse, but rather provided additional protection even during the postexposure period. In each of six experiments, some or all of the animals lived through the test even though they were exposed to greater than lethal levels of HCN plus lethal levels of NO_2. In addition, in four tests, some of the animals lived through the postexposure period even though the animals were exposed to combined levels of HCN, NO_2, and CO_2 that would be equivalent to 4.7–5.5 times the lethal concentrations of these gases. One possible reason that CO_2 seems to provide an additional degree of protection is that NO_2 in the presence of 5% CO_2 produces four times more MetHb than does NO_2 alone.

Mixtures of CO, CO_2, NO_2, O_2, and HCN. The initial design of these experiments was to look for additivity of the CO/CO_2, HCN, and NO_2 factors keeping each at about one-third of its toxic level, while keeping the O_2 concentration above 19%. When these initial experiments produced no deaths, we started to increase the concentrations of CO up to one-third of the LC_{50} of CO alone (6600 ppm), HCN was increased to 1.3 or 1.75 times its LC_{50} depending on whether the within-exposure LC_{50} (200 ppm) or the within- and postexposure LC_{50} (150 ppm) is being considered, and NO_2 was increased up to a full LC_{50} value (200 ppm). The results indicated that just adding an NO_2 factor (e.g., [NO_2]/LC_{50} NO_2) to eqn (1)] would not predict the effect on the animals. A new mathematical model was developed and is shown as eqn (3). In this model, the differences between the within-exposure predictability and the within-exposure and postexposure predictability are: (1) the LC_{50} value used for HCN is 200 ppm for within-exposure or 150 ppm for within-exposure and postexposure, and (2) the HCl and HBr factors are not used to predict the within-exposure lethality, only the within-exposure and postexposure lethality. According to eqn (3), animal deaths will start to occur when the *N*-gas value is above 0.8% and 100% of the animals will die when the value is above 1.3. The results indicated that in those few cases where the values were above 0.8 and no deaths occurred, the animals were severely incapacitated

(close to death) as demonstrated by no righting reflex or eye reflex.

$$N\text{-gas value} = \frac{m[\mathrm{CO}]}{\mathrm{CO_2} - b} + \frac{21 - [\mathrm{O_2}]}{21 - \mathrm{LC_{50}(O_2)}} + \left(\frac{[\mathrm{HCN}]}{\mathrm{LC_{50}(HCN)}} \times \frac{0.4[\mathrm{NO_2}]}{\mathrm{LC_{50}(NO_2)}}\right) + 0.4\left(\frac{[\mathrm{NO_2}]}{\mathrm{LC_{50}(NO_2)}}\right) + \frac{[\mathrm{HCl}]}{\mathrm{LC_{50}(HCl]}} + \frac{[\mathrm{HBr}]}{\mathrm{LC_{50}(HBr)}} \quad (3)$$

The N-gas model including NO_2. For an explanation of these terms, see the paragraphs following eqn (1). Equation (3) should be used to predict the within-exposure plus postexposure lethal toxicity of mixtures of CO, CO_2, HCN, reduced O_2, NO_2, HCl, and HBr. The LC_{50} values will be the same as those given for eqn (1) using 150 ppm for HCN and 200 ppm for NO_2. If one wishes to predict the deaths that will occur only during the exposure, the LC_{50} value used for HCN should be 200 ppm and the HCl and HBr factors should not be included. To predict the lethal toxicity of atmospheres that do not include NO_2, eqn (1) is to be used.

Combustion Toxicity Test Methods

The toxicity of the combustion products from any new material formulation or product containing additives or new combinations of additives needs to be examined. Material and polymer chemists are currently trying to develop new 'fire safe' materials. The terms 'fire safe' or 'fire resistant' are not the same as noncombustible. Unless these new materials are truly noncombustible, some thermal decomposition will occur when the materials are exposed to fire conditions. The toxic gases and the irritants that are present in all smoke need to be considered potential dangers. The toxic products can cause both acute and delayed toxicological effects. It is the acute and extremely short-term effects that prevent escape from burning buildings by causing faulty judgment, incapacitation, and death. The irritants in the smoke can also interfere with the passengers' ability to escape by causing severe coughing and choking and by preventing them from keeping their eyes open long enough to find the exits. In addition, the delayed effects, such as tissue or organ injury, mutagenicity, carcinogenicity, and teratogenicity, need to be studied since they may ultimately lead to permanent disability and postexposure deaths.

Toxicity screening tests for both the acute and delayed effects are, therefore, needed to evaluate the combustion products including any irritants that may be present in newly proposed materials and products. It is imperative that the materials and products be tested under experimental conditions that simulate realistic fire scenarios of concern (e.g., flash-over conditions emanating from first, smoldering and then, flaming of upholstered furniture in homes or smoldering fires in concealed spaces in aircraft). The ideal tests should be simple, rapid, inexpensive, use the least amount of sample possible (since, in many cases, only limited amounts of new experimental materials may be available), use a minimum number of test animals, and have a definitive toxicological end point for comparison of the multiple candidates. While faulty judgment and incapacitation are significant causes of worry since they can prevent escape and cause death, they are extremely difficult and complex end points to define and measure in nonhuman test subjects. Death of experimental animals (e.g., rats), on the other hand, is a more definitive and easily determined end point and can be used to compare the relative toxicities of alternate materials deemed suitable for the same purpose. The assumption made here is that if the combustion products of material X are significantly more lethal than those of material Y, the combustion products of X would probably cause more incapacitation and more impairment of judgment than Y as well. The number of experimental animals can be significantly reduced by utilizing one of the predictive mathematical models developed for combustion toxicology such as the *N*-gas models previously discussed in this chapter. The six-gas *N*-gas model is currently included in two national standards (ASTM E1678 and NFPA 269) and one international standard (ISO 13344).

Many test methods for the determination of the acute toxicity of combustion products from materials and products have been developed over the last two decades and continue to be developed and/or improved. In 1983, 13 of the methods published up to that time were evaluated by Arthur D. Little, Inc. to assess the feasibility of incorporating combustion toxicity requirements for building materials and finishes into the building codes of New York State. On the basis of seven different criteria, only two methods were found acceptable. These two methods were the flow-through smoke toxicity method developed at the University of Pittsburgh and the closed-system cup furnace smoke toxicity method developed at NIST (known at that time as the National Bureau of Standards (NBS)). Standard Reference Materials and protocols (SRM 1048 and SRM 1049) were developed at NIST and are available to the users of these methods to provide assurance that they are performing the methods correctly (see 'Relevant Websites'

section). Based on the results of the Arthur D. Little report, the state of New York under Article 15, Part 1120 of the New York State Fire Prevention and Building Code decided to require that building materials and finishes be examined by the method developed at the University of Pittsburgh and that the results be filed with the state. It is important to note, however, that although the results are filed, the state of New York does not regulate any materials or products based on the results of this or any other toxicity test. Although not regulated, the process of testing by the developer should prevent any unduly toxic products from appearing in the marketplace.

New methods that have been developed since 1983 to examine acute combustion toxicity include the University of Pittsburgh II radiant furnace method, a radiant furnace smoke toxicity protocol developed by NIST and SwRI, and the National Institute of Building Sciences (NIBS) toxic hazard test method. All three use radiant heat to decompose materials.

The NIST radiant test and the NIBS toxic hazard test use the same apparatus, consisting of three components: a radiant furnace, a chemical analysis system, and an animal exposure chamber. The chemical analysis system and animal exposure system are identical to that developed for the NBS cup furnace smoke toxicity method. Although the apparatus of both methods are essentially the same, they have different toxicological end points. In the NIST method, an approximate LC_{50}, based on the *mass* of material needed to cause lethality in 50% of the test animals during a 30 min exposure and/or a 14 days postexposure period, is the determinant of toxicity. The number of animals needed to run the test is substantially reduced by first estimating the LC_{50} by the six-gas *N*-gas model. This estimate is then verified with one or two animal tests to assure that no unforeseen gas was generated. The toxicological end point of the NIBS toxic hazard test is the IT_{50}, the irradiation *time* (the time that the material is exposed to the radiant heat) that is required to kill 50% of the animals during a 30 min exposure or 14 days postexposure time. The actual results of the NIBS test with 20 materials indicated that the test animals died in very short periods of time (personal communication) and the test was unable to discriminate very well between materials. These results substantiate the thesis that mass (the smaller the mass necessary for an LC_{50}, the more toxic the material) is a better indicator of acute toxicity than time.

Both the NIST and NIBS test procedures are designed to simulate a postflashover scenario. The premise for simulating a postflashover fire is that most people who die from inhalation of toxic gases in *residential* fires are affected in areas away from the room of fire origin. Smoke and toxic gases are more likely to reach these distant areas following flashover. This scenario may not be relevant in certain circumstances (e.g., aircraft interior fires, where a smoldering fire in a concealed space may cause significant problems if the plane is over a large body of water and unable to land for a considerable period of time or the Station Nightclub in the early 2000s fire in West Warwick (Rhode Island, USA) that killed 100 and injured over 200 people and resulted from a combination of the use of pyrotechnics inside the nightclub, the use of a highly flammable foam on the walls, no sprinkler system and outdated fire codes).

The NIST radiant test has been accepted by ASTM as a US national standard designated ASTM E1678 and entitled "Test Method for Measuring Smoke Toxicity for Use in Fire Hazard Analysis." In 1995, the International Organization for Standardization, Technical Committee 92, Subcommittee 3 (ISO/TC92/SC3) on Toxic Hazards in Fire published an international standard for combustion toxicity that was approved by 16 countries. This standard – ISO/IS 13344 entitled "Determination of the Lethal Toxic Potency of Fire Effluents" – describes the mathematical models (including the six-gas *N*-Gas Model) available for predicting the toxic potency of fire atmospheres based on the toxicological interactions of the main combustion gases present. In the international standard, investigators have the flexibility of designing or choosing a system that will simulate conditions relevant to their fire scenario, rather than having to accept a designated combustion system.

Toxicant Suppressants

Fire scientists are very familiar with fire-retardant chemicals, which are defined by ASTM as "chemicals, which, when added to a combustible material, delay ignition and combustion of the resulting material when exposed to fire." The discussion adds "a fire-retardant chemical can be a part of the molecular structure, an admixture or an impregnant." The term 'toxicant suppressant', however, is a new term arising from research at NIST which demonstrated that the addition of copper compounds to flexible polyurethane foam (FPU) significantly reduced the generation of hydrogen cyanide (HCN) as well as the toxicity of the combustion products when the foam was thermally decomposed. These experiments were designed to simulate the nonflaming and then flaming stages of a chair ignited by a cigarette (a two-phase heating system which simulates the fire scenario that results in the most fire deaths in the United States). The term 'toxicant suppressant' may be defined as a chemical, which, when added to a

combustible material, significantly reduces or prevents one or more toxic gases from being generated when that material undergoes thermal decomposition. The resultant gas effluent should be less toxic than that from the untreated material, that is, the toxic gas, whose concentration is being reduced, should not be converted to an equally or more toxic product.

The results of these studies at NIST indicated that:

1. Hydrogen cyanide concentrations in the thermal decomposition products from a flexible polyurethane foam were reduced ~85% when the foam was treated with 0.1% or 1.0% Cu_2O by weight and thermally decomposed via a two-phase heating system in the NIST Cup Furnace Smoke Toxicity Apparatus.
2. The copper or copper compounds could be added to the foams during or after the foams were formulated and still reduce the HCN yield and toxicity of the combustion products. (NIST added the copper after formulation; the BASF Corporation added the Cu_2O during formulation.) The addition of the copper or copper compounds during formulation did not affect the foaming process or the physical appearance on the foams except for a slight change of color.
3. Low levels of the copper compounds were effective. In particular, when cupric oxide (CuO) was used, the concentration of copper needed was only 0.08% by weight and when cuprous oxide (Cu_2O) was used, only 0.07% by weight was needed to significantly reduce the generation of HCN.
4. Full-scale room burns indicated that the presence of Cu_2O in the flexible polyurethane foam reduced the HCN generation by ~50–70% when the experimental plan was designed to simulate a realistic scenario (the foams contained 1.0% Cu_2O by weight, were covered with a cotton upholstery fabric and arranged to simulate a chair; smoldering was initiated with cigarettes and flaming occurred spontaneously).
5. Under small-scale conditions, less than 3 ppm of NO_x was generated from the untreated foams, whereas a range of 3–33 ppm of NO_x was measured from 0.1% to 1.0% by weight Cu_2O-treated foams. About 6% of the HCN appeared to be converted to NO_x. In the full-scale room tests, ~23% of the HCN appeared to be converted to NO_x. Since we have shown at NIST that NO_2 acts as an antagonist to HCN, this amount of NO_x may also act to counteract the immediate toxic effects of any residual HCN.
6. Since atmospheric oxygen (O_2) concentrations can reach very low levels in real fires, it was important to know whether the reduction of HCN by copper would occur under low O_2 conditions. Small-scale tests with the ambient O_2 concentrations as low as 6% indicated that the HCN levels were reduced by as much as 82% when the flexible polyurethane foam was treated with 0.1% Cu_2O by weight.
7. The toxicity of the gas effluent was also reduced (an indication that HCN was not being converted into some compound that was even more toxic). Fewer animal (Fischer 344 rats) deaths occurred during the 30 min exposures to the flexible polyurethane foam treated with the copper and copper compounds compared to the untreated flexible polyurethane foam. Toxicity based on LC_{50} values was reduced 40–70% in the small-scale tests with 0.1% Cu_2O-treated foams. The blood cyanide levels in the animals exposed to combustion products from the CuO-treated foams for 30 min were 1/2 to 1/4 of those measured in the animals exposed to the smoke from the same amount of untreated foam.
8. Postexposure deaths were also reduced in the animals exposed to the combustion products from the Cu and Cu_2O-treated FPU foams in the small-scale tests. These delayed postexposure deaths have *not* been observed in animals exposed to combustion products from flexible polyurethane foams decomposed in large-scale room fire tests. The specific cause of these postexposure deaths is not known.
9. No differences in flammability characteristics between the 0.1% Cu_2O-treated and untreated flexible polyurethane foam were observed. These characteristics were examined to assure that the positive effect on toxicity was not contradicted by negative effects on the flammability properties. The flammability characteristics examined were: (1) ignitability in three systems (the NIST Cup Furnace Smoke Toxicity method, the Cone Calorimeter, and Lateral Ignition and Flame Spread Test (LIFT)), (2) heat release rates under small-scale (Cone Calorimeter) and medium-scale (furniture calorimeter) conditions, (3) heats of combustion under small-scale (Cone Calorimeter) and medium-scale (furniture calorimeter) conditions, (4) CO/CO_2 ratios under small-scale (Cone Calorimeter) and medium-scale (furniture calorimeter) conditions, (5) smoke obscuration (Cone Calorimeter), and (6) rate of flame spread (LIFT).
10. Research conducted at the BASF Corporation indicated that the physical properties of the

1.0% Cu_2O-treated flexible polyurethane foam were not significantly different from the comparable untreated flexible polyurethane foam. The physical properties examined were tensile strength, elongation, tear strength, resilience, indentation force deflection, support factor, compression sets, and airflow.

11. One of the additives being used in combustion-modified flexible polyurethane foams is melamine. Small-scale tests conducted at NIST indicated that a melamine-treated flexible polyurethane foam generated six times more HCN than an equal amount of foam that did not contain melamine. The presence of Cu_2O reduced the HCN from the melamine foam by 90%. Melamine-treated flexible polyurethane foam is one of two flexible polyurethane foams currently allowed in Great Britain.

In the late 1970s, research by Jellinek *et al*. at Clarkson College of Technology also showed that the concentrations of HCN generated from the thermal decomposition of a polyurethane at 300°C and 400°C decreased when flowed through copper compounds. In their studies, the polyurethane films were usually 15 μm thick (50 mg). In some experiments, the metal powder was mixed with the polymer and, in others, copper metal films of 400–1000 Å were deposited on top of the polymer films. In most cases, the percentage of copper was 10% or greater. The lowest concentration that they tested was a 2.6% copper film, which inhibited the evolution of HCN by 66%. Their experiments indicated that copper probably acts as an oxidative catalyst that decomposes gaseous HCN into N_2, CO_2, H_2O, and small amounts of nitrogen oxides. Further research is needed to determine whether this is the actual molecular mechanism that allows copper to act as a HCN toxicant suppressant.

Research by Levin *et al*. at NIST differed from that of Jellinek in that much larger samples of flexible polyurethane foam (including full-scale room burns of cushions and simulated chairs), and much smaller concentrations of copper were used. The toxicity of the combustion products from the copper-treated flexible polyurethane foam was also examined.

Unpublished data of Levin *et al*. also indicated that a wool fabric treated with copper would generate 50% less HCN than the untreated fabric. These results demonstrate a more universal effect, namely that treating nitrogen-containing materials with copper compounds will probably reduce the HCN generated when that material is exposed to fire conditions. Taking these results one step further, one could develop other toxicant suppressants, which, when added to materials and products, would now prevent or significantly reduce the toxic effluents that are generated when they are thermally decomposed. Since 80% of fire deaths are the result of smoke inhalation, a less toxic smoke could significantly increase the time available for escape and reduce the number of injuries and deaths from fire.

See also: Carbon Dioxide; Carbon Monoxide; CCA-Treated Wood; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50).

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Relevant Websites

http://www.wolmanizedwood.com – Information on Wolmanized pressure-treated wood.

http://ts.nist.gov – NIST website providing information of standard reference materials and protocols developed.

Common Mechanism of Toxicity

Beth Mileson

Common mechanism of toxicity is a phrase used to characterize the toxicological actions of two or more agents that act by the same cellular and molecular mechanisms leading to a common adverse effect on the structure or function of a living organism. An understanding of all steps that comprise a common mechanism of toxicity for given toxicants is rarely achieved, but identification of the crucial events following chemical interaction with an organism can be sufficient to describe a common mechanism of toxicity. For example, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and its congeners are a group of toxicants that have been characterized as acting by a common mechanism of toxicity, mediated by binding to the aryl hydrocarbon (Ah) receptor in animals.

The concept of common mechanism of toxicity gained prominence in the United States after Congress passed the Food Quality Protection Act of 1996 (FQPA), which requires the US Environmental Protection Agency (EPA) to consider the effects of human exposure to all pesticides and other chemicals that act by a common mechanism of toxicity when they derive tolerances (acceptable levels) for pesticide use on crops. As a result of the FQPA, the term common mechanism of toxicity has a regulatory connotation in addition to a toxicological definition. The US EPA Office of Pesticide Programs (OPP) makes an official determination to identify specific pesticides that act by a common mechanism of toxicity. After the common mechanism group is identified, OPP conducts a cumulative risk assessment of exposure to all the pesticides in the common mechanism group when establishing, modifying, leaving in effect, or revoking a tolerance for a pesticide chemical residue, as specified in the FQPA.

The US EPA OPP has issued guidance to identify pesticides and other chemicals that act by a common mechanism of toxicity. The first step in this guidance is to determine whether a group of pesticides should be considered a 'preliminary grouping' of compounds that may act by a common mechanism of toxicity. Characteristics that OPP suggests may be an indication that substances act by a common mechanism of toxicity include: (1) compounds share a structural similarity; (2) pesticides have a similar mechanism of insecticidal action; (3) compounds act by the same general mechanism of mammalian toxicity, and/or (4) cause a particular toxic effect.

The EPA OPP refines the evaluation of a preliminary common mechanism group by considering the biochemical and toxicological actions of each toxicant to determine if they all act by a common mechanism of toxicity, or if they should be separated into more than one common mechanism group for cumulative risk assessment. To determine if a preliminary group of compounds all act by a common mechanism of toxicity, the actions of these chemicals are evaluated based on whether they cause the same critical effect, act on the same molecular target at the same target tissue, act by the same biochemical mechanism, and/or share a common toxic intermediate. To evaluate mechanisms of toxicity, the US EPA OPP relies on data from studies submitted in support of pesticide registration, data from the public literature, and data from government reports.

The Pest Management Regulatory Agency (PMRA) of Health Canada proposed to harmonize with the US EPA policy on common mechanism of toxicity. PMRA issued guidance for identifying pesticides that have a common mechanism of toxicity that was adapted from the US EPA guidance document.

An example of a group of toxicants that act by a common mechanism of toxicity is the organophosphorus (OP) pesticides. OP pesticides are an otherwise structurally diverse group of chemicals that all contain phosphate atoms that are pentavalent and tetracoordinate. The primary molecular mechanism of action of most of the OP pesticides is initiated by inhibition of acetylcholinesterase (AChE), a serine esterase that occurs throughout the central and peripheral nervous system of vertebrates. The normal physiological action of AChE is to hydrolyze the neurotransmitter acetylcholine (ACh) so that activation of cholinergic receptors is transient. Inhibition of AChE results in accumulation of ACh at the synapses, overstimulation of cholinergic receptors on muscle fibers, neurons, and autonomic end organs, and resultant signs of cholinergic toxicity. Clinical signs of cholinergic toxicity include: increased lacrimation and salivation, bronchoconstriction, bronchosecretion, miosis, gastrointestinal cramps, diarrhea, urination, bradycardia, tachycardia, hypertension, muscle fasciculations, tremors, and muscle weakness, among other signs. OP pesticides have been determined to act by a common mechanism of toxicity if they inhibit AChE by phosphorylation and elicit any spectrum of cholinergic effects in exposed animals or humans.

Many classes of pesticides are composed of structurally similar compounds that act by a common

mechanism of toxicity. Examples of pesticide groups that contain multiple compounds that act by a common mechanism of toxicity in addition to the OP pesticides include: methyl carbamates, triazines, and pyrethroids. The fact that groups of pesticides act by a common mechanism of toxicity is predictable since these chemicals were designed to resemble one another structurally and elicit similar pesticidal effects.

See also: Food Quality Protection Act, US; Pesticides; TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin).

Further Reading

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Relevant Website

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Comprehensive Environmental Response, Compensation, and Liability Act, US

Robert Kapp

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- TITLE: CERCLA; revised as the Superfund Amendments Reauthorization Act (SARA): also known as the Superfund
- AGENCY: US Environmental Protection Agency (EPA)
- YEARS ENACTED AND REVISED: CERCLA: December 11, 1980; SARA: October 17, 1986

Background

Until the 1970s, US citizens were mostly unaware of how the indiscriminate dumping of chemical wastes might affect public health and the environment. The common practice was to simply abandon waste chemicals on properties and in landfills with no consideration for their ultimate fate. This resulted in thousands of uncontrolled and abandoned hazardous waste sites throughout the nation. Although the Environmental Protection Agency (EPA) had been created in 1970, there were no specific provisions to deal with this ever-growing environmental problem. The Clean Water Act – CWA (1977), the Resource Conservation and Recovery Act – RCRA (1976), the Water Pollution Control Act (1972), and the Rivers and Harbors Act (1899) set the stage for more comprehensive legislation. However, in 1979, the events at Love Canal, New York brought to a head the fact that abandoned hazardous waste could be a serious threat to any community. Citizen concern was high over the magnitude of the ever-growing problem and that led Congress to establish The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) to locate, investigate and clean up the worst sites nationwide.

Overview of CERCLA (Superfund)

On December 11, 1980, Congress passed the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, also known as the Superfund). This law strengthened the EPA and State authorities to investigate and respond to the release of waste and hazardous materials into the environment. The Office of Emergency and Remedial Response at EPA administers the program in cooperation with states and tribal governments. This legislation essentially created a tax on the chemical and petroleum industries, and provided broad Federal authority to respond directly to chemical releases or potential releases of hazardous substances that are deemed threatening to public health or the environment. The taxes collected from inception through 1986 totaled US$1.6 billion.

The legislation was amended on October 17, 1986 with the enactment of the Superfund Amendments and Reauthorization Act (SARA). One of the changes in SARA was to increase the fund from $1.6 to $8.5 billion, and SARA required the EPA to make changes to the Hazard Ranking System to more accurately note the level of danger of sites to be placed on the National Priorities List (NPL). In addition, the NPL must be revised and republished every 2 years, and informally reviewed annually. The term ‘Superfund’ is derived from the fund of money that is collected by EPA to investigate sites and to

pay for cleanups where no responsible parties can be determined.

There are four basic components to the CERCLA/Superfund Legislation:

1. The legislation sets up an information-gathering and analysis system that enables federal, state and tribal governments to designate chemical dumpsites and develop priorities for cleaning them up. The EPA Administrator issues regulations that, in the Administrator's opinion, identify 'hazardous' or 'toxic' substances. The owners and operators of sites containing any listed chemical must notify the EPA of the amount and types of identified wastes their sites contain. This information assists the EPA in developing the NPL to plan responses.
2. The legislation also gives the EPA the authority to respond to toxic substance emergencies where immediate short-term response is deemed necessary for the public welfare.
3. The legislation further established a Hazardous Substance Trust Fund to pay for removing wastes and for remedial actions associated with the cleanup where no responsible parties can be determined. SARA revised and expanded the 1980 CERCLA legislation from its original $1.6 billion budget to $8.5 billion.
4. The legislation holds responsible persons and companies liable for toxic wastes cleanup and restitution costs. Unfortunately, the legislation was not clear on this point and not all federal courts have applied the same standards to determine parent corporation liability for CERCLA infractions.

Site Cleanups – Remediations and Removals

- Remediations are conducted according to the National Contingency Plan and refer to permanent cleanups.
- Removals are cleanups other than permanent, that is, emergency or temporary cleanups.
- EPA generally takes remedial actions only at sites listed on the NPL (which currently lists ~1300 sites).
- EPA assigns responsibility by looking at all potentially responsible parties (PRPs), including current owners/operators, previous owners/operators, facilities that generated the waste and transporters that delivered the waste.
- 'Strict' liability is defined as parties being responsible regardless of how careful they were in their practice of disposing of the waste.
- 'Joint and several' liability is defined as any one PRP is potentially liable for all costs of the cleanup no matter how much of the total contamination is directly due to their disposal activities.
- General remediation process is specified under the Superfund's National Contingency Plan as follows:
  - emergency removal to address immediate environmental problems;
  - a remedial investigation/feasibility study (RI/FS) to determine cleanup approaches;
  - a Record of Decision to document the approach EPA has selected for cleanup; and
  - the design, construction, operation and maintenance of the final cleanup.
- In addition, the cleanup process is required to meet all other environmental requirements during its operation. These are referred to as applicable or relevant and appropriate requirements.

New Releases

The CERCLA hazardous substance release reporting regulations in the US Code of Federal Regulations (40 CFR Part 302) are intended to minimize environmental releases of current manufacturing processes. A designated person at the facility must report the release of any hazardous material when it exceeds the reportable quantity to the National Response Center. The hazardous substances and reportable quantities are defined and listed in 40 CFR §302.4.

The National Response Center telephone number is: 1-800-424-8802

US EPA's RCRA/Superfund/UST Hotline to answer questions regarding guidance for the Superfund Program: 1-800-424-9346.

See also: Love Canal; National Environmental Policy Act, US; Pollution Prevention Act, US; Resource Conservation and Recovery Act, US; Valley of the Drums.

Relevant Websites

http://www4.law.cornell.edu – Cornell University Law School, US Code covering CERCLA.
http://www.epa.gov – US EPA CERCLA information.

Computational Toxicology

S Satheesh Anand and Harihara M Mehendale

Definition

Computational toxicology is the application of mathematical and computer models for prediction of effect of toxic agents and understanding the mechanism.

Background

Protecting human health from the possible hazardous effects of toxic chemicals is a challenging task. A number of scientific uncertainties exist along a 'source-to-adverse outcome' continuum, beginning with the presence of the chemical in the environment, frequency and duration of exposure, disposition of the chemical, the presence of the active chemical at a systemic target site, and the series of biological events that lead to the manifestation of an adverse outcome. Unexpected toxicity due to interaction and altered toxicity by lifestyle such as smoking, drinking, etc. are additional issues. Present risk assessment methods rely on laboratory testing of chemical-to-chemical basis to obtain toxicity data and the quantitative relationship between dose level and likelihood of toxic response to estimate human risk. The large number of chemicals in commerce coupled with time and expense limit the testing to a few chemicals. Moreover, the question pertaining to high to low dose and animal to human extrapolation still remains. In view of some 87 000 chemicals under consideration, it would be beneficial if rapid testing methods were developed to assist prioritization of chemicals for further testing and reduce the existing uncertainties in risk assessment.

Over the last several years, there has been increasing pressure to reduce the animal use in toxicology and to utilize novel technologies such as *in vitro* methods, and computational chemistry for rapid identification of chemical risks. The *in vitro* data are not sufficiently validated to address the uncertainties in risk assessment. Hence, the interest has shifted toward using computers, which are capable of performing a series of complex arithmetic or logical operations and have the ability to process, store, and retrieve data without human intervention. The use of computers in toxicology has increased significantly in recent years. Computational toxicology involves the application of various mathematical and computer models to predict effects and understand the cascade of events that result in an adverse response, or its mode of action. It would improve linkages across the source-to-outcome continuum, including the areas of chemical transformation and metabolism, better diagnostic/prognostic molecular markers, improved dose metrics, characterization of toxicity pathways, metabonomics, systems biology approaches, modeling frameworks, and uncertainty analysis.

Computational toxicology includes several computational disciplines including:

- Computational chemistry, which refers to physical–chemical mathematical modeling at the molecular level and includes such topics as quantum chemistry, force fields, molecular mechanics, molecular simulations, molecular modeling, molecular design, and cheminformatics.
- Computational biology or bioinformatics, which refers to development of molecular biology databases and the analysis of the data.
- Systems biology, which refers to the application of mathematical modeling and reasoning to the understanding of biological systems and the explanation of biological phenomena.

Three strategic objectives of the computational toxicology initiative are to:

1. improve understanding of the linkages in the continuum between the source of a chemical in the environment and adverse outcomes;
2. provide predictive models for screening and testing; and
3. improve quantitative risk assessment.

Computer in Contemporary Toxicology

Computational toxicology is a rapidly emerging and developing area, combining theoretical models with computers to investigate a variety of toxicological problems. In order to achieve the precise predictive methodology, it is necessary to link adverse outcomes to initiating events and the target organ's response to injury. Computational toxicology techniques have excellent promise to focus research on reducing uncertainties in both ecological and human health risk assessments. The use of computer in toxicology has steadily increased from literature survey, data mining, and statistical analysis to predicting toxic outcome and reducing uncertainties in risk assessment.

Several computer software programs are available for compartmental modeling of pharmacokinetic

data (such as WinNonlin, PK analyst, Summit, SAS). In general, concentration and time data are entered into a spreadsheet format. The operator then chooses a user-defined model or a specified model from a built-in library to fit curves to concentration versus time data. Program outputs include pharmacokinetic parameter estimations and descriptive statistical estimations. A wide variety of options and costs are available that can fit users' needs.

The following are the emerging approaches in toxicology in which use of computer is pivotal:

'Omics'

The unprecedented advances in molecular biology during the last two decades have resulted in a dramatic increase in knowledge about gene structure and function, an impressive set of efficient new technologies for monitoring genetic sequences, genetic variation, and global functional gene expression. These advances have led to a new subdiscipline of toxicology, 'toxicogenomics', which includes studies of the cellular products controlled by the genome (messenger RNAs, proteins, metabolites, etc.). The new 'global' methods of measuring families of cellular molecules, such as RNA, proteins, and intermediary metabolites, have been termed 'omic' technologies. The computer is an integral part in the 'omics' field and it is very difficult to handle the data generated by these technologies without computers. The development of 'omic' technologies has evolved into three scientific disciplines. Systems biology allows one to integrate the complex information developed by three areas of 'omics' at the organismic level.

Genomics

Genomics is the study of genes and their biological function. It has been done using the microarray technique (also called DNA chips), which contains many hundreds or thousands of short DNA strands, each in its own compartment. By washing a solution of a substance over the whole chip at once, the section of DNA affected can be made to fluoresce, thus indicating which genes are turned on or off by the substance and suggesting its likely effect on the body. Presently, each chip contains a particular number of genes and it may soon be possible to include the whole human genome on such a chip and test all of it at once for possible adverse effects. The advantage of using the genomic technology is that it will capture the changes in the genes, which are not originally under investigation. It is expected that genomics will greatly help in defining and characterizing sensitive subpopulations and producing signature profile for each toxin. There are number of software such as arraySCOUT, GeneSpring, Spotfire DecisionSite for Functional Genomics available to read and interpret the genomic data.

Proteomics

Proteins are involved in all biological processes and can therefore be considered the functionally most important biological molecules and are crucial for the description of biological systems. The systematic identification and characterization of proteins is called proteomics. A predominant technology platform in proteomics, two-dimensional gel electrophoresis, is used to separate complex protein mixtures allowing individual protein spots on the gel to be identified by computer-operated mass spectrometry. Mass spectrometric data are then processed through a series of computer algorithms such as Mass Lynx and ProteinLynx software to determine the sequence identity of the proteins.

Metabonomics

Metabonomics is a systems approach for studying *in vivo* metabolic profiles and is still a relatively new technology in comparison to the other 'omics'. Genomics and proteomics allow for the measurement of response to chemicals on the genetic and cellular protein levels, respectively; however, neither provides a complete description of metabolism and chemical toxicity. To fully understand the xenobiotics metabolism, it is crucial to understand the metabolic status of the whole organism. Metabonomics complements genome and proteome responses and provides connection between these and tissue function. The application of metabonomics to toxicity testing involves the elucidation of changes in metabolic pattern associated with chemical toxicity based on the measurement of component profiles in biofluids, cells, or tissues. Metabolites are assayed in biofluids using nuclear magnetic resonance spectroscopy. Like proteomics and genomics, metabonomics provides a fingerprint of the small molecules contained in a given biofluid. Software such as Eclipse and MATLAB are used for bioprofiling of metabolites.

Systems Biology

Systems biology is a new field, which integrates genomic, proteomic, and metabonomic information into a coherent picture. Systems biology is brought by joining computer science, biology, and medical programs and could lead to the development of virtual biological systems. Systems biology uses computational methods to reconstruct an integrated physiologic and biochemical model of an organism's

or cell's biology that allows validation and simulation experiments that build confidence in predictive ability of adverse effects. In this regard, it is targeted at studying how normal biological processes are governed, and how alterations can lead to diseases or other unwanted outcomes.

The 'omics' technology is a high throughput separation of genes, proteins, and metabolites and has the potential to be a powerful tool in risk assessment. However, it is still in its infancy. The advantage of using these approaches is to capture the effects of compounds at low doses, which are not possible in animal testing. These techniques generate huge amount of data and it is not easy to analyze. Bioinformatics aids in making meaningful conclusions from a deluge of data points. Advanced computer software are now able to interpret the data with an increasing degree of accuracy. In addition to reading and interpreting the data, computers are making it possible to apply complex analytical techniques used in 'omics'.

It is anticipated that these new technologies will: (1) lead to new families of biomarkers that permit characterization and efficient monitoring of cellular perturbations; (2) provide an increased understanding of the influence of genetic variation on toxicological outcomes; and (3) allow definition of environmental causes of genetic alterations and their relationship to human disease. With the development of 'omics', the discipline of toxicology is acquiring exciting new tools in safety screening, problem solving, and mechanistic investigation. Assuming a group of compounds induces similar changes in the gene, protein, or metabolic profile, it may be possible to classify compounds based on their profiles; i.e., it would provide a 'fingerprint'.

Quantitative Structure–Activity Relationships

Quantitative structure–activity relationship (QSAR) dates back to the nineteenth century and is a computer-based tool that attempts to correlate variations in structural or molecular properties of compounds with their biological activities. These physicochemical descriptors, which include parameters to account for hydrophobicity, topology, electronic properties, and steric effects, are determined empirically or, more recently, by computational methods. The premise is that the structure of a chemical determines the physiochemical properties and reactivities that underlie its biological and toxicological properties. Being able to predict potential adverse effects not only aids in the designed development of new chemicals but also reduces the need for animal testing. It may ultimately or potentially lead to better health and environmental protection through the strategic application of limited testing resources and existing information assets to help sort out or identify the most hazardous chemicals. These principles have already been successfully applied to the prediction of skin permeability coefficients, the skin corrosivity of organic acids, bases, phenols, and electrophilic organic chemicals, and the eye irritation potential of neutral organic chemicals. QSARs are currently being applied in many disciplines pertaining to drug design and development. Lately, interest has grown in utilizing this tool in environmental risk assessment to group the compounds of similar biological activity. Software such as TOPKIT and CaseTox are used in QSAR modeling.

Biological variability can be demonstrated quite readily by building a QSAR model, which discriminates between chemicals with different toxicological hazard classifications. Application of computer-based QSARs has resulted in developing novel predictive capabilities for representing chemical structures as a distribution of conformations and properties rather than discrete structures.

Physiologically Based Pharmaco (Toxico) Kinetics

Knowledge of the concentration–time relationships of toxicants in the biological systems is paramount in predicting toxicological effects. The commonly used pharmacokinetic models cannot be used for extrapolation due to the lack of actual anatomical, physiological, and biochemical realism. The extrapolation is possible when the mathematical descriptions of the uptake and disposition are combined with the knowledge of anatomy, physiology, and biochemistry and is termed physiologically based pharmaco (toxico) kinetics (PBPK) models. These models are used to gain insights into the dosimetry and mode of action of chemicals. A PBPK model is founded on known physiological processes (blood flow rates, tissues volumes, breathing rates, etc.), on chemical-specific processes (partition coefficients, chemical density, metabolic constants, molecular weight, etc.), and on species-dependent processes. Thus, a PBPK model can be developed and validated against the more readily available experimental animal data, and then extrapolated to predict concentrations of toxic compounds in human tissues following exposure. PBPK models can be used to extrapolate from a single acute to a chronic exposure, from a continuous to an intermittent exposure, from a high to a low exposure, and from a single chemical to a complex mixture exposure. Perhaps the greatest impact in using a PBPK model to convert exposure into a target

tissue dose is the flexibility to perform these calculations on an individual basis. Models can be developed to individually track each person's unique physiological and/or metabolic characteristics. The metabolic rates in a model can be individually adjusted to account for enhanced (or decreased) metabolism of a particular compound when the person is on a specific pharmaceutical agent. PBPK modeling is a powerful toxicological tool designed to convert an exposure (regardless of route) into a target tissue dose.

In PBPK models, the body is divided into compartments with blood flow to each compartment. Chemicals can be introduced into the body by several routes, metabolized, and excreted in breath, feces, and urine. The compartments and flows are described by a system of differential mathematical equations. In order to write and solve the mathematical equation and interpret the data, the help of a computer is essential. The PBPK model equations, along with the integration algorithms, can be written and solved using programming language (FORTRAN, BASIC, etc.), simulation software (ACSL, acslXtreme, Matlab, Simusolv, STELLA, etc.), or spreadsheets on many type of computers – mainframes, workstations, microcomputer, or minicomputers. The processing speed, hard disk space, and run time memory of the currently marketed computers are quite helpful in PBPK modeling.

Computational Toxicology in Quantitative Risk Assessment

Following are the areas in which computational toxicology is expected to reduce or eliminate the uncertainties:

Dose–Response Assessment

Computer-based technologies would help in determining the shape of the dose–response curve in the low dose range based upon *in vivo* and *in vitro* data, and to correlate with low dose adverse effects. These technologies may also result in the identification of useful biomarkers, adverse effect, and mode of action of the low dose range.

Cross-Species Extrapolation

One of the major challenges in regulatory toxicology is the prediction of toxicity of a chemical across the species; presently, the effects in humans are predicted from animal studies. The concept of extrapolation is based on the knowledge that all the species arise from common evolutionary ancestors. However, it is not trivial because chemicals vary as a function of an animal's physiology and its environment and pathways of metabolism can differ significantly across species, even closely related animals. These can be addressed by biologically based dose–response models. Although there is remarkable similarity in basic biology among animals, there are also significant species-specific differences in genes, proteins, biochemistry, and physiology. These differences lead to uncertainties in extrapolation. To address this, characterizing the toxicity pathways is critical.

Chemical Mixtures

Multichemical exposures are ubiquitous. A number of uncertainties exist from the mode of action, type of interaction to toxic outcome in the mixture risk assessment. Computer-based technologies have huge potential to elucidate the mechanisms and predict the outcome for real-world mixtures rather than the defined mixtures at high-dose concentration.

Sensitive Population

There is an increasing concern that some people are more susceptible to toxic effects of one chemical compared to others. This group includes children, pregnant women, elderly, genetic background, lifestyle such as smoking, alcohol consumption, diet, and existing disease conditions. With application of computational toxicology it is expected that we will be able to fine-tune our ability to predict the mechanisms behind the susceptibility and reduce the uncertainty.

Conclusions

Protecting human health and the environment carries with it the challenge of assessing the risk that is posed by tens of thousands of chemicals. The large numbers of chemicals, various uncertainties and difficulties associated with the regulation have made it impossible to evaluate the data on all the chemicals. Today, however, toxicology is getting a facelift with the help of recent computer-based approaches (e.g., genomics, proteomics, metabonomics, QSAR, and PBPK), and scientists may have the ability to develop a more detailed understanding of the risks posed by a much larger number of chemicals. When the biology is complex and the database is large (modern technologies generate prodigious amounts of data), then these models play an essential role in organizing data, identifying key data gaps, and generating predictions of dose–response behaviors. Computational

toxicology is expected to increase the accuracy or precision of risk assessment based on science. It is designed to increase the capacity to prioritize, screen, and evaluate chemicals by enhancing the predictive understanding of toxicities. Success will be measured by the ability to improve risk assessments by understanding the potential of chemicals to affect molecular and biochemical pathways of concern. Computational methods will not assure 100% accuracy in the prediction of toxicity, and they will not eliminate the need for testing, but these methods will dramatically slash the time, choice, and expense of testing chemicals and make regulatory decision-making quicker and science-based.

See also: Bioinformatics; Risk Assessment, Human Health; Safety Testing, Clinical Studies; Toxicity Testing, Validation.

Further Reading

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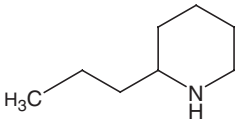
Relevant Websites

http://www.niehs.nih.gov – National Center for Toxicogenomics (NCT).
http://www.epa.gov – US EPA. Computational Toxicology.

Coniine

Murali Badanthadka and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 458-88-8
- Synonyms: S-2-Propylpiperidine; Cicutine; Conicine; *N*-Methylconine; Conhydrine; Pseuedoconhydrine
- Chemical/Pharmaceutical/Other Class: Piperidine alkaloid
- Chemical Formula: $C_8H_{17}N$
- Chemical Structure:



Background Information

Poison hemlock (*Conium maculatum*) and dog parsley (*Aethusa cynapium*) are poisonous plants of the parsley family, which contain coniine.

Exposure Routes and Pathways

The most common route of coniine exposure is by ingestion, although there are reports of dermal and eye irritations upon direct contact.

Toxicokinetics

Coniine is rapidly absorbed from the gastrointestinal tract.

Mechanism of Toxicity

Coniine acts on the autonomic ganglia to produce initial stimulation of skeletal muscle followed by neuromuscular blockade. The actions of coniine are similar to those of nicotine but produce paralysis of greater numbers of central nervous system (CNS) and skeletal muscle nerve endings.

Acute and Short-Term Toxicity (or Exposure)

Animal

Certain small birds (skylarks, chaffinches, and robins) are not susceptible to coniine poisoning. Coniine toxicity has been reported in cows, goats, horses, pigs, sheep, ewes, rabbits, and chickens. The oral LD_{50} of coniine in the mouse is $\sim 100\,mg\,kg^{-1}$. There are limited data in cattle, goats, and sheep suggesting developmental abnormalities to the musculoskeletal system of offspring when pregnant mothers are exposed orally to coniine ($70\,mg\,kg^{-1}$ for cattle and $484\,mg\,kg^{-1}$ for goats and sheep).

Human

Toxic doses of the plant extract are difficult to determine due to differing concentrations of eight piperidinic alkaloids in the plant. The concentrations of alkaloids vary with the age of the plant. Plants up to ~1-year-old have very low alkaloid content in roots, ~0.15% in stems and 0.3–0.6% in the leaves. Plants in their second year have an alkaloid content of ~1% in all parts of the plant. Geographic latitude and drying will also affect the coniine content of the plant. A toxic dose of coniine is estimated to be

60 mg and a lethal dose is estimated to be 100–300 mg for an adult person.

The principal manifestations of coniine poisoning are nausea and vomiting, salivation, fever, and gradually increasing muscular weakness followed by paralysis with respiratory failure.

Clinical Management

No antidote exists for coniine. Treatment is directed at removing ingested toxin and providing supportive care. Gastric lavage may be used to remove the ingested plant or plant extract. However, this method may not effectively remove large pieces of plant material. Intragastric administration of activated charcoal is recommended to reduce absorption in the gastrointestinal tract. Due to the rapid onset of CNS depression and seizures, emesis is generally not recommended. Difficulty to breath is treated by artificial respiration with oxygen. Convulsions are controlled with diazepam.

See also: Hemlock, Poison; Plants, Poisonous.

Further Reading

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Consumer Products

Nancy Linde

Introduction

There are products that we use everyday and products that we use only occasionally. Among both categories, there are products that are very tempting for children by the nature of the way the look, smell, sound, feel, or simply because they can be reached. According to the Federal Hazardous Substances Act of 1960, labeling of hazardous products must contain instructions for special handling and storage, as well as warning statements for accidental exposure prevention and treatment. Several common household products are briefly discussed here for their most common toxicological concerns.

Specific Categories and Concerns

Air Fresheners/Deodorizers

These products commonly contain formaldehyde. Accidental exposures, depending on the amount, may lead to eye, nose, throat and skin irritation, as well as nausea, nosebleeds, headaches, dizziness, memory loss, and shortness of breath. While these symptoms are typically reversible, formaldehyde is a regulated as a carcinogen.

Ammonia

Ammonia is a respiratory irritant when inhaled, and large doses may affect the nervous and circulatory system with rapid heart beat, weak pulse, bluish lips and fingers, restlessness, and temporary blindness. When ingested it can cause severe pain in the mouth, chest, throat, and stomach. Dermal contact can cause severe irritation, burning, and even permanent blindness.

Antifreeze

Consumption of small amounts of antifreeze can be deadly. Poisonous constituents are typically ethylene glycol and methanol. There is no home treatment aside from standard first-aid and cardiopulmonary resuscitation (CPR) for signs of shock or cardiac arrest. Gastric treatment and dialysis may be immediately necessary for survival depending on the dose, and long-term kidney and brain damage are possible.

Bleach

Powdered and liquid household bleach contain sodium hypochlorite. This is a respiratory irritant and can cause burning from inhalation, ingestion, or dermal contact. Contact with other chemicals can

lead to chlorine fumes, and when mixed with ammonia can result in formation of a nonodorous deadly methane gas. The extent of damage will depend on the amount consumed and how rapidly it is diluted and neutralized. Accidental ingestion should be followed by immediate consumption of milk or water to dilute, and vomiting should not be induced due to the burning potential. Long-term damage to the mouth, throat, eyes, lungs, esophagus, nose, and stomach are possible, some of which may continue to occur for weeks after exposure.

Detergents

Household detergents generally contain simple soaps and corrosive alkalis. Ingestion may lead to respiratory irritation, severe abdominal pain, vomiting, hypotension, and dangerous change in blood pH. Ingestion should be followed by immediate consumption of milk or water if possible, then immediate medical attention. The prognosis will depend on how rapidly the alkali was diluted and neutralized. Extensive damage to the mouth, throat, eyes, lungs, esophagus, nose, and stomach are possible, and depending on the severity of the damage, death could occur up to 1 month after ingestion.

Disinfectants

Common household disinfectants contain sodium hypochlorite, phenols, or ammonia.

Drain Cleaners

Drain cleaners may contain sodium or potassium hydroxide (lye), hydrochloric acid, or trichloroethane. Sodium or potassium hydroxide is a caustic irritant that can affect the central nervous system (CNS) inhibiting reflexes, cause burns to skin and eyes, and is poisonous if swallowed due to severe tissue damage. Hydrochloric acid is a corrosive irritant, causes damage to the kidneys, liver, and digestive system. Trichloroethane is a skin and eye irritant, causes central nervous system depression, and liver and kidney damage when ingested.

Dyes

Household dyes for cloth are typically nontoxic mixtures of pigments, salts, and mild soaps. Some contain small amounts of corrosive alkali detergent that can only be hazardous if consumed in large quantity.

Fertilizers and Household Plant Foods

Plant fertilizers are mildly toxic in small doses, and may lead to gastrointestinal upset and or skin irritation. Larger doses can be more harmful, especially to children, and may cause severe burns, therefore, vomiting should not be induced, but milk or water should be given to dilute poisonous ingestions. Poisonous ingredients are typically nitrates and nitrites.

Glue

Household glues such as Elmer's and Krazy Glue are generally nontoxic and would require large consumption to cause abdominal pain and obstruction of the gastrointestinal tract. Elmer's glue can be easily washed off the skin with soap and water, whereas Krazy Glue and other super glues (containing cyanoacrylates) generally require rinsing with nail polish remover or acetone. For eye contact, rinsing should be done only with water and/or an eye doctor should be seen if symptoms persist or the eyelids cannot be opened. Symptoms are generally treatable with full recovery. For effects related to inhalation of glue solvents, see section on Inhalants.

Hydrogen Peroxide

Household hydrogen peroxide is typically sold in a 3% solution. Short-term dermal exposures may whiten the skin temporarily. Ingestion can lead to burns in the oral cavity and throat, and abdominal pain. Dermal and ocular exposures should be immediately rinsed, and vomiting should not be induced.

Inhalants

Inhalant abuse is a drug abuse problem. Inhalants can be any substances that have volatile hydrocarbons as their base. Examples of hydrocarbons are acetone, benzene, toluene, turpentine, and gasoline. Volatile hydrocarbons can be classified into several groups. They range from high volatility, minimal viscosity substances such as methane, butane, benzene and petroleum ether to minimal volatility, high viscosity products such as lubricating oil, mineral oils and asphalt. The inhalation of these substances, especially those with high and intermediate volatility, can rapidly displace alveolar gas, causing difficulty in breathing. In addition, they can easily cross the capillary membrane of the lungs and affect the CNS. Most hydrocarbons are CNS depressants and the early effects of inhalation resemble alcohol intoxication. Continued inhalation however may lead to increased symptoms of intoxication, confusion, hallucination, and aggressive behavior.

As most of the harmful effects of inhalant abuse are not felt immediately, chronic abuse of inhalants is associated with a variety of medical problems with a real risk of death. There are a number of solvents that have become the target for abuse. A great number of them are known to be toxic (see **Table 1**).

Table 1 Common targets for consumer product inhalation abuse

Products	*Major volatile compounds*
Adhesive/glues	Acetone, ethyl acetate, butanone, toluene, cyclohexane, trichloroethylene, *n*-hexane, xylene
Aerosol propellants	Liquid petroleum gas, dimethyl ether, fluorocarbon
Anesthetics and analgesics	Nitrous oxide, cyclopropane, diethyl ether, halothane, enflurane, isoflurane
Commercial dry cleaning and degreasing agents	Dichloromethane, methanol, trichloroethane, toluene
Domestic spot removers and dry cleaners	Dichloromethane, trichloromethane, tetrachloroethylene
Fire extinguishers	Bromochlorodifluoromethane
Cleaning solutions	Trichloroethylene, petroleum products, carbon tetrachloride
Fuel gases	Liquid petroleum gas, propane, butane
Nail varnish and nail varnish remover	Acetone and esters
Paint and paint thinners	Acetone, butanone, esters, hexane, toluene, trichloroethylene, xylenes
Typewriter correction fluid	Trichlorethane
Industrial solvents	*n*-Hexane
Lighter fluids	Naphtha, aliphatic hydrocarbons

Source: Razak Lajis, pharmacist at the National Poison Centre, Universiti Sains Malaysia, Penang, Malaysia.

Table 2 The health hazards of some solvents used in substances that are inhaled

Solvent	*Health hazard summaries*
Acetone	Vapors mildly irritating to eyes and respiratory tract. A CNS depressant at high levels. Ataxia and seizures have been reported
Chloroform	Vapors slightly irritating to eyes and respiratory tract. A CNS depressant. Mild to moderate systemic toxicity include headache, nausea, vomiting, confusion, and drunkenness. More severe exposures may cause respiratory arrest and coma. A carcinogen in animals
n-Hexane	Vapors mildly irritating to eyes and respiratory tract. Light-headedness, giddiness, nausea, and headache. Greater exposure may cause unconsciousness and death
Toluene	Acute exposure results in euphoria, excitement, dizziness, headache, nervousness, ataxia, convulsion, and coma. Deaths have been recorded from acute exposure to toluene in 'sniffers'
Trichloroethane	A respiratory and CNS depressant. The symptoms of acute inhalation may include nausea, euphoria, ataxia, dizziness, agitation, and lethargy. Severe exposure will lead to respiratory arrest, seizures, and coma
Xylene	Dizziness, excitement, flushing of the face, drowsiness, incoordination, tremor, confusion, respiratory depression, and coma

Taken in smaller doses, they can cause euphoria, delusions, and hallucinations. Higher doses may lead to convulsions and coma. Chronic abuse of certain substances such as toluene-containing products can produce severe organ damage involving the liver, kidneys, and brain. Inhalation of glues remains hazardous and can be fatal. Glues containing *n*-hexane and toluene (see **Table 2** for a list of health hazards posed by solvents used in substances that are inhaled) have been associated with the development of muscle weakness and atrophy. Three major clinical presentations are common with people who sniff toluene-containing glues. They will experience muscle weakness, gastrointestinal complaints, and neuropsychiatric disorders. Glue sniffers may also develop signs of renal toxicity. The euphoria of mild intoxication may be accompanied by nausea and vomiting. Some of the signs and symptoms of acute intoxication are breathing difficulties, chest pain and discomfort, eye irritation, double vision, ringing ears, diarrhea, and muscle and joint pain. After prolonged inhalation or rapid inhalation of highly concentrated vapor, the sniffer may experience a phase of excitement followed by loss of consciousness and coma.

Inks and Dyes

Artistic inks and dyes, as well as those used in textiles are generally nontoxic, though occasionally irritating or allergenic to some. They are known to have high contents of heavy metals, such as cobalt, lead, mercury, and others, many of which are common skin irritants, and some of which are carcinogens and reproductive toxicants therefore large and frequent

ingestions should be avoided. Dermal and ocular contact should be followed by rinsing with water to prevent irritation. Eye irritation and staining of skin and other mucous membranes is generally reversible.

Pesticides

Typical household pesticides can be moderately toxic with inhalation, but are generally nontoxic for human dermal contact or low-level ingestion (carbon, sulfur, potassium nitrate). Revenge Rodent Smoke Bombs, for example, contain a variety of volatile gases and solids that will become airborne and respirable upon ignition. After igniting, cartridge produces foul-smelling smoky mixture of gases (including carbon monoxide, carbon dioxide, and nitrogen) and solids (including potassium carbonate, potassium sulfate, sulfides, and uncombined carbon). Fumes may be harmful if inhaled. If inhaled, and person has poison symptoms (headache, nausea, dizziness, and weakness), the victim should be transferred to fresh air. The victim should be made to lie down and kept warm. If response is adequate recovery will be rapid. If breathing has stopped, artificial respiration should be provided. A physician should be contacted immediately.

Pesticide residues are common to many types of food as they protect against molds, fungi, and insects. The actual types of pesticides, and residuals found on foods are tightly regulated by the US Department of Agriculture. While allowed pesticides residues already have low in toxicity, rinsing/washing fresh produce in cold water is recommended.

Herbicides

Common household herbicides such as Ortho Weed B Gon Crabgrass Killer generally contain pesticides that are relatively nontoxic to humans, such as calcium acid methanearsonate at 8–10%. Eye and skin irritation may be mild with quick recovery, and small ingestions and inhalation are nonirritating.

Fungicides

Common household fungicides, such as Scotts Lawn Fungus Control and Ortho Multi Purpose Fungicide Daconil 2787 Plant Disease Control generally contain carbamates that are moderately toxic, cause skin and eye irritation upon exposure, may be harmful if swallowed, and with prolonged or excessive inhalation may cause respiratory tract irritation.

Insecticides

Typical household bug sprays are generally mixtures of pyrethrins. These are generally not highly toxic to humans; however, inhalation may cause asphyxiation. Industrial insecticides occasionally found in households, garages, and greenhouses may contain more toxic organophosphates, carbamates, and paradichlorobenzenes. Exposure to these compounds may result in breathing difficulty, gastrointestinal upset, multiple nervous system effects such as weakness, sweating, convulsions, and other treatable symptoms. Dermal exposures should be followed by immediate rinsing, CPR should be administered if breathing stops, and all exposures should be immediately treated by a health professional. Some multipurpose landscaping/yard insecticides, such as Ortho Bug B Gon Multi Purpose Insect Killer Ready may contain permethrin at 2.5%, or other pyrethroids. Canine flea products contain higher amounts, such as 45–65% permethrin. Agricultural insecticide applications of pyrethrins are in the range of 0.14–0.25 lb per acre on various nuts and vegetables. In most mammals, there is generally a rapid metabolic breakdown of pyrethroids by the liver; therefore, no signs of toxicity are likely to occur. Permethrin is detoxicified by ester hydrolysis or oxidation, followed by hepatic hydroxylation and conjugation to either glucuronides or sulfates. Because cats are generally deficient in metabolizing substances through hepatic glucuronidation, they are limited in their ability to metabolize permethrin quickly. Cats will exhibit sensitivity to high concentration of this insecticide. The clinical signs of permethrin toxicosis in cats may include muscle tremors, hyperexcitability, depression, ataxia, vomiting, seizures, anorexia, and death. Signs may develop within a few hours to 3 days following exposure. Treatment of permethrin toxicosis in a cat consists of tremor and/or seizure control, dermal decontamination, and supportive care.

Other household garden insecticides, such as Ortho Bug B Gon Ready Spray contain dizainon, a common pesticide that is relatively non-toxic when inhaled or ingested in small accidental amounts, though are moderately irritating to the eyes and skin. Larger ingestions can cause cholinesterase inhibition with symptoms occurring within 12 h. Effects may include headache, dizziness, weakness, nausea, vomiting, diarrhea, pupil constriction, blurred vision, excessive salivation or nasal discharge, profuse sweating, and abdominal cramps. Incontinence, unconsciousness, convulsions and breathing difficulties are indicative of severe poisoning.

Human dermally applied insecticides such as Cutter, DEET, Off Skintastic and others may contain *N*,*N*-diethyl-*m*-toluamide up to 35%. They are generally safe at the recommended doses. Several case examples have reported symptoms in children receiving higher and frequent doses, especially of DEET, of disorientation, slurred speech, flexing of

fingers and dorsiflexing of toes, shaking, and convulsions. As these products are sold at diluted concentrations, the active compound is generally mildly irritating to mucous membranes and if it gets into the eyes, can be immediately relieved by rinsing with water. Few have reported skin irritation with frequent use, especially those with other skin ailments such as psoriasis or severe acne.

Flea Powder

Flea powders may contain carbaryl, dichlorophene, chlordane, and other chlorinated hydrocarbons. Carbaryl is very toxic, interfering with the CNS, respiratory system, and cardiovascular system, and may also cause skin damage. Dichlorophene is a skin irritant and may cause damage to the liver, kidneys, spleen, and central nervous system. Chlordane and other chlorinated hydrocarbons are biocumulative, and may damage the eyes, lungs, liver, kidneys, and skin.

Mineral Spirits

Mineral spirits are solvents. Ingestion, skin contact, or inhalation of fumes may lead to breathing difficulty, severe pain in the throat, burning in nose, eyes, ears, lips, or tongue, loss of vision, abdominal pain, vomiting, blood in vomit and/or stool, hypotension, collapse, skin irritation including burns and necrosis (holes) in skin and underlying tissues. Accidental skin exposures should be rinsed immediately, accidental ingestions should be followed by immediate consumption of milk or water, and all exposures should follow emergency treatment of the symptoms by a health professional.

Oven Cleaners

Oven cleaners contain corrosive alkalis that can cause burning of the skin and respiratory tract if inhaled. Large doses may lead to breathing difficulty, pain in throat, nose, eyes, ears, lips, and tongue, abdominal pain, vomiting, blood in vomit and stool, hypotension, collapse, skin irritation, burns, and necrosis (holes) in skin and underlying tissue, and severe changes in blood pH. Dermal contact should be treated with immediate washing, ingestion with consumption of milk or water, inhalation with fresh air, and in all cases treatment of the symptoms by a health professional.

See also: Arts and Crafts Materials and Processes; Consumer Product Safety Commission; Corrosives; Deodorants and Antiperspirants; Detergent; Food Additives; Food, Drug, and Cosmetic Act, US; Glyphosate; Mouthwash; Pesticides.

Relevant Websites

http://www.nlm.nih.gov – National Institutes of Health and the National Library of Medicine. Household Products Database. Provides information on health and safety of everyday products.

http://www.cpsc.gov – United States Consumer Product Safety Commission (CPSC). Find recalled products by product type.

http://www.kidsource.com – KidSource – Education and Healthcare Information.

Copper

Shayne C Gad

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- Chemical Abstracts Service Registry Number: CAS 7440-50-8
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Cu^{2+}

Uses

Copper is an essential trace element. Adequate daily requirements are 2–3 mg day^{-1}. It is widely distributed in nature and extensively used in industry. It is used as an electrical conductor, as a component in a variety of alloys (including gold and silver alloys), and as a constituent in paints and ceramic glazes. Because it corrodes at a very slow rate, it is used extensively for water pipes. In addition, copper sulfate mixed with lime is used as a fungicide.

Medicinally, copper sulfate is used as an emetic. It has also been used as an antihelminthic (antiparasitic agent) based on its astringent and caustic actions.

Background Information

Copper and its compounds are naturally present in the earth's crust. Natural discharges to air and water

may be significant. Therefore, it is important to consider the background levels that are commonly found and distinguish these from high levels that may be found as a result of anthropogenic activity.

Copper is emitted into the air naturally from windblown dust, volcanoes, and anthropogenic sources, the largest of which are being primary copper smelters and ore processing facilities. It is associated with particulate matter. The mean concentration of copper in the atmosphere is 5–200 $ng\,m^{-3}$.

Exposure Routes and Pathways

The primary exposure pathway for copper is ingestion (e.g., food and water). Many foods contain copper, especially legumes, organ meats, and oysters. Water carried through copper pipes is also a source of this element. Inhalation is only a significant exposure pathway in industrial settings (e.g., near copper refineries).

Many workers are exposed to copper in agriculture, industries connected with copper production, metal plating, and other industries. Little information is available concerning the forms of copper to which workers are exposed. Copper has been identified at many National Priorities List hazardous waste sites in the United States.

Toxicokinetics

Approximately 50% of ingested copper is absorbed from the stomach. Although copper can be absorbed from the gastrointestinal tract, a modifying biological mechanism regulates total copper absorbed. Copper is transformed in the blood by first binding to albumin and then to a copper-specific protein (ceruloplasmin). Copper also binds to metallothionein more firmly than zinc or even cadmium. Copper is stored in the liver and bone marrow as the metallotheionein.

Copper-dependent enzymes include tyrosinase (which is involved in melanin pigment formation) and the various oxidases (i.e., cytochrome oxidase, superoxide dismutase, amine oxidase, and uricase). Copper plays a major role in the incorporation of iron into the heme of hemoglobin. Copper deficiency is characterized by hypochromic, microcytic anemia resulting from defective hemoglobin synthesis.

Copper levels in the human body vary with age. Copper levels in the brain increase with age, whereas in some tissues (e.g., liver, lungs, and spleen), copper levels are higher in newborns than in adults. Tissue levels gradually decline up to age 10 and remain relatively constant thereafter. Copper is normally excreted in bile, which plays a primary role in copper homeostasis.

Mechanism of Toxicity

Copper reduces glutathione, which is necessary for normal cell viability. The amino acid transferases are inhibited in the presence of excess copper; lipid peroxidation also occurs. Copper combines with thiol groups, which reduces the oxidation state II to I in copper and oxidizes the thiol groups to disulfides, especially in the cell membrane.

Acute and Short-Term Toxicity (or Exposure)

Animal

Copper produces lung damage by inhalation. Intratracheal administration of copper has produced lung damage in rodents; macrophages increased with degenerative membrane structure and hemoglobin values decreased. In larger animals, excess copper intake resulted in iron-deficient anemia and gastric ulcers.

Human

Although copper is an essential element, it is much more toxic to cells than such nonessential elements as nickel and cadmium. Acute poisoning from ingestion of excessive amounts of copper salts, most frequently copper sulfate, results in nonspecific toxic-symptoms, a metallic taste, nausea, and vomiting (with vomitus possibly a blue-green color). The gastrointestinal tract can be damaged by ulceration.

Chronic Toxicity (or Exposure)

Animal

No statistically significant increases in tumor formation were noted in mice fed copper for $\sim$1 year. Subcutaneous and intramuscular injection of copper compounds showed a low incidence of sarcomas. The current data are adequate to assess the carcinogenicity of copper.

Human

Severe symptoms include hypotension, coma, jaundice, and death. Liver necrosis has also been observed. In some cases, copper toxicity can result in an inability to urinate. Treatment with copper compounds can induce hemolytic anemia.

It is believed that the increased susceptibility to copper toxicity seen in infants and children is due to

the normally high hepatic copper levels in early life and the fact that homeostatic mechanisms are not fully developed at birth.

Copper is associated with two genetic inborn errors of metabolism. The first, Menke's disease or Menke's kinky-hair syndrome is associated with severe copper deficiency, due to a defect in an ATPase gene resulting in the inability of the gastrointestinal tract to absorb copper. It is a sex-linked trait characterized by peculiar hair, failure to thrive, severe neurological degradation in the brain, and death before 3 years of age. The cerebral cortex and white matter degenerates; mental retardation ensues before death. The second disease, Wilson's disease or heptolenticular degeneration, is associated with severe copper excess, due to a defect in another ATPase gene resulting in the inability of the liver to excrete copper in the bile. It is characterized by an unusual concentration of copper in the brain, kidneys, cornea, and especially in the liver (which may become abnormally large). Mental retardation is not associated with this disease. This disease is usually treated with a chelating agent such as penicillamine or triethylene tetramine.

In Vitro Toxicity Data

Mutagenesis results are dependent on the bacterial strain and copper compound evaluated. Mammalian cell tests indicate a positive mutagenic response.

Clinical Management

For acute toxicity, emesis is recommended. Treatment is symptomatic. A combination of BAL (British AntiLewisite; 2,3-dimercaptopropanol) and calcium-ethylene diamine tetraacetic acid has been used successfully in a poisoned infant. Penicillamine has also been used. Recently, oral administration of 2,3-dimercapto1-propane sulfonate was found to be effective in experimental rodents. Electrolyte balance must be maintained when gastric lavage is indicated. Potassium ferrocyanide should be added to precipitate the copper.

Environmental Fate

The largest release of copper by far will be to land, and the major sources of release are mining and milling operations, agriculture, solid waste, and sludge from publicly owned treatment works. Sediment is an important sink and reservoir for copper. In relatively clean sediment, the copper concentration is <50 ppm; polluted sediment may contain several thousand ppm of copper.

Copper is released to water as a result of natural weathering of soil and discharges from industries and sewage treatment plants. Copper compounds may also be intentionally applied to water to kill algae. Of special concern is copper that gets into drinking water from the water distribution system.

The bioconcentration factor (BCF) of copper in fish obtained in field studies is 10–100, indicating a low potential for bioconcentration. The BCF is higher in molluscs (i.e., oysters), where it may reach 30 000, possibly because they are filter feeders. There is a good deal of evidence that there is no biomagnification of copper in the food chain.

The major species of soluble copper found in freshwater, seawater, and a combination of the two over a range of pHs is Cu^{2+}, $Cu(HCO_3)^+$, and $Cu(OH)_2$. At the pH values and carbonate concentrations characteristic of natural waters, most dissolved Cu(II) exists as carbonate complexes rather than as free (hydrated) cupric ions.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value time-weighted average is $0.2\,mg\,m^{-3}$ for copper fume and $1\,mg\,m^{-3}$ for copper dusts and mists.

The Environmental Protection Agency drinking water limit is 1.3 ppm. The median concentration of copper in natural water is 4–10 ppb.

Daily intakes of copper and other essential minerals are estimated and can be found as part of the Food and Drug Administration's Total Diet Study.

See also: Metallothionein; Metals; Pollution, Water.

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Copper.

Coprine *See* Mushrooms, Coprine.

Corrosives

Greene Shepherd

- Representative Chemicals: Glacial acetic acid; Oxalic acid; Acetic acid; Nitric acid; Hydrofluoric acid
- Synonyms: Irritants; Acids/bases

Acute and Short-Term Toxicity (or Exposure)

Human

After ingestion, corrosive injury to the esophagus and stomach are commonly found. With skin contact, the symptoms are severe pain and brownish or yellow stains. Burns usually penetrate the full thickness of the skin, have sharply defined edges, and heal slowly with scar formation. With eye contact, conjunctival edema and corneal destruction is prevalent. Symptoms include pain, tearing, and photophobia.

The Occupational Safety and Health Administration permissible exposure limits for various corrosives are as follows: glacial acetic acid, 10 ppm; acetic anhydride, 5 ppm; hydrofluoric acid, 3 ppm; sulfuric acid, 1 mg m^{-3}; oxalic acid, 1 mg m^{-3}; nitric acid, 2 ppm; bromine, 0.1 ppm; chlorine, 1 ppm; fluorine, 1 ppm; hydrochloric acid, 5 ppm.

Chronic Toxicity (or Exposure)

Human

Long-term exposure to acid fumes (inhalation exposure) may cause erosion of the teeth followed by jaw necrosis. Bronchial irritation with chronic cough and frequent attacks of bronchial pneumonia are common.

Clinical Management

In case of ingestion, neither gastric lavage nor emesis should be used. Activated charcoal is unlikely to bind significant amounts of corrosive agents and can make endoscopic evaluation difficult. Ingested corrosives may be diluted by drinking 4–6 oz (113.4–170.1 ml) of water or milk. If vomiting is persistent, do no attempt to administer additional fluids. Avoid neutralization therapies as the resultant exothermic reaction may cause additional tissue injury.

In case of eye contact, the corrosive should be diluted by irrigating the area with tap water or saline for 30–60 min. With alkali exposures prolonged irrigation may be necessary.

In case of skin contact, the corrosive should be removed by flooding the affected area with water for at least 15 min. With alkali exposures prolonged irrigation may be necessary. If the exposure is in the form of a powder rather than a liquid the excess powder should be brushed off prior to irrigation.

See also: Acetic Acid; Bromine; Chlorine; Fluorine; Hydrochloric Acid; Hydrofluoric Acid; Sulfuric Acid.

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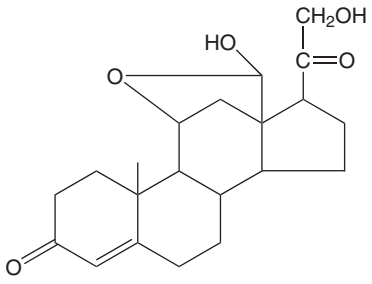
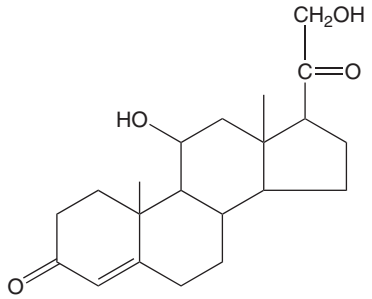
Corticosteroids

Prathibha S Rao

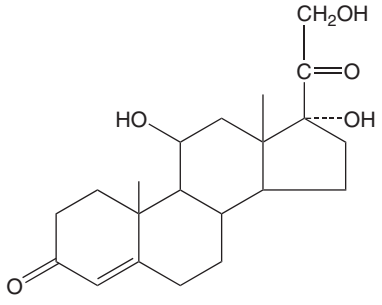
- Representative Chemicals: Mineralocorticosteroids (e.g., aldosterone); Glucocorticoids (e.g., corticosterone)
- Chemical/Pharmaceutical/Other Class: Natural or synthetic hormones
- Chemical Structures:



Aldosterone



Corticosterone



Cortisol

Uses

Corticosteroids cause a wide range of physiological effects, including impacts on protein and lipid metabolism; electrolyte and water balance; and functions of the cardiovascular system, kidneys, skeletal muscle, the nervous system, and other organs and tissues. Corticosteroids are used to treat a wide variety of clinical conditions including adrenal insufficiency, asthma, allergic disorders, and collagen and autoimmune diseases.

Exposure Routes and Pathways

Corticosteroids are administered orally, parenterally, and topically. A certain degree of absorption into the systemic circulation occurs with all forms of topical administration. With respiratory aerosols, the total absorption is similar to that from parental or oral administration.

Toxicokinetics

Generally, the biological half-lives of corticosteriods can be classified as short (8–12 h), intermediate (12–36 h), or long (36–72 h). Cortisone and cortisol are examples of short-lived corticosteroids. Prednisone, prednisolone, and triamcinolone are of the intermediate class. Dexamethasone and β-methasone are associated with the longer-lived class.

The adrenocortical steroids and their synthetic congeners require a double bond in the 4,5 position and a ketone group at C3 for biological activity. The reduction of the 4,5 double bond, resulting in an inactive compound, occurs by both hepatic and extrahepatic metabolisms. Most of the ring A-reduced metabolites can be conjugated at the 3-hydroxyl position with sulfate or glucuronic acid forming water-soluble metabolites enhancing excretion.

Mechanism of Toxicity

The corticosteroids, like other steroid hormones, act by altering the nature of protein synthesis in target tissues. Corticosteroids interact with specific receptor proteins found in the cytoplasm of cells in many tissues to form a steroid–receptor complex. This complex then translocates into the nucleus, where it combines with DNA sequences within the regulatory region of affected genes (termed glucocorticoid response elements). Subsequently, target genes are expressed and appropriate proteins are synthesized. Although there are many similarities between the mechanisms of action of the glucocorticoids and mineralocorticoids, several processes have been identified, such as tissue-restricted receptors for

mineralocorticoids, to explain differences in effects of these two major corticosteroid classes.

Acute and Short-Term Toxicity (or Exposure)

Animal

No relevant information is available on acute toxicity of corticosteroids in animals.

Human

Two categories of toxic effects are observed in the therapeutic use of corticosteroids: those resulting from withdrawal and those resulting from continuous use of large doses. Too rapid withdrawal causes (1) acute adrenal insufficiency and (2) fever, myalgia, arthralgia, and malaise.

The use of corticosteroids for days or few weeks does not lead to adrenal insufficiency but prolonged therapy may result in suppression of pituitary–adrenal function.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to high serum levels of corticosterone induced a significant impairment of inhibitory avoidance learning in rats. In another study, corticosterone elevated over a period of 21 days impaired the formation of a longer-term form of memory, most likely reference memory. Impairments in spatial working memory were seen only after longer durations of corticosterone administration.

Human

In addition to pituitary–adrenal suppression, prolonged therapy with corticosteroids can cause fluid and electrolyte disturbances, hypertension, hyperglycemia, and glycosuria. It also increases the susceptibility to infections including tuberculosis; causes peptic ulcers, osteoporosis, behavioral disturbances, posterior subcapsular cataracts, growth arrest, Cushing's habitus, 'buffalo hump', enlargement of supraclavicular fat pads, 'central obesity', striae, ecchymoses, acne, and hirsutism.

Clinical Management

Acute overdose probably would not result in toxicity. Should oral overdosage occur, standard emergency and supportive care procedures should be followed. If anaphylaxis should occur, epinephrine may be given as 0.3–0.5 ml of a 1:1000 solution for adults (children should receive 0.01 ml kg^{-1}). Mild anaphylaxis may be treated with antihistamines alone. If chronic toxicity should occur, it is important to reduce the dosage of corticosteroid to a minimal maintenance dose at the first sign of toxicity.

Environmental Fate

Not much information is available.

Ecotoxicology

Not much information is available.

See also: Anabolic Steroids; Lipid Peroxidation.

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Corticosterone *See* Corticosteroids.

Cortisone *See* Corticosteroids.

Cosmetics and Personal Care Products

Paul Sterchele

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Introduction

Cosmetics are natural or synthetic toiletry products that are used to maintain hygiene and include externally applied products used to enhance appearance. This class includes dental products, bath supplies (e.g., bubble baths, body washes, and bath beads), powders, lotions, lipsticks, perfumes, colognes, shampoos, depilatories, and hair coloring/waving products. Most of these products contain alcohols, aromatic hydrocarbons, perborates, and anionic and nonionic surfactants. Use of cosmetics is as old as civilization itself. Centuries ago wealthy women would apply the white lead pigment known as ceruse to their faces to appear fashionably pale – sometimes with lethal consequences. Women also used belladonna alkaloids like atropine to dilate pupils to enhance the attractiveness of the eyes in the late nineteenth century.

Product Formulations and Human Toxicity

Most cosmetics are nontoxic, although composition and the magnitude and route of exposure are important determinants. In general, accidental ingestion of small quantities might be expected to elicit some minor, transient gastrointestinal distress, but frank toxicity is rare. However, caution should be exercised to prevent children from being overexposed to these products.

Hair-Coloring Products

Permanent hair colors contain an oxidizer (usually 6% hydrogen peroxide) and a dye intermediate (*p*-phenylenediamine, resorcinol, aminophenols along with water, ammonia, glycerin, isopropanol, and propylene glycol). Semipermanent hair colors contain propylene glycol, isopropanol, fatty acids, fragrance, alkanolamines, and dyes. Some Grecian hair formulations contain lead in the form of lead acetate.

Large ingestions of hydrogen peroxide may produce mild gastritis due to decomposition resulting in release of oxygen.

Hair-Waving Products

Waving lotions contain thioglycolic acids and ammonia sulfides, and neutralizer solutions contain hydrogen peroxide, sodium bromate, or perborate in mildly acidic solutions. Some permanent wave fixatives contain 2–8% (weight/volume) mercuric chloride.

Sodium borate decomposes into borate and peroxide and is less toxic than potassium bromate. From 3 to 6 g and from 15 to 30 g boric acid is potentially fatal to children and adults, respectively. Cutaneous manifestations include desquamating, erythematous rash commonly over palms, soles, buttocks, and scrotum. The lesion may progress to exfoliation. Central nervous system (CNS) effects range from irritability, restlessness, and headache to coma and convulsions in severe cases. Gastrointestinal symptoms include anorexia, nausea, vomiting, and diarrhea. Acute renal tubular necrosis may lead to renal failure in moderate to severe cases.

Bromate salts are extremely toxic; they are capable of causing deafness and renal failure at doses between 240 and 500 mg kg^{-1}. Potassium bromate, also used as neutralizer in cold waves, is an extremely toxic compound that produces nausea, vomiting, diarrhea, deafness, acute renal failure, hypotension, CNS depression, and hemolysis. Both otic symptoms and renal impairment may be permanent. Primary tubular damage can progress to interstitial fibrosis and glomerular sclerosis.

Hair-Straightening Products

Hair straighteners contain 1–3% sodium hydroxide solution. The solution is highly caustic.

Depilatories

Similar to other hair preparations that are formulated to modify the molecular configuration of hair, hair-removal products typically contain thioglycolate salts to dissolve the keratin protein. Because of the caustic nature of these products, care should be taken to minimize irritation to the surrounding skin.

Hair Sprays and Conditioners

Hair sprays contain ethanol as a solvent with resin polymers composed of vinyl acetate, acrylamide, and methyl vinyl ether. Hair conditioners contain cationic surfactants, perfumes, and alcohols.

Bath Preparations

Bubble baths usually contain anionic and nonionic surfactants along with alcohols and preservatives. Bath salts may contain borax, while bath oils contain vegetable and mineral oils.

Nail Polish and Removers

Nail polish contains hydrocarbon solvents (xylene, toluene, and acetone), alcohol solvents, plasticizers, and resins. Nail polish removers are solvents containing acetone or ethanol.

Colognes, Perfumes, Toilet Waters

Colognes, perfumes, and toilet waters usually contain ethanol (at concentrations ranging from 50% to 95%) and volatile or essential oils.

Volatile or Essential Oils

Sage, eucalyptus, turpentine, pine, pennyroyal, and cinnamon contain hydrocarbons, ethers, alcohols, esters, and ketones. These components can cause allergic contact dermatitis, which begins 12 h within sensitization and peaks at 48–72 h. Essential oils are mucosal irritants leading to gastrointestinal distress and salivation. Concentrated formulations of essential oils can cause convulsions and CNS depression at 10 ml doses. Aspiration can cause chemical pneumonitis. Alcohol produces intoxication, which may be complicated by hypoglycemia, especially in children.

Dental Products

Toothpastes, powders, and tooth liquids contain calcium phosphates, alumina, abradants, and anionic surfactants. Mouthwashes usually contain alcohol, flavoring (essential oils), and sweeteners. (For mouthwash toxicity information, see section on Colognes, Perfumes, Toilet Waters.) Denture cleaners contain bicarbonates, borates, phosphates, and carbonates. (For toxicity information on borates, see section on Hair-Waving Products.) Acrylic denture material contains methacrylate.

Deodorants

Deodorants contain aluminum and zinc salts, and fragrance to mask the smell of perspiration.

Clinical Management

Since most ingested cosmetics are nontoxic, only supportive care and dilution are required.

1. Induction of emesis depends on product toxicity, quantity, time since exposure, patient's weight, and the presence of symptoms. Cationic surfactants, perborates, and substantial ingestion of essential oils may benefit by administration of syrup of ipecac. Syrup of ipecac can be used in hydrocarbon ingestion only if the total dose of hydrocarbons exceeds 1 or 2 ml kg^{-1}.
2. Potassium borate: Lavage with 2% sodium bicarbonate solution and administer 10–50 ml of 10% sodium thiosulfate solution intravenously at the rate of 3 ml min^{-1} to reduce the bromate to the less toxic bromide ion. An alternative therapy is the administration of 100–500 ml of 1% sodium thiosulfate. Patients should be observed for development of renal toxicity and ototoxicity.

See also: Acetone; Acrylamide; Alkalies; Ammonia; Atropine; Belladonna Alkaloids; Boric Acid; Deodorants and Antiperspirants; Ethanol; Food, Drug, and Cosmetic Act, US; Fragrances and Perfumes; Hydrogen Peroxide; Isopropanol; Lead; Surfactants, Anionic and Nonionic; Surfactants, Perfluorinated; Toluene; Xylene.

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Relevant Websites

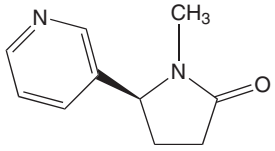
http://www.ctfa.org – US Cosmetic, Toiletry, and Fragrance Association (CTFA).

http://hpd.nlm.nih.gov – US National Library of Medicine, 'Household Products Database' and 'Tox Town'. The Household Products Database links several thousand US Consumer brands to Health effects from Material Safety Data Sheets (MSDSs) provided by manufacturers, and allows scientists and consumers to research products based on chemical ingredients.

Cotinine

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 486-56-6
- Synonyms: 1-Methyl-5-(3-pyridinyl)-2-pyrrolidinone; *N*-Methyl-2-(3-pyridyl)-5-pyrrolidone
- Chemical/Pharmaceutical/Other Class: Research chemical; Antidepressant
- Chemical Structure:



Uses

Cotinine is primarily used in research.

Background Information

Cotinine is the major metabolite of nicotine. Nicotine is rapidly metabolized and has a short half-life, but cotinine is metabolized and eliminated at a much lower rate. This results in higher cotinine-to-nicotine ratio in various tissues, including the brain. In studies on conscious, freely moving rats, intravenous administration of either nicotine or cocaine induced the release of dopamine in the nucleus accumbens, as assayed by microdialysis. Prior intravenous administration of a high dose of cotinine ($500\,\mu g\,kg^{-1}$) inhibited this nicotine- or cocaine-induced dopamine release. The effect of cotinine is not due to altered metabolism of nicotine or its binding at the receptor site, because cotinine, unlike nicotine, does not affect the binding of the nicotinic ligand cystine. The findings suggest that cotinine affects a putative component of the reward mechanism, and as such could have therapeutic value.

Exposure Routes and Pathways

Cotinine is a viscous liquid. Dermal or ocular contact is the most common exposure pathway. Because it is a predominant metabolite of nicotine, systemic exposure occurs after consumption of tobacco products.

Toxicokinetics

Cotinine is formed as a major metabolite of nicotine after tobacco smoking. The average half-life of cotinine is 19 h. It can be detected in plasma, urine, and saliva. Cotinine is also formed in the body after intake of some vegetables (e.g., eggplant, tomato, and green pepper) primarily of the family solanaceae. These vegetables contain nicotine as their natural defense mechanism against fungi, bacteria, insects, and animals. It had been thought that the presence of cotinine in human urine could be used as evidence of smoking or tobacco use. However, because cotinine can also arise from consumption of some vegetables, use of cotinine as a biomarker of exposure to tobacco smoke or other forms of tobacco products may not be reliable.

Mechanism of Toxicity

Cotinine stimulates the nicotinic receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity in animals is similar to that observed in humans. The intraperitoneal LD_{50} in mice is $930\,mg\,kg^{-1}$; the oral gavage LD_{50} in mice is $1604\,mg\,kg^{-1}$.

Human

Symptoms of acute toxicity include nausea, salivation, abdominal pain, vomiting, diarrhea, cold sweat, headache, dizziness, and disturbed hearing and vision.

Chronic Toxicity (or Exposure)

Animal

Reported to be a carcinogen.

Human

Reported to be a carcinogen.

Clinical Management

Vomiting should be induced with syrup of ipecac or gastric lavage should be performed. Respiratory assistance and treatment of shock may be necessary.

See also: Nicotine; Tobacco; Tobacco Smoke.

Further Reading

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Stevens KR and Munoz LR (2004) Cigarette smoking: Evidence to guide measurement. *Research in Nursing & Health* 27: 281–292.

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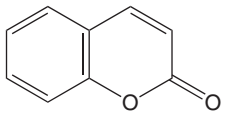
Coumarins

Betsy D Carlton

- Chemical Abstracts Service Registry Number: CAS 91-64-5
- Synonyms: Coumarin; 2*H*-1-Benzopyran-2-one; 1,2-Benzopyrone; *cis*-*o*-Coumaric acid lactone; Coumarinic anhydride; 2-Oxo-1,2-benzopyran; Tonka bean camphor (*note*: coumadin and warfarin are not synonyms for coumarin)
- Chemical/Pharmaceutical/Other Class: Benzo-α-pyrone
- Chemical Formula: $C_9H_6O_2$
- Chemical Structure:



Uses

Coumarin is a naturally occurring benzo-α-pyrone compound that is most often used as a fragrance ingredient, where it functions as a fragrance, as a fragrance enhancer, and as a stabilizer. Coumarin is widely used in perfumes, hand soaps, detergents, and lotions at concentrations from 0.01% to 2.4%. It is used to give pleasant aromas to household products or to mask unpleasant odors. The conservative estimate for systemic exposure of humans by using cosmetic products is $0.13\,mg\,kg^{-1}\,day^{-1}$, disregarding any corrections that should be made for absorption that is $<100\%$. Coumarin is used as a pharmaceutical for the treatment of high protein lymphedema, for improved venous circulation, and has been in clinical trials as an antineoplastic. Unlike coumadin (or warfarin), coumarin has no anticoagulant activity and is not used clinically as an anticoagulant or as a rodenticide.

Coumarin is found in a large number of plants belonging to many different families including tonka beans, woodruff, lavender oil, cassia, melilot (sweet clover), and other plants. It is found in edible plants such as strawberries, cinnamon, peppermint, green tea, carrots, and celery, as well as in partially fermented tea, red wine, beer, and other foodstuffs. Although coumarin's use in foods is allowed via naturals such as cinnamon, at the present time coumarin is not permitted for use as a direct food additive, although it is used as a tobacco flavor.

Coumarin is also used in the electroplating industry.

Exposure Routes and Pathways

Due to its common use in fragrances and fragrance-containing products, dermal exposure to coumarin is common. Coumarin is readily absorbed dermally, a fact that makes dermal dosing for lymphedema treatment a consideration. Human exposure to coumarin also occurs orally via natural foodstuffs, from pharmaceutical use, and from tobacco products.

Toxicokinetics

The absorption, metabolism, and excretion of coumarin have been widely studied for many years. Advances in synthetic and analytical chemistry techniques in recent years have allowed a significant revision to our understanding of how coumarin is handled in the body, particularly in rodents and humans. While coumarin is readily available by both the oral and dermal routes in animals and humans, blood levels and toxicity profiles are influenced by the specific exposure mode. Plasma levels more than 20×higher than those observed following exposure via the diet have been reported following a bolus oral dose at similar milligram coumarin per kilogram body weight levels.

Dermal exposure by-passes the 'first-pass' effect of initial metabolism by the liver. Coumarin in the blood first passes through the lung, where significant amounts can be exhaled, prior to being metabolically processed by the liver. Metabolic pathways are highly species- and, sometimes, strain-specific. DBA/2J mice have been reported to have a high level of coumarin hydroxylase activity, resulting in metabolism mainly to 7-hydroxycoumarin. CH3/HeJ mice, on the other hand, have been reported to have very little hydroxylase activity.

In rats and many strains of mice other than the DBA/2J, oral coumarin exposure results in hepatic metabolism of coumarin, with the formation of the coumarin 3,4-epoxide (CE), which spontaneously rearranges extremely rapidly to the *o*-hydroxyacetaldehyde (*o*-HPA), the toxic metabolite. The *o*-HPA is then further metabolized to the nontoxic *o*-hydroxyacetic acid (*o*-HPAA) and *o*-hydroxyethanol (*o*-HPE). Rodents also metabolize coumarin by several lesser pathways, to the nontoxic 3-hydroxycoumarin and several other more minor metabolites. It is the balance between the formation of the toxic *o*-HPA and the nontoxic *o*-HPAA and *o*-HPE that is critical to the determination of hepatotoxicity at high exposure levels of coumarin. Mice form more *o*-HPA than do rats, but detoxify it much more rapidly and efficiently than do rats. The result is that hepatotoxicity at doses $\geqslant 150\,\text{mg coumarin kg}^{-1}$ body weight is observed in rats, but not mice. Similarly, when high doses of coumarin result in high plasma levels, mice demonstrate pulmonary toxicity whereas rats do not. This is the result of the formation of higher levels of CE and *o*-HPA in the Clara cells in the lungs of mice, which is not observed in rats.

In contrast to rodents, humans primarily metabolize coumarin not to the epoxide and *o*-HPA, but rather to the nontoxic metabolite, 7-hydroxycoumarin. Very high levels of coumarin are required to generate any *o*-HPA in human liver, and what is formed is rapidly detoxified. Humans have very few Clara cells in the lungs and do not generate CE and *o*-HPA in the lung, even at high coumarin doses. In a study using human hepatic microsomes with various CYP2A6 7-hydroxylation capacities, those samples that demonstrated a low capacity to utilize the 7-hydroxylatation pathway also showed a decreased capacity to form CE.

Mechanism of Toxicity

Coumarin toxicity is a function of blood and target tissue levels of coumarin relative to the metabolic capacity of the target organ. Cellular toxicity results when the formation of the toxic moieties exceeds the capacity of the cell to detoxify. This can have significant impact when comparing dosing by gavage to dietary exposure (see section 'Toxicokinetics').

Acute and Short-Term Toxicity (or Exposure)

Animal

LD_{50} values ranging from 160 to $780\,\text{mg kg}^{-1}$ body weight have been reported. The differences may relate to the species/strain of animal used and whether the animals were fasted at the time of dosing. Coumarin can be slightly irritating to the eye and skin.

In rat studies, dietary exposure at levels $\geqslant 2500$ ppm for 4 weeks or more may result in decreased food consumption, with resulting decreased body weight, and microscopic changes in the liver. Doses as high as 1–2% in the rodent's diet (10 000–20 000 ppm) have been given, usually resulting in a refusal of food and mortality.

In contrast to coumadin, coumarin is not teratogenic. In rats exposed to doses that are sufficiently high to significantly effect food consumption, coumarin can decrease reproductive success. At lower dose levels, no adverse effects on reproduction or development have been reported.

Human

Coumarin exposure is common via cinnamon, green tea, sweet clover honey, and other foodstuffs and adverse effects have not been reported. When used as a pharmaceutical, doses have ranged from 70 to $7000\,\text{mg day}^{-1}$. The most common pharmaceutical dosage appears to be 200 mg once or twice per day. Infrequently, hepatotoxicity has been reported following pharmaceutical use. Hepatic enzyme changes have been reported to be reversible following cessation of administration, and occasionally are reversible despite continued use. The incidence rates reported have ranged from $<0.1\%$ to 6%, depending on the study population and, to a lesser extent, on the dose administered. Some deaths have been reported, but confounding factors such as preexisting medical conditions have precluded interpretation in most cases. Studies of CYP2A6 polymorphism in humans have not shown an association with coumarin-associated liver dysfunction.

Coumarin has been tested for its ability to cause sensitization in several test systems including dermal application in guinea pigs, the mouse ear swelling test, and the local lymph node assay (LLNA). In all cases where pure coumarin was tested in animals, results were negative, including when tested at up to 50% in four recent LLNA studies. Also in LLNA studies, a chlorinated impurity (6-chlorocoumarin) and less-pure coumarin derived from *o*-cresol have been shown to be sensitizers, confirming reports of sensitization from various substituted derivatives of coumarin. In humans already sensitized to certain other substances such as Balsam of Peru, coumarin has been reported to cross-react. While laboratory species are not likely to have been exposed to coumarin or cross-reacting substances before being tested, the human population is likely to have been

previously exposed. This can make testing in humans more difficult to interpret.

Coumarin has no anticoagulant activity, is not used to prevent blood clots, and does not interact with the vitamin K-dependent clotting factors. It is not teratogenic in humans (does not cause hemorrhagic syndrome) and has no reported adverse effects on human reproduction. In contrast to overexposure to coumadin, coumarin overexposure does not result in bruising and hemorrhage.

Chronic Toxicity (or Exposure)

Animal

Numerous long-term toxicity and/or carcinogenicity studies have been conducted. In general, the primary effects reported are decreased food consumption with resulting decreased body weight and liver toxicity (in rats). At doses that produce a significantly decreased body weight gain ($\geqslant 150\,mg\,kg^{-1}$ body weight), liver toxicity and liver tumors are reported in rats. These tumors are nonmetastatic and nonlethal. Lung tumors have been reported in mice exposed by bolus (via stomach tube) administration at doses $\geqslant 150\,mg\,kg^{-1}$ body weight, but not in mice exposed to comparable doses in the diet.

Human

In workers, dusts may be irritating to the respiratory system. Coumarin may be a weak dermal sensitizer in sensitive individuals. Purity of the material can play a significant role in this regard. No other long-term effects of coumarin have been reported in humans. The International Agency for Research on Cancer (IARC) reviewed coumarin in 2000 and classified it in group 3, not classifiable as a carcinogen in humans.

In Vitro Toxicity Data

Coumarin is not mutagenic and does not bind to DNA. It is not clastogenic (i.e., it has no significant effect on chromosomes).

Environmental Fate

Coumarin is readily biodegradable. Coumarin is unlikely to bind to soil. Coumarin does not bioaccumulate; the bioconcentration factor has been determined to be <10–40. Various environmental fate studies have shown that coumarin in the environment would biodegrade and be lost to volatilization. Losses resulting from photolysis may also occur.

Ecotoxicology

Coumarin is not very environmentally toxic, with 96 h LC_{50} values in fish of $56\,mg\,l^{-1}$ and a 24 h EC_{50} in *Daphnia magna* of $55\,mg\,l^{-1}$. Algal respiration was depressed at laboratory test concentrations of $50\,\mu mol\,l^{-1}$. Coumarin in the environment will readily degrade.

Miscellaneous

Coumarin is a white crystalline solid. Its odor has been described both as vanilla-like and as having a note of 'newly mown hay'. Odor thresholds of 0.33–2 ppb have been reported. Coumarin occurs naturally in many plants such as tonka beans, lavender, and cassia and in many natural food stuffs such as cinnamon, green tea, peppermint, and sweet clover honey. Concentrations range from 87 000 ppm in cassia and 40 000 ppm in cinnamon to 20 ppm in peppermint and 5 ppb in tangerines.

See also: Fragrances and Perfumes.

Further Reading

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Born SL, Hu JK, and Lehman-McKeeman LD (2000) *o*-hydroxyphenylacetaldehude is a toxic metabolite of coumarin. *Drug Metabolism and Disposition* 28(2): 218–223.

Burian M, Freudenstein J, Tegtmeier M, *et al.* (2003) Single copy of variant CYP2A6 alleles does not confer susceptibility to liver dysfunction in patients treated with coumarin. *International Journal of Clinical Pharmacology and Therapeutics* 41(1): 141–147.

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Lake B (1999) Coumarin metabolism, toxicity and carcinogenicity: Relevance for human risk assessment. *Food and Chemical Toxicology* 37: 423–453.

Crafts Materials *See* Arts and Crafts Materials and Processes.

Creosote

William S Utley

- Chemical Abstracts Service Registry Number: CAS 8001-58-9 (Coal tar creosote); CAS 8021-39-4 (Wood creosote)
- Synonyms: Coal tar oil; Brick oil; Heavy oil; Naphthalene oil; Liquid pitch; Wood creosote
- Chemical/Pharmaceutical/Other Class: Phenolic Compound
- Chemical Formula: A distillate of coal that contains an estimated 162 different compounds. Estimated make-up is as follows: aliphatic hydrocarbons (7%), polycyclic aromatic hydrocarbons (69%), and nitrogen containing polycyclic aromatic hydrocarbons (11%). Some of the polynuclear aromatic hydrocarbons identified in creosote are: anthracene, benz(*a*)anthracene, benzo(*a*)pyrene, and pyrene

Uses

Creosote is primarily used as a wood preservative in the United States. It has been used as a disinfectant, antiseptic, and a germicide, as a hop defoliant antifungal preparation, and as an animal or bird repellent. The leaves of the creosote bush may be used in herbal remedies or dietary supplements.

Exposure Routes and Pathways

The primary route of exposure is dermal through handling treated wood or inhalation, particularly when treated wood is burned in a poorly ventilated area.

Toxicokinetics

Absorption

Creosote is readily absorbed through the skin and the gastrointestinal tract. As creosote is diluted the absorption rate may actually increase.

Distribution

The K_{ow} (log of the octonal to water partition coefficient) is 1 and therefore is not expected to bioconcentrate.

Elimination

Creosote appears to be primarily excreted in the urine. Conjugation with sulfuric and hexuronic acids as well as oxidation leads to a 'smoky' appearance of the urine.

Clinical Management

Acute episodes are treated similar to phenolic poisonings with initial stabilization of breathing and cardiac monitoring. Dermal decontamination is accomplished by swabbing the affected area with olive oil. For ingested material the preferred method is administration of activated charcoal followed by a cathartic. Phenol and phenolic substances tend to exhibit an increased absorption rate at dilute concentrations and have a rapid onset of acute symptoms; therefore, there is a potential for seizures.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cattle have been poisoned by licking treated lumber, the estimated dose being 4–6 g kg^{-1}. A mixture of fuel oil and creosote was once widely distributed as a cure for ringworm. Excessive application of this material has caused poisoning of animals. Skin painting studies with mice have produced skin tumors. Creosote was mutagenic in the Ames test when metabolically activated with S9. The acute LD_{50} to rats is 725 mg kg^{-1}.

Human

Toxicity is expressed either via general depression with cardiac collapse or via the irritating/corrosive nature by irritation and burns of the skin and eyes. Brief exposures via inhalation may cause respiratory irritation. Oral exposure to larger quantities of creosote may result in stomach pains and burning of the mouth. Large doses (7 g for adults and 1–2 g for children) have been associated with death 14–17 h after ingestion. Cardiovascular collapse appears to be the primary cause of death. Nonlethal symptoms include salivation, vomiting, thready pulse,

headache, and loss of pupillary reflexes. Reports of long-term self-medication have indicated symptoms of intoxication and visual disturbances.

Chronic Toxicity (or Exposure)

Animal

Animal studies have demonstrated that creosote oils derived from coal tar are capable of producing skin carcinomas and papillomas when applied directly to the skin.

Human

Creosote is carcinogenic to humans x-udd occupational studies that show an increased incidence in scrotal cancer in workers exposed to creosote from wood and coal burning fire places.

Environmental Fate

Given the low water content and persistence of creosote, the material will tend to end up in the soil and sludge columns of the environment.

Ecotoxicology

Creosote is highly toxic to aquatic organisms with typical acute values of 76 ppb for *Daphnia magna* (48 h LC_{50}) and 600 ppb for rainbow trout (96 h LC_{50}).

Regulatory Levels

Creosote is regulated as a combustible/flammable liquid for transport. The US Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) classify creosote as a probable human carcinogen, class B 1 and 2A, respectively. The 8 h time-weighted average for creosote established by the EPA is 0.2 mg m^{-3}.

Regulations

- Creosote is included in the EPA high production volume program.
- Creosote is listed as an RCRA hazardous waste U051.
- IARC 2A carcinogen based on limited human and sufficient animal data.
- EPCRA SARA 313 reportable substance.
- CERCLA reportable quantity (RQ) of 1 pound.
- Regulated as a California Proposition 65 carcinogen.
- Classified in the European Union as toxic, may cause cancer (T: R-45).

See also: Coal Tar; Petroleum Distillates.

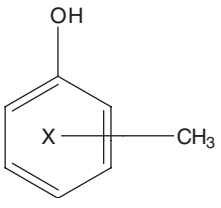
Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Creosote.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Creosote.

Cresols

Murali Badanthadka and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 1319-77-3
- Synonyms: Cresol; Tricresol
- Chemical Formula: C_7H_8O
- Chemical Structure:



Uses

It is used as a disinfectant, fungicide, bactericide, wood preservative, local antiseptic, parasiticide, and insecticide.

Background Information

Pure cresol is colorless, yellowish, brownish-yellow, or pinkish liquid. *o*-Cresol, *m*-cresol, and *p*-cresol are the three structural isomers of cresol. The names of the three compounds indicate which of the hydrogens on the benzene ring portion of the molecule have been replaced. They are obtained from coal tar or petroleum. Because the boiling points of these three compounds are nearly the same, a separation of a mixture of the three into its pure components is impractical.

The mixture of cresols obtained from coal tar is called cresylic acid, an important technical product used as a disinfectant and in the manufacture of resins and tricresyl phosphate. Cresols are useful as raw materials for various chemical products, disinfectants, and synthetic resins. The isomer *o*-cresol is a starting material for the herbicides 4,6-dinitro-*o*-cresol and 2-methyl-4-chlorophenoxyacetic acid. The isomers *m*-cresol and *p*-cresol are used in phenol–formaldehyde resins and are converted to tricresyl phosphate (a plasticizer and gasoline additive) and to di-*t*-butyl cresols (antioxidants called BHT).

Exposure Routes and Pathways

Cresols are released to the atmosphere in auto and diesel exhaust, during coal tar and petroleum refining, wood pulping, and during its use in manufacturing, metal refining, etc. Wastewater from these industries as well as municipal wastewater treatment plants contains cresols. Exposure may occur through inhalation, drinking water, dermal contact, food, and beverage ingestion.

Toxicokinetics

Cresols are absorbed across the respiratory and gastrointestinal tracts, and through the skin. Gastrointestinal and dermal absorption are rapid and extensive. Cresols are distributed to all the major organs. The primary metabolic pathway for cresols is conjugation with glucuronic acid and inorganic sulfate. Minor metabolic pathways include hydroxylation of the benzene ring and side-chain oxidation. The major route for elimination is renal excretion in the form of conjugate metabolites.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cresols are highly irritating to the skin and eyes of rabbits, rats, and mice. Short-term exposure to inhaled mixtures of cresol aerosol and vapor results in irritation of the respiratory tract, small hemorrhages in the lung, body weight reduction, degeneration of heart muscle, liver, kidney, and nerve cells.

Short-term oral exposure resulted in decreased body weight, organ weight, histopathological alterations in the respiratory and gastrointestinal tracts of rats. More severe effects were reported in mice. At the highest concentrations death resulted from exposure to *o*-, *m*-, and *p*-cresols but not from exposure to cresol itself.

Human

Cresols are highly irritating upon dermal contact, eye contact, and contact with any mucous membranes. Ingestion of cresols results in burning of the mouth and throat, abdominal pain, and vomiting. The target tissues/organs affected are the blood, kidneys, lungs, liver, heart, and central nervous system (CNS). In acute exposures, severe burns, anuria, coma, and death may result. Dermal exposure has been reported to cause severe skin burns, scarring, systemic toxicity, and death. Very few data are available regarding reproductive effects and there are no data on carcinogenicity in humans. At concentrations normally found in the environment, cresols do not pose any significant risk for the general population. However, under conditions of high exposure, people with renal insufficiency or enzyme deficiency will develop potential adverse health effects.

Chronic Toxicity (or Exposure)

Animal

Exposure to vapors of *o*-, *m*-, and *p*-cresol resulted in weight loss, reduced locomotor activity, inflammation of nasal membranes and skin, and changes in the liver. Thirteen-week oral exposures to mice, rats, and hamsters resulted in mortality, tremor, reduced body weights, hematological effects, increase in organ weight, hyperplasia of nasal and forestomach epithelium. Oral and inhalation exposures to cresol isomers result in lengthened estrus cycle, histopathological changes in the uterus and ovaries of rats as well as mice. No adverse effects on spermatogenesis are observed. Mild fetotoxic effects have been reported upon exposure of pregnant mice. Some evidence of genotoxicity has been reported from *in vitro* experiment using sister-chromatid exchange assay. However, cresol is not genotoxic after *in vivo* exposure.

Human

Prolonged or repeated absorption of low concentrations of cresol through the skin, mucous membranes, or respiratory tract may cause chronic systemic poisoning. Symptoms and signs of chronic poisoning include vomiting, difficulty in swallowing, salivation, diarrhea, loss of appetite, headache, fainting, dizziness, mental disturbances, and skin rash. Death may result if there has been severe damage to the liver and kidneys.

Clinical Management

Oral exposure: Liquid intake should be avoided because dilution may enhance absorption. Immediate

administration of activated charcoal is recommended to limit systemic toxicity. Ipecac-induced emesis is not recommended because of the potential for CNS depression, seizures, and aspiration. Gastric lavage is effective only within 1 h after ingestion. Patients should be treated symptomatically. Convulsions are controlled with diazepam.

In case of inhalation exposure victims should be removed to fresh air. Respiratory distress should be monitored and healthcare personnel consulted.

Dermal exposure: Decontamination with water is necessary. Copious dilution with room temperature water is appropriate after dermal and eye exposures.

Environmental Fate

Atmospheric fate: Cresols are not expected to persist in the atmosphere because: (1) cresols have low estimated half-lives (less than 1 day); (2) they are sensitive to photolysis; and (3) the water solubility of cresols may cause transport of cresols from the atmosphere to the soil or aqueous environment. The photodegradation half-life of cresol isomers during the daytime is 8–10 h while at night it is ~2–4 min. Daytime half-lives would be reduced under smog conditions. Cresols are highly soluble compounds, and gas scavenging will be an efficient removal process as is reflected by high concentrations in rain.

Terrestrial fate: While there is substantial release of cresols to the soil, this route of environmental exposure is not expected to be a problem. Cresols are readily biodegraded by soil microflora and move to lower layer of soil. Therefore, cresols will not persist in soils and will probably be leached, due to their water solubility, into the aquatic environment where they will be degraded by microorganisms. The degradation rates of cresols in soil may decrease at lower temperatures (-2°C to 5°C).

Aquatic fate: Cresols do not contain any functional groups that are hydrolyzable. Therefore, hydrolysis of these compounds in aquatic media is unlikely. However, it will degrade primarily due to biodegradation in eutrophic waters although photolysis may make a contribution in oligotrophic lakes based on modeling studies. Biodegradation generally occurs within 8 h after several days of acclimation, except in oligotrophic lakes, estuarine, and marine waters where degradation takes several days. Degradation is much slower under anaerobic conditions especially for the *o*-isomer.

Exposure Standards and Guidelines

Occupational Safety and Health Administration permissible exposure limit is 5 ppm (22 mg m^{-3}) for 8 h time-weighted average (TWA). The threshold limit value for cresol and its isomers is 5 ppm for 8 h TWA. National Institute for Occupational Safety and Health recommended exposure limit is 2.3 ppm (10 mg m^{-3}) for 10 h TWA for all isomers.

See also: Coal Tar; Pesticides.

Further Reading

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Lesaffer G, De Smet R, D'Heuvaert T, *et al.* (2003) Comparative kinetics of the uremic toxin *p*-cresol versus creatinine in rats with and without renal failure. *Kidney International* 64: 1365–1373.

Morinaga Y, Fuke C, Arao T, and Miyazaki T (2004) Quantitative analysis of cresol and its metabolites in biological materials and distribution in rats after oral administration. *Legal Medicine (Tokyo)* 6: 32–40.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Cresols.

Criminal Enforcement of Environmental Laws

Grant R Trigger

Scope of Potential Environmental Tort Criminal Liability

Initial environmental laws in the United States were focused on reducing the amount of contamination released to the environment. Expanding wastewater treatment systems and requiring improved pollution control equipment with attendant regulatory permit requirements were the primary vehicles for improving the environment. Over time those who failed to comply with these requirements became the focus of enforcement officials and the desire to encourage more widespread compliance resulted in pressuring individual corporate officials with the threat of

personal liability to ensure greater compliance. As discussed below, these trends have eroded traditional principles of criminal law such that there may actually be greater jeopardy of being convicted of an environmental related crime than of a drug or robbery related offense. The 'shrinking' *mens rea* requirement of knowingly causing a violation of law is a potential issue for any environmental criminal litigation matter according to Marshall, Sims, and Castella (see Further Reading section). In other words, traditional criminal law punished the defendant for knowingly causing harm; however, the trend in environmental crimes is to convict due to the consequences rather than intent. For example, if a plant operator allows a discharge, he may be potentially criminally liable even if he did not know the content of the discharge was hazardous. For a contrary view see the note authored by Escobar listed in the 'Further Reading' section.

From an international perspective, growing attention to chemical use and waste disposal practices has resulted in a proposal by the European Union to enact new legislation, which would require the registration and control of certain chemicals. Under the proposed Registration, Evaluation, and Authorization of Chemicals Act (REACH) chemical producers would be required to provide authorities, the public, and customers with basic toxicity and exposure information. If that information is not supplied the chemical will not be allowed on the market. The more dangerous chemicals cannot be used without permission and the user must demonstrate that there is no alternative to the use of that chemical. Interestingly, REACH as proposed does not specify civil or criminal penalties but instead defers to the member states for selection and enforcement of specific penalties. In the meantime, potential criminal investigations and prosecutions of environmental matters are underway in international forums such as Paris (1999 Erika oil spill) and Malaysia (industrial sludge from Taiwan imported under falsified import documents). More information on these matters can be found in the 'Further Reading' section.

Evolution of Environmental Criminal Liability

Tampering with monitoring equipment and falsifying consumer certifications has been the basis of criminal convictions. In May, 1998, Louisiana-Pacific Corp. pleaded guilty to Clean Air Act (CAA) and consumer fraud violations at its strand board manufacturing plant near Montrose, Colorado, agreed to pay a $5.5 million criminal fine, $31 million for consumer fraud violations, and make a $500 000 donation to seven groups working to improve air quality. The criminal fine was the largest ever received in the CAA's 28 year history. The company pleaded guilty to tampering with emissions monitoring equipment, lying to the Colorado Department of Public Health about the number of times the mill violated its permits, creating nonrepresentative samples for the American Plywood Association that were used in quality assurance testing, and misrepresenting to customers, through use of the Association's quality assurance certification mark, that the product met the requirements of the Association. The investigation began when a former employee filed suit against the company when he was discharged after refusing to tamper with the equipment. *US v. Louisiana-Pacific Corp.*, No. 95-CR-215 (D. Colo. 1998).

In addition, inadequate resources devoted to environmental compliance can lead to criminal liability. In May 1991, United Technologies Corp. pleaded guilty to six felony violations of Resource Conservation and Recovery Act (RCRA) and agreed to pay a $3 million fine for hazardous waste violations. The violations were the result of improper disposal of cleaning solvents at a Stratford, Connecticut site, discovered during an Environmental Protection Agency (EPA) inspection of the facility. The company's in-house environmental compliance officer became aware of the illegal disposal, but it was not discontinued until the following year. The company had only one full-time person responsible for environmental compliance for all of its facilities in the United States. The US Attorney issued a statement that "companies creating hazardous wastes have a clear duty to aggressively devote adequate manpower and financial resources to protecting our environment." The EPA Regional Administrator also issued a statement that "it should now be abundantly clear that criminal sanctions are not reserved only for the flagrant and deliberate violations of the environmental laws, but also for violations that result from a company's plain or institutional indifference to meet its legal responsibility." *US v. United Technologies Corp.*, No. 2:91CR00028 (D. Conn. 1991).

Liability exposure to individual officers or employees has expanded the scope of criminal liability and responsibility for not only the actions of individuals but also their respective corporate employers. In the first conviction for the newly created Multi-Agency Environmental Task Force in the US District Court for the Eastern District of Michigan, the owner of an environmental laboratory pled guilty to mailing falsified environmental test results and bills for tests his company never performed. Jerry Martin, owner of Martin Environmental

Laboratories, was sentenced to 1 year in prison and payment of $16 781 to former customers. The company was fined $5000. *US v. Martin Envt'l Labs.*, No. 01-90040 (E.D. Mich. May 2, 2002). Because no environmental statutes prohibited laboratory fraud, Mr Martin was charged with mail fraud, a federal felony with sentencing guidelines that include prison. The case started with a tip from a former employee to the Task Force. The Task Force consists of the US Attorney for the Eastern District of Michigan, the Michigan Attorney General, the Federal Bureau of Investigation, the US Coast Guard, and the US Customs Service.

Summaries of Selected Environmental Criminal Actions Involving Individuals

Expanding the 'reach' of the federal racketeering statute to environmental matters has broadened the basis for criminal environmental liability. On August 10, 2001, the US District Court for the Eastern District of Michigan, in the first successful case for convictions under the federal racketeering law for environmental crimes, sentenced the president/owner and operations manager of Hi-Po, Inc. to multiple-year prison terms. They were charged with dumping materials such as diesel fuel into streams and sewers so that their company would win contracts to clean up the polluted waters. Aaron Smith, president and owner, was sentenced to 33 months in prison, for violation of the Racketeer Influenced and Corrupt Organizations Act (RICO), followed by 3 years of supervised release. He was also ordered to pay $505 000 in restitution and to forfeit $500 000. Stephen Carbeck, the company's operations manager, was sentenced to 27 months in prison, for violation of RICO, followed by 3 years of supervised release, and was ordered to pay $430 000 in restitution. The company itself was fined $50 000 for pleading guilty to two counts of violating the Clean Water Act (CWA). *US v. Smith*, No. 00-80528 (Aug. 10, 2001).

In a case that further widened the scope of environmental criminal liability the court concluded that a defendant did not have to know that his conduct violated the law to be held criminally liable. On September 16, 1998, the US Court of Appeals for the Sixth Circuit upheld a trial court's conviction of a Louisville, Kentucky paint manufacturer and its vice president for violations of RCRA for the illegal storage and disposal of hazardous waste materials. The court found that government prosecutors were not required to prove that the paint manufacturers and its vice president knew that materials stored or dumped at its facility were hazardous substances or that such storage or disposal required a permit and distinguished this case from the *Ahmad*, *infra*, case because the *Ahmad* case did not discuss the requirement for knowledge of the law and dealt with the issue of mistakes in facts. The government prosecutors were only required to prove that the defendant had knowledge of the storage or disposal, that the material was waste, and that it was harmful to others or the environment. The court held that the vice president did not have to know the materials were RCRA hazardous wastes to support a 'knowing' violation under RCRA. The trial court's 21 month prison term and $5000 fine for the vice president was upheld. The company had not appealed its $225 000 fine. The violations were discovered in 1992 during a Kentucky Department of Environmental Protection inspection. The vice president was in charge of manufacturing operations and had responsibility for environmental compliance at the company's facilities. *US v. Kelley Technical Coatings, Inc.*, No. 96-6282, 157 F.3d 432 (6th Cir. 1998).

On May 29, 1998, four executives of the former LCP Chemicals plant in Brunswick, Georgia, were indicted on 42 counts of violating federal environmental laws. The plant had been placed on the National Priorities List (NPL) and was closed in 1994 as state officials were in the process of revoking the facility's permits. Each defendant was charged with one count of conspiring to violate the CWA, RCRA, the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), and the Endangered Species Act. Christian Hansen, chairman and CEO of the corporate owner of the company and *de facto* plant manager, and Randall Hansen, COO of the corporate owner, and Alfred Taylor, plant and operations manger of the plant, were charged with 20 counts of violating the CWA. Brent Hanson, technical and environmental manager of the corporate owner, was charged with 17 counts of violating the CWA. The CWA violations were for alleged discharges of mercury and chlorine into a creek in violation of the facility's NPDES permit. The RCRA charges were for illegal storage of mercury-contaminated hazardous waste in buildings and tanks at the plant and for filing false and misleading reports. Christian Hansen was sentenced to 9 years in prison; Randall Hansen was sentenced to 46 months. *US v. Hansen*, No. CR-298-23 (S.D. Ga. Jan. 1999). The Hansens appealed and on August 24, 2001 the US Court of Appeals for the Eleventh Circuit upheld the convictions. *US v. Hansen*, No. 99-11638, 262 F.3d 1217 (11th Cir. Aug. 24, 2001). On June 3, 2002, the US Supreme Court declined to overturn the convictions or to review the limits of the responsible corporate officer doctrine.

On July 11, 1997, a US appeals court upheld the 1996 conviction of Timothy Sinskey, a former vice president and plant manager, and Wayne Kumm, the plant engineer, of a John Morell & Co. meat packing facility in Sioux Falls, South Dakota. *US v. Sinskey*, No. 96-3962, 119 F.3d 712 (8th Cir. 1997); *US v. Kumm*, No. 96-3965 (8th Cir. 1997). The court distinguished this case from *Ahmad, infra*, and held that while *Ahmad* was based on a 'mistake of fact' defense, this case was based on a 'mistake of law' and stated, "We have repeatedly held that, in other statutes with similar language, the word 'knowingly' refers only to knowledge of the relevant activities" and that knowledge of the illegal nature of the acts need not be proved. 119 F.3d at 715. The two employees were charged with manipulating the flow of discharge material and selectively sampling the effluent to minimize the number of exceedances at the plant. In resolution of criminal charges against the company, it had previously agreed to pay a $2 million fine and $1 million to establish an environmental cleanup fund. *US v. John Morrell & Co.*, No. 96-CR-40004 (D.S.D. May 28, 1996). Mr Sinskey was convicted on felony counts that included conspiracy, rendering a discharge monitoring method inaccurate, illegally discharging hazardous waste, and falsifying discharge monitoring reports and was sentenced to 24 months in prison, placed on probation for 2 years, and ordered to pay a $5000 fine and $600 to a federal victim witness fund. *US v. Sinskey*, No. 96-400/001 (D.S.D. Nov. 4, 1996). Mr Kumm was convicted on one felony count of rendering a monitoring device inaccurate and was sentenced to 6 months in jail, 6 months of home confinement, a $2000 fine, and $8000 to an environmental group. *US v. Kumm*, No. 96-400/002 (D.S.D. Nov. 4, 1996).

In *US v. Ahmad*, 101 F.3d 386 (5th Cir. 1996), a federal appeals court held that the government must meet 'traditional' intent requirements of criminal law to obtain a conviction under the CWA. The court reversed and remanded the lower court's conviction and held that the law required the government to prove defendant's knowledge of each actual element of an offense and that the defendant had to know all the facts that made his actions illegal. The court also narrowed the 'public welfare doctrine' that allows the government to dispense with traditional intent requirements in prosecuting some environmental regulatory crimes and held that serious felonies should not fall within the public welfare exception absent a clear statement from Congress. Mr Ahmad was convicted of discharging gasoline into a city sewer when, in fact, he thought it was water – a mistake in fact which, he claimed, meant that he lacked the 'knowledge' required for a criminal conviction. Later decisions have distinguished themselves from *Ahmad* as being convictions based on 'mistakes in law' and not 'mistakes in fact'.

On April 3, 1991, the US Court of Appeals for the First Circuit upheld the conviction and 2-day prison sentence of David Boldt, a chemical engineering manager employed for 6 months by Astro Circuit Corporation, for knowingly discharging wastewater containing excessive amounts of copper into a city sewer. Mr Boldt was one of four defendants charged in a 52-count indictment for numerous violations of the CWA and he was found guilty of preparing false written statements to authorities for two incidents. *US v. Boldt*, No. 90-1454, 929 F.2d 35 (1st Cir. 1991).

On August 16, 1999, the US District Court for the Middle District of Florida sentenced the owner of a company to 13 years in prison and fined him $14 000 for intentionally and continuously dumping toxic waste into the Tampa, Florida sewer system over a period of 9 years in violation of the CWA and RCRA. Gary Benkovitz, also known as Gary Blake, owned Bay Drum and Steel, Inc., and admitted that he directed employees to empty drums into a storm sewer that empties into McKay Bay. The prison sentence is the maximum allowed under the federal sentencing guidelines for environmental crimes. One of the reasons Mr Benkovitz received the maximum sentence is because he allegedly continued the violations while he was awaiting sentencing for an earlier felony charge for illegal dumping. The company is estimated to have discharged 3 million gallons of contaminated wastewater and more than 450 000 lb of hazardous solid waste into the sewer system. *US v. Benkovitz*, Nos. 97-331, 98-349 (M.D. Fla. Aug. 16, 1999).

Criminal Convictions Related to Toxic Tort Exposures

In the context of personal injury toxic tort liability a defendant's apparent intentional disregard for the safety of his employees led to a criminal conviction. On June 26, 1998, a five-count indictment was lodged against the owner of an Idaho fertilizer company who allegedly exposed workers to cyanide in 1996 without protective equipment, causing permanent brain damage to one employee. Allan Elias, the owner, was charged with endangering the safety and health of employees by ordering employees to clean out a 25 000 gal storage tank that contained cyanide. Count one was for knowing endangerment; and counts two through five were for illegal disposal of hazardous waste on three occasions and making a

false statement by fabricating and backdating a safety plan on worker entry of the tank. Mr. Elias ordered the workers to continue after they complained about sore throats and stopped work inside tank. Mr. Elias informed emergency workers that the tank contained water and that he did not know what caused the injury to the 20 year old employee who ultimately sustained permanent brain damage. Mr Elias was sentenced to 17 years of imprisonment and ordered to pay $6 million in restitution to the family of one of his employees who suffered brain damage as a result of cleaning the tank without wearing appropriate protective gear. *US v. Allan Elias*, No. CR-98-070-BLW (D. Idaho June 2, 1998). On October, 23, 2001, the US Court of Appeals for the Ninth Circuit upheld the conviction, but remanded the case to the district court to amend the sentence by deleting the restitution provision because the particular provision under which Mr. Elias was ordered to pay restitution (18 U.S.C. § 3663) did not allow the imposition of restitution. *US v. Elias*, No. 00-30145, 27 Fed. Appx. 750 (9th Cir. Oct. 23, 2001).

In 1995, a Florida jury awarded $500 million to the parents of one of two children who died in 1992 from acute toluene exposure while playing in an unlocked dumpster where the chemical was illegally discarded. *Perez v. William Recht Co.*, No. 92-8983-B (Hillsborough County Cir. Ct., Sept. 1995). As a result of the incident, a plant manager and a shop foreman received federal prison sentences, and the company pleaded no contest to criminal charges of knowing endangerment and received a $1.5 million fine. *US v. Whitman*, No. 94-70-CRT-1B (M.D. Fla. 1994). On July 28, 1998, a federal grand jury indicted the owner of William Recht Co. for eight counts of criminal hazardous waste storage without a permit. The indictment alleges that Mr Recht refused at least four times to provide funds for proper disposal of drums of hazardous and nonhazardous waste after the death of the children. *US v. Recht*, No. 98-280-CR-T-24A (M.D. Fla. 1998).

The potential liability for corporate officials has expanded even to those who are not in direct management control of environmental compliance activities. In *Doe Run Resources Corp. v. Neill* No. SC85451, 123 S.W. 3d 502 (Mo. S. Ct Feb. 10, 2004), the Missouri Supreme Court concluded that a lead smelter's chief financial officer likely would have had enough knowledge of the company's alleged polluting activities that he could have stopped or influenced those activities by his control over the company's finances and budget. As a result the court held he could be sued personally under applicable Missouri law for harm caused by the company's breach of state and federal environmental laws. Although this case did not address criminal liability it suggests that if a CFO can be held liable for environmental non-compliance of his company, that a criminal case based on little more may under case specific circumstances lead to potential criminal liability.

US Environmental Protection Agency Criminal Enforcement Activities

As of the end of 2003, the US EPA had charged an average of about 330 criminal defendants per year (1998–2003) with no trends suggesting a decline in prosecutions. An average of over 183 years of sentences were issued and an average of over $84 million dollars in fines were collected each year during this same time period. While these numbers do not reflect any assessment of tort claim recoveries they do demonstrate the fact that criminal matters are not insignificant on a national scale and must be considered in reviewing any potential toxic tort matter.

See also: Chemicals of Environmental Concern; Clean Air Act (CAA), US; Clean Water Act (CWA), US; Resource Conservation and Recovery Act.

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Cromolyn

F Lee Cantrell

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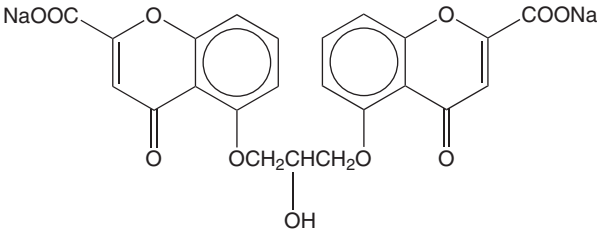
- Chemical Abstracts Service Registry Number: CAS 16110-51-3
- Synonyms: Cromolyn sodium; Disodium cromoglycate; Disodium salt of cromolyn; Nasalcrom; Intal; Crolom; Gastrocrom
- Chemical/Pharmaceutical/Other Class: Mast cell stabilizing antiallergic agent
- Chemical Formula: $C_{23}H_{14}Na_2O_{11}$
- Chemical Structure:



Cromolyn sodium

Uses

Cromolyn is used primarily for the prophylaxis of various types of asthma and in the treatment of mastocytosis and vernal conjunctivitis.

Exposure Routes and Pathways

For use in asthma, cromolyn is administered by inhalation using solutions delivered by aerosol spray or nebulizer as well as a powdered drug mixed with lactose and delivered by a turbo inhaler. For use in mastocytosis, cromolyn is ingested in a liquid form. Cromolyn is available in ocular drop form for the treatment of vernal conjunctivitis.

Toxicokinetics

Oral absorption of cromolyn is less than 1%, although up to 10% of an inhaled dose of cromolyn can be absorbed systemically. After complete absorption, cromolyn is excreted unchanged in urine and bile in about equal proportions. Peak plasma concentrations occur 15 min after inhalation. The distribution of cromolyn in the lung and the extent of systemic absorption are enhanced by bronchodilation during drug delivery. The biological half-life following inhalation ranges from 45 to 100 min.

Mechanism of Toxicity

The major prophylactic effect of cromolyn is centered on inhibition of the degranulation of pulmonary mast cells causing a reduction in histamine release, reduced leukotriene production, and inhibition of release of inflammatory mediators from several cell types.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of cromolyn, measured as the LD, has been determined in the rat ($>2150\,mg\,kg^{-1}$ orally and $6000\,mg\,kg^{-1}$ subcutaneously) and the mouse ($3300\,mg\,kg^{-1}$, intravenous; $1000\,mg\,kg^{-1}$, intraperitoneal; and $4400\,mg\,kg^{-1}$, subcutaneous).

Human

None reported.

Chronic Toxicity (or Exposure)

Animal

Studies of reproduction in mice, rats, and rabbits have not demonstrated fetal toxicity at doses up to 338 times the usual human dose.

Human

Because of its low toxicity, cromolyn is generally well tolerated. Adverse side effects, such as bronchospasm, cough, wheezing, laryngeal edema, joint swelling, joint pain, angioedema, headache, rash, and nausea, are rare (less than 1 in 10 000 patients). Documented instances of anaphylaxis are also been rare.

In Vitro Toxicity Data

Peripheral blood mononuclear cells obtained from allergy-dependent asthmatics demonstrated antigen-specific anti-allergic inflammatory effects.

Clinical Management

Toxicity is unlikely, but should adverse effects occur, general emergency management and supportive care procedures are indicated.

See also: Aerosols.

Further Reading

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Crude Oil *See* Oil, Crude.

CS Gas

Harry Salem, Bryan Ballantyne, and Sidney A Katz*

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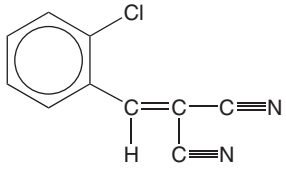
- Military Designation: CS
- Chemical Name: Chlorobenzylidene malononitrile
- Chemical Abstracts Service Registry Number: CAS 2698-41-1
- Synonyms: Tear gas; Less-than-lethal; Nonlethal; Lacrimator, Harassing agent; Incapacitant; (2-Chlorophenyl) methylene; Propanedinitrile, (*o*-Chlorobenzylidine) malononitrile; 2-Chlorobenzalmalononitrile
- Chemical Formula: $C_{10}H_5ClN_2$
- Chemical Structure:



Pharmacological Action

Riot control agents such as CS are those that cause disabling physiological effects when they come into contact with the eyes or skin, or when inhaled. They have the capacity to cause intense sensory irritation of the skin and mucus membranes of the eye and respiratory tract. They are peripheral sensory irritants that pharmacologically interact with sensory nerve receptors in skin and mucosal surfaces at the site of contamination resulting in local pain and discomfort sensations with associated reflexes. The reflex associated with the inhalation exposure of irritants is the Kratschmer reflex. This reflex causes apnea, bradycardia, and a biphasic fall and rise in aortic blood pressure.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Pharmacological Class

CS is a peripheral sensory irritant, lacrimator, sternutator, and an incapacitant.

Uses

CS is used as a nonlethal or less-than-lethal chemical in riot control situations, to distract, deter, incapacitate, disorient, or disable disorderly people, to clear facilities, areas, deny areas, or for hostage rescue. It can also be used in peacekeeping operations. It is also used in military training as a confidence builder for the protective mask. In addition to the nonpersistent form of CS, two hydrophobic variations were created, CS1 and CS2. CS1 is a micronized powder formulation containing 5% hydrophobic silica aerogel, which can persist for up to 2 weeks in normal weather conditions, and CS2 is a siliconized microencapsulated form of CS1 with a long shelf life, persistence, resistant to degradation, and ability to float on water, which could restrict or deny the use of water for military operations. CS is commonly used as a riot control agent and a simulant for training. Members of military organizations and law enforcement agencies are routinely exposed to heated CS during training. The heat vaporizes the CS for dispersion, which then condenses to form an aerosol.

Exposure Routes and Pathways

CS at room temperature is a white solid, stable when heated and with a low vapor pressure. The vapor is several times heavier than air. It can be dispersed as a fine powder, or as a jet or stream of solution from small or large spray tanks, as well as aerosols or smokes by pyrotechnic generation. Its solubility in water is limited, but it is soluble in organic and chlorinated organics. High-temperature dispersion may produce a number of organic thermal degradation

products through rearrangements and loss of cyano and chlorine substituents on CS, possibly HCN and HCl. CS is rapidly hydrolyzed in water with a half-life of ~15 min at room temperature at pH 7. In alkaline solution (pH 9), the half-life is ~1 min. Therefore, CS can be easily inactivated by a water/alkaline solution, or by washing with soap and water.

Toxicokinetics

CS reacts covalently with plasma proteins to form compounds that may be antigenic. On contact with water, it hydrolyses into *o*-chlorobenzaldehyde and malononitrile. The kidney excretes *o*-chlorobenzaldehyde as the metabolites *o*-chlorohippuric acid (major) and *o*-chlorobenzoic acid (minor). The malononitrile is metabolized to thiocyanate. The cyano groups of 2-chlorobenzylidene malononitrile are unlikely to cause systemic cyanide toxicity since no significant amounts of free cyanide appear in the plasma.

Mechanism of Toxicity

These agents are considered less than lethal and nonlethal because they have a very large safety ratio. That is, their effective dose or concentration ECt_{50} is very low compared to their lethal dose or concentration (LCt_{50}).

CS as well as CN is an SN2-alkylating agent with activated halogen groups that react readily at nucleophilic sites. The prime targets include sulfhydryl-containing enzymes such as lactic dehydrogenase. In particular, CS reacts rapidly with the disulfhydryl form of lipoic acid, a coenzyme in the pyruvate decarboxylase system. It has been suggested that tissue injury may be related to inactivation of certain of these enzyme systems. CS causes the release of bradykinin, which can cause pain without tissue injury. The initial response to the inhalation of CS or other sensory irritants is consistent with the Kratschmer reflex and the Sherrington pseudoaffective response. These aerosols stimulate the pulmonary irritant receptors to produce bronchoconstriction and increased pulmonary blood volume by augmenting sympathetic tone. The chlorine atoms released from CS on contact with skin and mucus membranes are reduced to hydrochloride acid that can cause local irritation and burns.

Human Toxicity

When CS was disseminated using spray nozzles from a 10% solution in acetone or in methylene dichloride, or from a miniature M8 thermal grenade, the mass median diameter of CS produced was 3.0 μm for the CS in acetone, 1.0 μm for the CS in methylene dichloride, and 0.5 μm for the miniature M18 CS thermal grenade. When properly fitted, protective masks fully protected against exposure to CS. In those who were unable to mask rapidly, panic was evident. Concentrations of 9–10 $mg\,m^{-3}$ forced 50% of the subjects to leave the chamber within 30 s, 99% left when exposed to ~17 $mg\,m^{-3}$, and 100% left and were considered incapacitated at 40 $mg\,m^{-3}$ or greater.

Persons who had been exposed previously to a high concentration developed a fear of the agent, and even though subsequently exposed to lower concentration, the time to incapacitation for trained men was shorter than expected. There were no significant differences noted in the time to incapacitation in subjects exposed to CS at 0–95°F, although it was apparent that the subjects appeared unable to tolerate the agent as well as those exposed at ambient temperature. At 95°F and a relative humidity of 35% or 97%, the skin-burning effects were much more prominent, possibly because of the excessive diaphoresis. Hypertensive subjects reacted similarly to and tolerated CS as well as normotensive individuals. However, their blood pressure elevation was greater and lasted longer than in normotensives, possibly because of the stress of exposure. The hypertensive subjects recovered as rapidly as normotensives.

Subjects with a history of peptic ulcer, jaundice, or hepatitis, and those between the ages of 50 and 60 years reacted similarly to normal subjects. Persons with a history of drug allergy, hay fever, asthma, or drug sensitivity were able to tolerate CS exposure as well as the normal subjects; however, a higher percentage of this group had more severe chest symptoms than the normals. Although many of these lay prostrate on the ground for several minutes, no wheezing or ronchi were heard on auscultation, and recovery time was slightly prolonged, but only by 1 to 2 min. Although not significantly different, subjects exposed to CS disseminated from methylene dichloride appeared to tolerate the agent for a slightly longer period than those subjected to CS in acetone solution, nor was there many differences from CS disseminated from the miniature M18 CS smoke grenade. CS was effective within seconds. Although high concentrations for prolonged exposure in closed spaces can produce severe effects, no validated deaths in humans have been reported for CS. These effects were acute laryngo-tracheobronchitis in an infant, reactive airway dysfunction syndrome, hemoptysis and hypoxia, and erythroderma in adults, which were all treated successfully. Ingestion

of CS may lead to abdominal cramping, pain, and diarrhea.

Clinical Management

Ocular exposure to CS produces intense blepharospasm, pain, lacrimation, conjunctival erythema, periorbital edema, and a rise in intraocular pressure. These effects generally diminish within 30 min postexposure. CS also produces rhinorrhea, nasal irritation and congestion, bronchorroea, sore throat, cough, sneezing, unpleasant taste, and burning of the mouth immediately after exposure. These effects rapidly resolve within minutes postexposure. Symptomatic treatment of ocular irritation consists of use of a topical solution to relieve the irritation with topical antibiotics. The eyes should be examined for corneal abrasions. Treatment with oral analgesics, topical antibiotics, and mydriatics should be considered. Since CS is a solid, it is possible for a particle or clump to become embedded in the cornea or conjunctiva and cause tissue damage. Medical care for eye pain after exposure should include thorough decontamination of the eyes and a thorough ophthalmologic examination. The eye with ocular injuries should be carefully irrigated with isotonic saline and the remaining powder removed with a cotton swab. Any remaining stromal particles should be removed with a needle tip under slit lamp illumination. Airway problems may occur in individuals with lung disease, especially if exposed to higher than average field use concentrations. If these occur, the immediate priority is the removal from the exposure and to ensure a potent airway.

Severe and prolonged erythema or serve dermatitis may occur several hours after exposure that is then followed by vesiculation. These are generally second-degree burns and should be treated like second-degree chemical burns.

If the release of irritant incapacitants is in a confined, unventilated space, exposure may be to very high concentrations. Some individuals may be more susceptible to high concentrations, possibly because of an existing medical condition such as asthma, and will require intensive supportive medical treatment postexposure.

Animal Toxicity

Various experimental animal species were exposed to aerosols of CS generated by various methods from exposure from 5 to 90 min. The toxic signs observed in mice, rats, guinea pigs, rabbits, dogs, and monkeys were immediate, and included hyperactivity, followed by copious lacrimation, and salivation within 30 s of exposure in all species except the rabbit. The initial level of heightened activity subsided, and within 5–15 min following initiation of the exposure, exhibited lethargy and pulmonary stress, which continued for about an hour following cessation of the exposure. All other signs had disappeared within 5 min following removal from the exposure. When toxic signs were observed, they occurred following exposure by all of the dispersion methods.

Lethality estimates were expressed by calculation of LCt_{50} values. From acute exposures to CS dispersed from a 10% CS in methylene dichloride the LCt_{50} values (in mg min m^{-3}) were as follows: mice, 627 000; rats, 1 004 000; and guinea pigs, 46 000. No deaths occurred in rabbits exposed to up to 47 000 mg min m^{-3}. CS at dosages up to 30 000 mg min m^{-3} did not cause any deaths in any of the monkeys with pulmonary tularemia. The combined LCt_{50} for mice, rats, guinea pigs, and rabbits was calculated to be 1 230 000 mg min m^{-3} for CS dispersed from methylene dichloride. Goats, pigs, and sheep did not exhibit hyperactivity on exposure to CS, and they were also resistant to its lethal effect. Therefore, no LCt_{50} values could be calculated for goats, pigs, or sheep. However, a combined LCt_{50} was calculated for all of the species tested, including mice, rats, guinea pigs, rabbits, dogs, monkeys, goats, pigs, and sheep, and was estimated to be 300 000 mg min m^{-3}. LCt_{50} values were also calculated for CS dispersed from M18 and M7A3 thermal grenades. These were (in mg min m^{-3}): 164 000 for rats and 36 000 for guinea pigs exposed to the M18 thermal grenade dissemination; for the M7A3 thermal grenade they were (in mg min m^{-3}) as follows: rats, 94 000; guinea pigs, 66 000; rabbits, 38 000; goats, 48 000; pigs, 17 000; dogs, 30 000; monkeys, 120 000.

All of the acute exposure results were combined and LCt_{50} values were calculated for all rodents to be 79 000 mg min m^{-3}, and for all nonrodent species tested to be 36 000 mg min m^{-3}, and for all the species it was 61 000 mg min m^{-3}. The LCt_{50} values for CS2 were also calculated. CS2 is 95% CS, 5% Cal-o-Sil R, and 1% hexamethyldisilazane, and the LCt_{50} values are: rats, 68 000 mg min m^{-3}; guinea pigs, 49 000 mg min m^{-3}; dogs, 70 000 mg min m^{-3}; and monkeys, 74 000 mg min m^{-3}. The lethal effects in animals following inhalation exposures are caused by lung damage leading to asphyxia and circulatory failure, or bronchopneumonia secondary to respiratory tract injury. Pathology involving the liver and kidneys following inhalation of high dosages of CS is also secondary to respiratory and circulatory failure.

The acute inhalation toxicity of CS, generated in smoke and as an aerosol, was studied in several

Table 1 Acute inhalation toxicity LCt_{50} (mg min m^{-3})

Animal	*CS smoke*	*CS aerosol*
Guinea pig	35 800	67 000
Rabbit	63 600	54 090
Rat	69 800	88 480
Mouse	70 000	50 110

animal species, and the LCt_{50} data are presented in **Table 1**.

Repeat exposures of thermally dispersed CS were conducted in rats and dogs. They were exposed from 4 to 5 min per day, 5 days a week for 5 weeks. The 25 day cumulative dosage (Ct) to which the rats were exposed was 91 000 mg min m^{-3} (3640 mg min m^{-3} per day), while the dogs were exposed to a cumulative dosage of 17 000 mg min m^{-3} (680 mg min m^{-3} per day). No lethality occurred in the dogs, while the rats became hyperactive and aggressive, biting noses and tails of other rats, and scratching their own noses. No changes were found in blood values for sodium, potassium, protein, albumin, or creatinine throughout the tests. Five of the 30 rats exposed died, two following the cumulative dosage of 25 000 mg min m^{-3}, and three died after 68 000 mg min m^{-3}. Gross pathological examinations of the rats that died were negative, as were those of six other rats that were sacrificed after 5 weeks' exposure. The exposed rats lost ~1% of body weight, while unexposed rats gained ~20% during the 5 weeks. There were no significant differences in organ to body weight ratios for heart, kidneys, lungs, liver, or spleen following the 5 week exposures. It was concluded that repeated exposures did not make the animals more sensitive to the lethal effects of CS. The animals that died after exposure to CS showed increased numbers of goblet cells in the respiratory and gastrointestinal tracts and conjunctiva, as well as necrosis in the respiratory and gastrointestinal tracts, pulmonary edema, and occasionally hemorrhage in the adrenals. Death appeared to result from poor transfer of oxygen from the lungs to the blood stream, probably because of edema, and hemorrhage in the lungs, and obstruction of the airways.

The effects of repeated exposures to CS in mice, rats, and guinea pigs to neat CS aerosols for 1 h per day, 5 days per week for 120 days demonstrated that high concentrations of CS were fatal to the animals after only a few exposures, while mortality in the low and medium concentrations did not differ significantly from the controls. It was concluded that CS concentrations below 30 mg m^{-3} were without deleterious effects.

The effects of CS inhalation were studied on embryonic development in rats and rabbits at concentrations consistent with those expected in riot control situations (~10 mg m^{-3}). Although the concentrations were low and the duration of exposure (5 min) may not have been adequate to assess the fetotoxic and teratogenic potential of CS, no significant increase in the numbers of abnormal fetuses or resorptions were noted.

CS2 was evaluated for carcinogenicity in the US National Toxicity Program (NTP) 2 year rodent bioassay. Compound related non-neoplastic lesions of the respiratory tract were observed. The pathologic changes observed in the exposed rats included squamous metaplasia of the olfactory epithelium, hyperplasia, and metaplasia of the respiratory epithelium. In mice, hyperplasia and squamous metaplasia of the respiratory epithelium was observed. Neoplastic effects were not observed in either rats or mice. It was concluded that the findings suggests that CS2 is not carcinogenic to rats and mice. CS in methylene chloride was also tested in mice and rats for carcinogenicity in a 2 year study, and no tumorigenic effects were observed in the CS-exposed animals.

In Vitro Toxicity Data

The mutagenic potential of CS and CS2 were studied in microbial and mammalian bioassays. CS was positive in the Ames Assay, while others reported questionable genotoxicity for *S. typhimurium*, and negative when tested in *S. typhimurium* strains TA 98, TA 1535, and TA 1537 with and without metabolic activation. The mutagenic potential for CS and CS2 in mammalian assays such as the Chinese hamster ovary test for the induction of sister chromatid exchange (SCE) and chromosomal aberration (CA), and the mouse lymphoma L5178Y assay for induction of trifluorothymidine (Tfi) resistance indicated that CS2 induced SCE, CAs, and Tfi resistance. The Committee on Toxicology of the (US) National Research Council (1984) reported that, taken in their totality, the test of CS for gene mutation and chromosomal damage provide no clear evidence of mutagenicity. Although most of the evidence is consistent with nonmutagenicity, in the committee's judgment, it is unlikely that CS poses a mutagenic hazard to humans.

Decontamination

Contaminated clothing should be removed and sealed in a plastic bag. Disposable rubber gloves should be used when handling contaminated clothes.

The eyes should be irrigated copiously with saline for 15–20 min. Contaminated skin should be washed thoroughly with copious amounts of water, alkaline soap and water, a mildly alkaline solution (sodium bicarbonate or sodium carbonate) or mild liquid soap and water. The use of sodium hypochlorite solution will exacerbate the skin lesions and should not be employed. Only a saline irrigation should be used over vesiculated skin.

Decontamination of material/clothing after contamination with CS can be done with sodium bicarbonate or carbonate 5–10% solution. If this means of decontamination cannot be accomplished (e.g., contaminated rooms and furniture), then the only other means is by intensive air exchange – preferably with hot air.

If clothing is to be washed, cold water should be used because hot water will cause any residual CS to volatilize leading to symptoms in attending staff.

See also: CN Gas; Non-Lethal Weapons, Chemical; Riot Control Agents.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

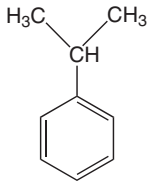
http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Culture and Toxicology *See* Toxicology in the Arts, Culture, and Imagination.

Cumene

Ralph Gingell

- Chemical Abstracts Service Registry Number: CAS 98-82-8
- Synonyms: Isopropyl benzene; (1-Methylethyl)-benzene; 2-Phenylpropane
- Chemical/Pharmaceutical/Other Class: Alkyl aromatic family of hydrocarbons
- Chemical Formula: C_9H_{12}
- Chemical Structure:



Uses

Cumene is an industrial intermediate in the manufacture of phenol and acetone via cumene hydroperoxide. It also has minor applications as a solvent.

Exposure Routes and Pathways

Cumene is a naturally occurring constituent of crude oil and may be released to the environment from a number of anthropogenic sources, including processed hydrocarbon fuels; for example, diesel fuel contains 0.86 wt.% of cumene; furnace oil (no. 2) contains 0.60 wt.%. Humans can be exposed to cumene via industrial emissions, petrol station or motor vehicle emissions, and accidental releases. The general population would be exposed to cumene primarily by inhalation, although occupational populations may be exposed by the dermal route. Minor exposure may result from contact with refined petroleum products and ingestion of contaminated foods and possibly drinking water.

Toxicokinetics

Cumene is absorbed readily via the inhalation route in man and animals, and is metabolized efficiently, within the body, to water-soluble metabolites that are excreted into the urine. The secondary alcohol 2-phenyl-2-propanol and its conjugates are major metabolites. Neither cumene nor its metabolites are likely to accumulate within the body. Based on controlled studies in humans, the average retention of inhaled cumene in the respiratory tract was 50%.

Mechanism of Toxicity

The signs of toxicity after exposure to cumene vapors are consistent with central nervous system (CNS) depression, and eye and respiratory irritation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cumene has low toxicity to laboratory animals by inhalation, oral, or dermal routes of exposure. A 4 h inhalation LC_{50} of 39 200 mg m^{-3} (8000 ppm) in rats was reported by several investigators. Acute oral LD_{50} values for rats range from 1400 to 2900 mg kg^{-1} body weight. Acute dermal LD_{50} values for cumene applied undiluted to rabbit skin range from 3160 mg kg^{-1} body weight to 12.3 g kg^{-1}. In acute exposures, animals exhibit damage to the spleen and fatty changes in the liver, but no renal or pulmonary effects. The concentration of cumene causing a 50% reduction in the respiratory rate in mice (a measure of respiratory irritation) was determined to be 2058 ppm (10 117 mg m^{-3}).

Cumene is a CNS depressant characterized by slow induction and long duration of effects. Acute behavioral effects following a single 20 min inhalation exposure to cumene at 2000–8000 ppm were short-lived and completely reversible. CNS depressant effects were reported only at quite high concentrations (>500 ppm).

In Fischer 344 rats, exposure to cumene vapor for 13 weeks resulted in mild toxicity at 1200 ppm, minimal effects at 500 ppm, and no-observed effects at 50 and 100 ppm; the main effects were reversible decreased activity, reversible organ weight changes, and male rat renal hyaline droplet formation, which is not believed to be relevant to humans. Neurotoxicological effects were not observed in this study, which included complete batteries of functional and motor activity tests and neurohistopathology.

Cumene is not a primary developmental toxicant. Exposure of rats to cumene vapor during organogenesis resulted in clinical signs of maternal irritation and toxicity at 500 and 1200 ppm with a no-observed-effect level of 100 ppm. No developmental toxicity was observed at any dose level tested. Exposure of New Zealand rabbits to cumene vapor during organogenesis also resulted in clinical signs of irritation and maternal toxicity at 2300 ppm with less severe effects at 1200 and 500 ppm; the no-observed-effect level was <500 ppm. No developmental toxicity was observed at any dose level tested.

Human

The main hazard with low volatility, low viscosity hydrocarbons such as cumene is aspiration pneumonitis, which may occur after vomiting accidentally ingested material. Cumene is irritating to the eyes and skin. Prolonged skin contact may result in skin drying, defatting, and rashes. Exposure to vapor concentrations may cause CNS depression indicated by dizziness, slight incoordination, and unconsciousness.

Chronic Toxicity (or Exposure)

Animal

Cumene is being tested for carcinogenicity by inhalation in rats and mice by the US National Toxicology Program; reports are not available. Cumene is not expected to be a genotoxic carcinogen.

Human

No information is available regarding the toxicity of cumene in humans following acute, subchronic, or chronic exposure. No epidemiology, case reports, or clinical studies of humans were located.

In Vitro Toxicity Data

Cumene was negative with or without activation in the Ames *Salmonella*/mammalian-microsome preincubation mutagenicity assay. Cumene was negative, with or without metabolic activation, in the HGPRT mutation assay with Chinese hamster ovary (CHO) cells, and the CHO chromosome aberration assay. Cumene was negative in the unscheduled DNA synthesis test using rat primary hepatocytes. Cumene was negative in the BALB/3T3 mouse embryo cell morphological transformation assay.

Clinical Management

As with other petroleum hydrocarbon products, management in most cases is symptomatic. Attention should be paid to possible aspiration pneumonitis after ingestion exposure; vomiting should not be induced. Oral or high concentration vapor exposure may cause CNS depression; the patient should be removed to fresh air. Liquid may cause skin or eye irritation; contaminated clothing should be removed, and skin and eyes should be flushed with water.

Environmental Fate

Cumene is a volatile liquid and exists mainly in the vapor phase in the atmosphere. It degrades in the atmosphere via reaction with hydroxyl radicals. Although small amounts of cumene may be removed from the atmosphere by precipitation, cumene is not expected to react with ozone or directly with light. In water, cumene can be volatilized, undergo biodegradation, or adsorb to sediments. It is

expected to biodegrade rapidly in soil under aerobic conditions; in water, it can readily adsorb to soil or volatilize.

Ecotoxicology

Although cumene is considered moderately toxic to aquatic organisms under rigorous laboratory conditions, its volatility and biodegradability greatly reduce its hazard to the aquatic environment. The 96 h LC_{50} values for rainbow trout, sheepshead minnow, and mysid shrimp, based on mean measured concentrations, were 4.8, 4.7, and 1.3 mg l^{-1}, respectively. The 48 h daphnid EC_{50} was 4.0 mg l^{-1}. Because of cumene's high volatility (vapor pressure, 3.2 mmHg at 20°C), all tests were conducted under flow-through conditions using a proportional diluter system.

Other Hazards

Cumene is flammable and vapors may result in explosive mixtures.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (time-weighted average) is 50 ppm (246 mg m^{-3}) based on irritation and CNS depression. The (US) Occupational Safety and Health Administration permissible exposure limit, (US) National Institute for Occupational Safety and Health recommended exposure limit, and Deutsche Forschungsgemeinschaft (DFG) MAK are all also 50 ppm, with skin notations. The odor threshold is 0.039 ppm.

Miscellaneous

Vapors are heavier than air and may travel across the ground and reach remote ignition sources causing a flashback fire danger. Electrostatic charges may be generated during pumping and may cause fire. Exposure prevention includes proper eye, skin, and face protection and a cartridge-type of self-contained breathing apparatus.

See also: Fuel Oils; Oil, Crude; Petroleum Distillates.

Further Reading

Seymour FK and Henry JA (2001) Assessment and management of acute poisoing by petroleum products. *Human and Experimental Toxicology* 20: 551–562.

Relevant Websites

http://ntp-server.niehs.nih.gov – NTP, National Toxicology Program (2003).

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Cumene.

Cumulative Risk Assessment

Jeffrey H Driver

Human health risk assessments with chemicals and other agents (biological, physical) typically follow a paradigm that involves four steps – hazard identification, dose–response assessment, exposure assessment, and risk characterization. The process was recommended by the US National Research Council in the 1980s, and is usually applied to a single agent and exposures associated with one or more routes (oral, dermal, inhalation). This has been more recently referred to as aggregate exposure and risk assessment.

It has been recognized that humans may be exposed, on a daily basis, to a plethora of synthetic and natural agents, by different routes of exposure. Concerns have been raised regarding the possibility that exposures to multiple agents, for example, chemical mixtures, could cause unanticipated adverse effects on human health through a variety of toxicological interactions. Various researchers and regulatory agencies have evaluated chemical mixtures previously, with respect to toxicity testing, exposure assessment, or risk estimation. However, only recently, in part because of the US Food Quality Protection Act (FQPA) of 1996, have cumulative risk assessments been developed for integrated food, water, and residential safety evaluations of agricultural chemicals. The FQPA requires that cumulative risk assessment should be considered in situations where there is exposure to two or more chemicals acting through a common mechanism of toxicity. This also implies that a determination must be made regarding the likelihood of concurrent exposure, for given subpopulation, to the chemical mixture of interest during a toxicologically relevant

time period (e.g., daily, 30 day moving average period).

General principles for defining the existence of a common mechanism of toxicity have been addressed by an expert working group convened by the International Life Sciences Institute (ILSI). The working group proposed that a common mechanism might exist if two or more chemicals cause the same critical effect, act on the same molecular target at the same target tissue, act by the same pharmacological mechanism of action, and may share a common toxic intermediate. With the exception of a few groups of chemicals, such as the organophosphate and carbamate insecticides, precise mechanistic information on the animal and/or human effects of chemical agents is limited. Common mechanism determinations will therefore be difficult to establish with the degree of rigor implied by the ILSI working group.

Concurrent exposure, a critical component of cumulative risk assessment, refers to coexposure to two or more chemicals, presumed to act via a common toxicological mechanism. It is important to distinguish between simultaneous concurrent 'external' exposure (timing of oral, dermal and inhalation exposures) and 'internal' exposure or the actual absorbed dose attained in a given biological compartment (e.g., plasma) or at a specific target tissue, as a function of time. It is the temporal dose profile at target tissues that provides the most accurate exposure assessment accounting for purposes of health risk estimation. In the case of chemical mixtures, variations in the timing and frequency of exposure and subsequent absorption, distribution, metabolism, and excretion of different chemical agents will result in differences in dose–response interactions. Therefore, assessing potential temporal patterns of concurrent exposure (via multiple pathways and routes) become a critical underpinning of a credible cumulative risk assessment.

The major steps required for cumulative risk assessment include:

1. Development and application of methodology (and associated data) for determination of the probability that an individual person in a reference population will actually be concurrently exposed to two or more chemicals with a presumed common mechanism of toxicity.
2. Development of an appropriate absorbed dose metric (including time scale) to cumulative toxicologically equivalent doses across the chemicals of interest.
3. Development of an appropriate cumulative risk metric to characterize the potential health risks of the mixture of interest.

It is important to emphasize that a significant degree of scientific uncertainty exists regarding the methodology, underlying data sources, and interpretation of cumulative exposure and risk assessments. Consequently, efforts are being taken to evaluate assessments that have been conducted to date, conduct uncertainty analyses, and then target the areas of data development (e.g., product use, time–activity, and biological monitoring surveys that include demographic, geographic, and temporal specificity for a representative reference population). These efforts will improve the quality of cumulative risk assessments and provide a basis for more scientifically sound risk management decision-making.

See also: Exposure Assessment; Risk Assessment, Ecological; Risk Assessment, Human Health.

Further Reading

CDC (US Centers for Disease Control) (2003) Second National Report on Human Exposure to Environmental Chemicals. Department of Health and Human Services, Centers for Disease Control and Prevention. NCEH Publication Number 02-0716.

ILSI (International Life Sciences Institute) (1999) *A Framework for Cumulative Assessment: An ILSI Risk Science Institute Workshop Report*. Washington, DC: ILSI Press. ISBN 1-57881-055-8.

Mileson BE, Chambers JE, Chen WL, *et al.* (1998) Common mechanism of toxicity: A case study of organophosphorus pesticides. *Toxicological Sciences* 41: 8–20.

NRC (National Research Council) (1983) *Risk Assessment in the Federal Government: Managing the Process*. Washington, DC: National Academy Press.

NRC (National Research Council) (1988) *Complex Mixtures: Methods for In Vivo Toxicity Testing*. Washington, DC: National Academy Press.

Safe SH (1998) Hazard and risk assessment of chemical mixtures using the toxic equivalency factor approach. *Environmental Health Perspectives* 106(suppl. 4): 1051–1058.

Seed J, Brown RP, Olin SS, and Foran JA (1995) Chemical mixtures: Current risk assessment methodologies and future directions. *Regulatory Toxicology and Pharmacology* 22: 76–94.

US EPA (US Environmental Protection Agency) (1999) *Guidance for Conducting Health Risk Assessment of Chemical Mixtures*. Washington, DC: Risk Assessment Forum.

US EPA (US Environmental Protection Agency) (1999) *Guidance for Performing Aggregate Exposure and Risk Assessments*, October 29. Washington, DC: US EPA, Office of Pesticide Programs.

US EPA (US Environmental Protection Agency) (2002) *Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity*, January 14. Washington, DC: US EPA, Office of Pesticide Programs.

US EPA (US Environmental Protection Agency) (2002) *Organophosphate Pesticides: Revised OP Cumulative Risk Assessment*, June 10. Washington, DC: US EPA, Office of Pesticide Programs.

Wilkinson CF, Christoph GR, Julien E, *et al.* (2000) Assessing the risks of exposures to multiple chemicals with a common mechanism of toxicity: how to cumulate? *Regulatory Toxicology and Pharmacology* 31: 30–43.

Curare

Susan M Stejskal

- Chemical Abstracts Service Registry Number: CAS 8063-06-7
- Synonyms: Intocostrine; Ourari; Urari; Woorali; Woorari; Wourara
- Chemical/Pharmaceutical/Other Class: Toxic alkaloid (D-tubocurare) found in South American woody vines including *Strychnos toxifera*, *S. castelnaei*, *S. crevauxii*, and *Chondodendron tomentosum*

Uses

Curare is often used as a general term to describe a wide variety of highly toxic plant extracts. Curare was originally used by South American Indians as an arrow poison that caused paralysis of skeletal muscle of prey being hunted. Curare was first used medically as a muscle relaxant in 1912. An extract from *Chondrodendron tomentosum* has been used clinically to reduce spasms in patients with tetanus and those treated with shock therapy, and to treat muscular rigidity and spastic paralysis. Curare is also used as an adjunct to general anesthesia.

Exposure Routes and Pathways

Only effective when enters the bloodstream.

Toxicokinetics

Curare is effective only when it enters bloodstream, but does not cross the blood–brain barrier. It is easily broken down following ingestion.

Mechanism of Toxicity

Curare mimics acetylcholine by binding to receptor at muscle synapses, preventing nerves from stimulating muscular contraction and causing death by respiratory paralysis. It is a neuromuscular nondepolarizing agent and a nicotinic antagonist.

Acute and Short-Term Toxicity (or Exposure)

Animal

As a potent muscle relaxant, curare can cause death quickly by inducing asphyxia due to rapid relaxation of diaphragmatic muscles. According to one source, death from respiratory arrest can take place within a few minutes in birds and small prey, and up to 20 min in larger mammals. Curare is considered to be highly toxic. The LD_{50} values are as follows:

- intravenous LD_{50}, dog: $1200\,\mu g\,kg^{-1}$;
- intravenous LD_{50}, mouse: $140\,\mu g\,kg^{-1}$;
- intravenous LD_{50}, rabbit: $1300\,\mu g\,kg^{-1}$;
- intraperitoneal LD_{50}, mouse: $3200\,\mu g\,kg^{-1}$; caused flaccid paralysis;
- subcutaneous LD_{50}, mouse: $500\,\mu g\,kg^{-1}$;
- subcutaneous LD_{50}, rabbit: $2700\,\mu g\,kg^{-1}$; and
- oral LD_{50}, rabbit: $270\,mg\,kg^{-1}$.

Human

Curare is acutely toxic. The lowest published lethal dose, route unreported: $375\,\mu g\,kg^{-1}$.

Clinical Management

Respiratory failure should be treated supportively until the effect subsides.

See also: Neurotoxicity.

Further Reading

Baden M (1992) *Unnatural Death: Confessions of a Medical Examiner.* Ivy Books.

Sumner J (2000) *The Natural History of Medicinal Plants*. Portland, OR: Timber Press.

Relevant Website

http://rain-tree.com – Curare (*Chondrodendron tomentosum*) (from the Raintree Rainforest Database).

Cuyahoga River

Lee R Shugart

The Cuyahoga River, flowing through the city of Cleveland in northwest Ohio, was so polluted that it caught fire on June 22, 1969. The cause of the fire was undetermined but investigations pointed to the discharge of highly volatile petroleum derivatives as the possible cause. The fire was not entirely unexpected as a fireboat patrolled the Cuyahoga River daily checking for oil slicks and clearing them away.

The ignited floating oil slick was extinguished in less than half an hour but not before the fire scorched two key railroad trestles as it passed under them. Flames from the burning oil slick reached heights of roughly five stories and were battled by a fireboat from the river and three fire battalions along the shore. Damage to the trestles was estimated to be ~$50 000. There had been previous fires in 1936 and one in 1952 that caused ~30 times the amount of damage as the 1969 fire.

Cleveland mayor Carl Stokes and others felt that the polluted state of the Cuyahoga was "a long-standing condition that must be brought to an end" and used the river fire as a reminder of the importance of continued support for cleanup of The Cuyahoga River and Lake Erie. *Time Magazine* published an article in August of the same year that dramatized the state of the Cuyahoga River and focused national attention on the deteriorating plight of our nation's waterways. The 1969 Fire was an event that mobilized America's commitment to cleanup its rivers and became the rallying point for the passage of the Clean Water Act of 1972.

At the time of the 1969 Fire, the river was characterized as being 'Chocolate-brown, oily, bubbling with subsurface gases, it oozes rather than flows. The river has no visible life, not even low forms such as leeches and sludge worms that usually thrive on wastes'. The Clean Water Act of 1972 was responsible for repairing many of these problems. Today, the areas around the Cuyahoga River are being revitalized. Pleasure boats crowd the river and restaurants pack the riverbank in downtown Cleveland. The greatest testament to the improvement of the Cuyahoga River is the return of fish and other organisms.

See also: Clean Water Act (CWA), US; Environmental Protection Agency, US.

Relevant Website

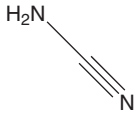
http://www.epa.gov – Cuyahoga River Area of Concern (from the US Environmental Protection Agency).

Cyanamide

Leonard I Sweet

This article is a revision of the previous print edition article by Heriberto Robles, volume 1, pp. 386–387, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 420-04-2
- Synonyms: Amidocyanogen; Carbamonitrile; Carbimide; Cyanoamine; Carbodiamide; Carbodiimide; Cyanogenamide; Hydrogen cyanamide; Cyanogen nitride
- Chemical/Pharmaceutical/Other Class: Cyanamides
- Chemical Formula: CH_2N_2
- Chemical Structure:



Uses

Cyanamide is used in the production of synthetic rubber, cyanide, fumigants, and metal cleaners. It has also found use as an intermediate for pesticides, herbicides, detergents, medicines (e.g., antihistamines, hypertension, sedatives), in the photography industry, as an additive for fuels, lubricants, and cements, and as a paper preservative. The dimer (dicyandiamide) is a raw material for melamine and guanidine.

Exposure Routes and Pathways

Exposure to cyanamide can occur via inhalation, ingestion, skin or eye contact, and there is potential for skin absorption. Inhalation and dermal contact is expected to be the primary route of occupational exposure.

Toxicokinetics

The major urinary metabolite of cyanamide is n-acetylcyanamide. *In vitro* studies suggest that cyanamide is metabolized to cyanide; however, results *in vivo* suggest this biotransformation pathway is irrelevant in humans. Rapid absorption is anticipated, though bioavailability is incomplete with estimates ranging from 53% to 70%. The estimated half-life in humans after oral administration is expected to be less than 2 h.

Mechanism of Toxicity

Cyanamide is not expected to release cyanide as part of its mechanism of toxicity. The principal toxicological mechanism of cyanamide is inhibition of aldehyde dehydrogenase. Cyanamide can produce acetaldehyde syndrome with concurrent exposure to alcohol, resulting in symptoms that include vomiting, parasympathetic hyperactivity, difficulty in breathing, and confusion.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cyanamide causes irritation of the eyes and mucous membranes, as well as inhibition of the liver enzyme aldehyde dehydrogenase. Cyanamide is very toxic by the oral exposure route and moderately toxic by the dermal route. The oral LD_{50} for rats is $125\,mg\,kg^{-1}$, and the dermal LD_{50} in the same species is $84\,mg\,kg^{-1}$. The intravenous LD_{50} for rats is $56\,mgkg^{-1}$. For rabbits, the dermal LD_{50} value is $590\,mg\,kg^{-1}$. Instillation of 100 mg of cyanamide into the eyes of rabbits resulted in severe irritation. Cyanamide induced ulceration on contact with the moist skins of experimental animals.

In rats, cyanide poisoning produces overactivity of the parasympathetic nervous system causing miosis, salivation, lacrimation, and twitching. Symptoms of severe poisoning in rats include constricted pupils followed by markedly dilated pupils, congested vessels of the iris and retina, and possibly papilledema.

Human

Cyanamide is irritating and caustic to the eyes, skin, mucous membranes, and respiratory and gastrointestinal tracts of humans. Effects after a single mild overexposure are expected to be transient, and diminish after a few hours. The typical signs and symptoms of acute overexposure to cyanamide include the following: flushing of the face and upper body, nausea, fatigue, difficulty in breathing, swelling, lacrimation, skin and eye burns, constricted pupils, excessive salivation, twitching, shivering, vasodilatation, tachycardia, bradycardia, and hypotension. Cyanamide poisoning produces overactivity of the parasympathetic nervous system. The most serious effects associated with acute, high-dose exposure are coma and cardiovascular collapse. The estimated fatal dose in humans ranges from 40 to 50 g cyanamide. Contact with cyanamide in dust or liquid form can cause severe irritation of the eyes and ulceration of moist skin.

Chronic Toxicity (or Exposure)

Animal

The reproductive toxicity of cyanamide has been studied in a two-generation study of reproduction-fertility in rats, involving oral daily doses of 2, 7, or $25\,mg\,kg^{-1}$ cyanamide. The highest dose level resulted in decreases in weight, number of corpora lutea, number of implantations, and numbers of neonates.

Cyanamide has been studies in mice for carcinogenicity, at dose levels administered in drinking water of 0, 70, 200, and 600 ppm for up to 104 weeks. Findings suggest elevated incidence of benign granulosa theca tumors of the ovary at the 600 ppm dose level, as compared to controls. Pathological findings indicated presence of ovarian hyperplastic lesions.

Hydrogen cyanamide has been investigated for genotoxicity, and has generally been shown to lack significant activity.

Human

Cyanamide also acts as a potent inhibitor of the enzyme aldehyde dehydrogenase, which results in a disulfiram-like reaction in individuals concomitantly exposed to alcohol. Potentiated by the ingestion of alcohol, the accumulation of acetaldehyde in the body presents as a syndrome of vasodilation characterized by facial flushing, headache, nausea, vomiting, difficulty in breathing, sweating, chest pain, hypotension, weakness, blurred vision, and confusion. Calcium cyanamide has been used in aversion therapy for alcoholism.

Chronic overexposure may produce the following: pneumonitis and pulmonary edema upon repeated inhalation; throat ulceration and esophageal irritation upon oral ingestion; dermal ulceration, allergic dermatitis, and sensitization upon skin exposure; and keratitis, conjunctivitis, or corneal ulceration upon

repeated contact with the eyes. Chronic overexposure may also affect the liver and nervous system.

Clinical Management

Management of individuals overexposed to cyanamide begins with removing those individuals from the source of exposure, flushing eyes and skin with copious amounts of water, and removing contaminated clothing. Skin contamination should be removed by washing with soap and water. Treat dermal irritation or burns with standard topical therapy. Patients developing dermal hypersensitivity reactions may require treatment with topical or systemic corticosteroids or antihistamines.

If ingested, vomiting should not be induced. If large doses have been ingested within an hour of exposure, gastrointestinal decontamination should be considered. If dosage was small or treatment is delayed, oral administration of activated charcoal and sorbitol may prove beneficial. Gastric lavage treatment may be given with caution and avoided if tracheal or esophageal ulceration is suspected.

Hypotension or 'antabuse'-type reactions should be treated by placing the patient in the Trendelenburg position, providing intravenous fluids, including plasma or blood if necessary, and vasopressor drugs.

In cases of respiratory overexposure, the victim should be moved to fresh air immediately and treated according to severity of irritation. The presence and severity of respiratory irritation, bronchitis, and pneumonitis should be evaluated. If respiratory tract irritation or respiratory depression is evident, arterial blood gases, chest X-ray, and pulmonary function tests should be monitored. For acute lung injury, ventilation and oxygenation should be maintained and evaluation should be done with frequent arterial blood gas or pulse oximetry monitoring.

Monitoring complete blood count, urinalysis, and liver and kidney functions test is suggested for patients with significant exposure. Assisted ventilation (100% humidified supplemental oxygen) should be provided as required, arterial blood gases should be monitored, and institution of basic life-support systems as necessary.

Environmental Fate

If released to the environment, cyanamide is expected to preferentially partition to the soil and water. Bioconcentration and bioaccumulation potential is expected to be low, based on the estimated bioconcentration factor and experimental octanol–water partition coefficient. Aerobic biodegradation is expected to occur. Volatilization is not expected to be an important fate and transport process based on the Henry's law constant and vapor pressure. When released into the air, vapor phase cyanamide is expected to have a half-life of less than 1 day.

Ecotoxicology

If released to the environment, cyanamide is expected to have a low potential for aquatic toxicity to invertebrates and fish (with estimated effective/lethal concentrations of $>1000\,\mathrm{mg\,l^{-1}}$).

Other Hazards

Cyanamide is a highly reactive chemical and is a dangerous explosion hazard. It can release toxic fumes of cyanides and nitrogen oxides when heated to decomposition, or contacted with acids, acid fumes, moisture, or 1,2-phenylenediamine salts. It is combustible when exposed to heat or flame. Cyanamide reacts with acids, strong oxidants, strong reducing agents, and water, causing explosion hazard.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for cyanamide include the following:

- American Conference of Governmental Industrial Hygienists ($2\,\mathrm{mg\,m^{-3}}$ ppm time-weighted average (TWA));
- Australia ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- Belgium ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- Canada ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- China ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- Denmark ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- France ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- Germany ($2\,\mathrm{mg\,m^{-3}}$ TWA inhalable fraction);
- Mexico ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- Sweden ($4\,\mathrm{mg\,m^{-3}}$ short-term exposure limit);
- United Kingdom ($2\,\mathrm{mg\,m^{-3}}$ TWA); and
- US Occupational Safety and Health Administration vacated permissible exposure limit ($2\,\mathrm{mg\,m^{-3}}$ TWA).

Miscellaneous

Cyanamide is a colorless, orthorhombic, hydrophilic, crystalline solid with a mild odor. It is commonly used in liquid solution, and is expected to be soluble in water, ether, benzene, acetone, phenols, amines, ketones, and alcohols.

See also: Acetamide; Cyanide; Formamide.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Cyanamide.

http://www.inchem.org – International Chemical Safety Card from the International Programme on Chemical Safety. Cyanamide.

http://www.state.nj.us – Hazardous Substance Fact Sheet from the New Jersey Department of Health and Senior Services. Cyanamide.

Cyanide

Zhengwei Cai

- Chemical Abstracts Service Registry Numbers: CAS 57-12-5 (CN); CAS 74-90-8 (Hydrogen cyanide); CAS 143-33-9 (Sodium cyanide); CAS 151-50-8 (Potassium cyanide)
- Synonyms: Carbon nitride ion; Cyanide anion; Cyanide ion; Cyanure (French); Hydrocyanic acid; Isocyanide; Hydrocyanic acid sodium salt; Hydrocyanic acid potassium salt
- Chemical/Pharmaceutical/Other Class: Cyanide is any one of a group of compounds containing the monovalent combining group CN. Inorganic cyanides are regarded as salts of hydrocyanic acid (hydrogen cyanide). Organic cyanides are usually called nitrites
- Chemical Formulas: HCN (hydrogen cyanide); NaCN (sodium cyanide); KCN (potassium cyanide); CH_3CN (acetonitrile)
- Chemical Structure: $-C\equiv N$

Uses

Cyanide compounds are widely used in industry. Sodium cyanide and potassium cyanide are used extensively in the extraction of gold and silver from low-grade ores. The cyanide ion can form a wide range of complex ions with metals. These complex metal cyanide ions are extensively used in electroplating. Cyanide compounds are also used in case-hardening of iron and steel, metal polishing, photography, and the fumigation of ships and warehouses. Organic cyanide compounds are used in ynthetic rubber, plastics, and synthetic fibers; they are also used in chemical synthesis. Cyanides are used in rodenticide and fertilizer production.

In addition, cyanides can be found in the seeds of the apple, peach, plum, apricot, cherry, and almond in the form of amygdatin, a cyanogenic glycoside. Amygdatin (Laetrile) has been used as an antineoplastic drug, but such beneficial effects have not been scientifically proven.

Background Information

Cyanide poisoning causes a high incidence of severe symptomatology and fatality. Between 1926 and 1947, death rates from cyanide poisoning in America ranged between 79 and 416 per 10 million population and gradually declined thereafter. The availability of the antidote kit may have contributed to this decreasing death rate. There are numerous sources of potential cyanide exposure. With the increased use of plastic building materials, the potential hazards of cyanide poisoning as a component of smoke inhalation in closed space fires still exist.

Exposure Routes and Pathways

Humans may be exposed to cyanide in a number of different forms. These include solids, liquids, and gases. Sources include industrial chemicals, natural products, medications, and combustion products. Inhalation of toxic fumes and ingestion of cyanide salts, cyanide-containing fruit seeds, and cyanide waste-contaminated drinking water are the most common exposure pathways. The respiratory route represents a potentially rapidly fatal type of exposure. Exposure to cyanides may also occur via the dermal route in industrial workers.

Toxicokinetics

Cyanide is rapidly absorbed from the skin and all mucosal surfaces; it is most dangerous when inhaled because toxic amounts are absorbed with great rapidity through the bronchial mucosa and alveoli. Once absorbed, distribution of cyanide through the body is rapid. Within a few minutes, cyanide is distributed through the body and its conversion to thiocyanate starts. The majority of cyanide in the body is protein-bound (60%). In sublethal doses, cyanide reacts with sulfane sulfur to form nontoxic

thiocyanate through an enzymatic reaction involving rhodanase and mercaptopyruvate sulfur transferase. Within 3 h, 90% of the dose of cyanide is converted to thiocyanate appearing in blood. Cyanide is also trapped as cyano of vitamin B_{12}, oxidized to formate and carbon dioxide, and incorporated into cysteine. In nonfatal cases, metabolized cyanide (thiocyanate) is excreted in the urine. Although cyanide is volatile, excretion through the lungs is not a significant route of elimination of cyanide.

Mechanism of Toxicity

Cyanide is described as a cellular toxin because it inhibits aerobic metabolism. It reversibly binds with ferric (Fe^{3+}) iron-containing cytochrome oxidase and inhibits the last step of mitochondrial oxidative phosphorylation. This inhibition halts carbohydrate metabolism from citric acid cycle, and intracellular concentrations of adenosine triphosphate are rapidly depleted. When absorbed in high enough doses, respiratory arrest quickly ensues, which is probably caused by respiratory muscle failure. Cardiac arrest and death inevitably follow.

For this reason, cyanide action has been described as 'internal asphyxia'. Although some cyanide combines with hemoglobin to form a stable nonoxygen-bearing compound, cyanhemoglobin, this substance is formed only slowly and in a small amount. Therefore, death is not due to cyanhemoglobin but to inhibition of tissue cell respiration.

Recent studies have shown that cyanide also inhibits the antioxidant defense enzymes (such as catalase, superoxide dismutase, and glutathione peroxidase) and stimulates neurotransmitter release. These effects of cyanide may also contribute to its acute toxicity. The prolonged energy deficit and the consequent loss of ionic homeostasis, which may result in activation of calcium signaling cascade and eventually cell injury, contribute to cyanide toxicity resulting from subacute exposure or in the postintoxication sequela.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cyanide toxicity varies with the animal species, type of cyanide compound, route of uptake, metabolic state, and other factors. The LD_{50} for cyanide has been ported in various species. Potassium cyanide, if injected, has a 24 h LD_{50} of 6.7–7.9 mg kg^{-1} in mice. The lethal dose of potassium cyanide infused at a rate 0.1 mg kg^{-1} min^{-1} is 2.4 mg kg^{-1} in dogs breathing room air. When hydrogen cyanide is inhaled by mice, the LD_{50} is 177 ppm with a lethal time of 29 min. The time to death is greater than 17 min for exposure to less than 266 ppm, but falls to 40 s at 873 ppm. The LD_{50} for sodium cyanide is 4.6–15 mg kg^{-1} in rats. Male gerbils are 50-fold more sensitive to methacrylonitrile, which is metabolized to cyanide in rodents, than Sprague–Dawley rats, and about fivefold more sensitive than albino Swiss mice. Single and repeated low-dose cyanide intoxication can result in demyelinating lesions of the cerebral white matter in monkeys, but high doses of cyanide are required to produce similar brain lesions in rat.

Human

Cyanide is a chemical asphyxiant, which renders the body incapable of utilizing an adequate supply of oxygen. Exposure to high dose of cyanide is often lethal. The lethal dose of cyanide in humans is 0.5–1.0 mg kg^{-1}. The lethal dose of hydrocyanic acid is $\sim$50 mg for an adult and the lethal dose of the potassium or sodium salt is 200–500 mg. The threshold limit value (TLV) of HCN for inhalation is 4.7 ppm. This is defined as the maximum safe average exposure limit for a 15 min period by the Occupational Safety and Health Administration. Exposure to 20 ppm of HCN in air causes slight warning symptoms after several hours; 50 ppm causes disturbances within an hour; 100 ppm is dangerous for exposures of 30–60 min; and 300 ppm can be rapidly fatal unless prompt, effective first aid is administered. The median lethal dose for skin contamination is $\sim$100 mg kg^{-1}.

Following the inhalation of toxic amounts of cyanide, symptoms usually appear within a few seconds, whereas it may take a few minutes for symptoms to appear following oral ingestion or skin contamination by the salts. The symptoms include a flushed skin, tachypnea, and tachycardia. Stupor, coma, and seizure immediately precede respiratory arrest and cardiovascular collapse. Death shortly occurs. If large amounts have been absorbed, collapse is usually instantaneous-the patient falling unconscious and dying almost immediately. With smaller doses, weakness, giddiness, headache, nausea, vomiting, and palpitation usually occur. With the rise of the blood cyanide level, ataxia develops and is followed by lactic acidosis, convulsive seizures, coma, and death. At higher cyanide doses, cardiac irregularities are often noted, but heart activity always outlasts the respiration.

Chronic Toxicity (or Exposure)

Animal

Ingestion of cyanogenic plants, such as cassava and sorghum, has been associated with development of

goiter and tropical pancreatic diabetes in both human and animals. However, results from animal studies indicate this association in animals is controversial. Chronic cyanide exposure has been reported to reduce memory along with reduction in the levels of dopamine and 5-hydroxytryptamine in the rat brain.

Human

Chronic low-level exposure to cyanide produces various signs and symptoms. Exposure to small amounts of cyanide compounds over long-term periods of time is reported to cause loss of appetite, headache, weakness, nausea, dizziness, and symptoms of irritation of the upper respiratory tract and eyes. The most widespread pathologic condition attributed to cyanide is tropical ataxic neuropathy associated with chronic cassava consumption. This is a diffuse degenerative neurological disease with peripheral and central signs. Cassava is the major staple food in various tropical areas; the plant has a high content of cyanogenic glycoside (linamarin). With continued ingestion over a period of time, tropical neuropathy gradually develops. The syndrome is characterized by optic atrophy, nerve deafness, and ataxia due to sensory spinal nerve involvement. Other signs include scrotal dermatitis, stomatitis, and glossitis. Chronic low-level exposure to cyanide may also lead to ultrastructural changes of heart muscle. In addition, with chronic cyanide ingestion, the thyroid may be affected due to enhanced formation of thiocyanate. Thiocyanate can block uptake of iodide by the thyroid gland, and myxedema, thyroid goiter, and cretinism may occur. This chronic effect of cyanide may pass to the fetus through maternal exposure.

Clinical Management

To be of any value, treatment of cyanide poisoning must be rapid and efficient. The rapid and early recognition of cyanide poisoning is usually difficult because most of the clinical manifestations are nonspecific. Potentially valuable cyanide blood levels are usually available for confirmation of diagnosis. Arteriolization of venous blood has been used as a significant symptom of cyanide poisoning. If cyanide was ingested, removing the unabsorbed poison by ravaging the stomach with copious amounts of water through a gastric tube is necessary. This should be continued until all odor of cyanide is gone from the lavage fluid. Artificial respiration with 100% oxygen is often used in the treatment of cyanide poisoning, although oxygen is not a specific antidote. It is theorized that oxygen therapy increases the rate of displacement of cyanide from cytochrome oxidase, and the increased intracellular oxygen tension nonenzymatically converts the reduced cytochrome to the oxidized species, enabling the electron transport system to function again. The nitrite-thiosulfate antidotal combination is still one of the most effective treatments of cyanide poisoning. If the victim is conscious and speaking, no treatment is necessary. If the victim is unconscious but breathing, an open ampoule of amyl nitrite can be placed under the victim's nose for 15 s and it can be repeated 5 to 6 times. A fresh ampoule should be used every 3 min until the victim regains consciousness. Amyl nitrite is a powerful cardiac stimulant and should not be used more than necessary. If the patient is not breathing, 0.3 g (10 ml of a 3% solution, adults) of sodium nitrite should be administered intravenously at the rate of 2.5 ml min^{-1} followed by 12.5 g (50 ml of a 25% solution) of sodium thiosulfate at the same rate. Inhalation of amyl nitrite should also be performed. Nitrite will convert hemoglobin to methemoglobin, which has higher affinity for cyanide than hemoglobin. A methemoglobin level of $\sim$25% is desired for maintaining normal hemoglobin function and detoxification. Thiosulfate is a sulfur donor for converting cyanide to nontoxic thiocyanate. For children weighing less than 25 kg, sodium nitrite should be dosed on the basis of their hemoglobin level and weight. The patient should be observed for the next 24–48 h and if the signs of intoxication persist or reappear, injection of nitrite thiosulfate at one-half of the recommended dose should be repeated. Hydroxocobalamine has been effectively used in France as an antidote for acute cyanide poisoning. Hydroxocobalamine (vitamin B_{12a}) is currently approved by Food and Drug Administration, but is not popularly used in the United States as an antidote for cyanide poisoning. Because of its extremely low adverse effect, hydroxocobalamine is ideal for out-of-hospital use in suspected cyanide intoxication. It is actively proposed to be used in the United States.

Ecotoxicology

The toxicity of cyanide in the aquatic environment or natural waters is a result of free cyanide, that is, as HCN and CN^-. Fish are extremely sensitive to cyanide. Most fish can tolerate a free cyanide stream concentration of 0.05 mg l^-, but some species are even more sensitive.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration permissible exposure limit: time-weighted average (TWA) 5 mg (CN) m^{-3}.
- American Conference of Governmental Industrial Hygienists TLV: CL 5 mg m^{-3} (skin)

- DFG MAK: 5 mg m^{-3}.
- National Institute of Occupational Safety and Health recommended exposure limit (cyanide) TWA CL 5 mg m^{-3} per 10 min.

See also: Cyanamide; Cyanogen Chloride.

Further Reading

Eckstein M (2004) Cyanide as a chemical terrorism weapon. *JEMS* 29(8): Suppl 22–31.

Gracia R and Shepherd G (2004) Cyanide poisoning and its treatment. *Pharmacotherapy* 24(10): 1358–1365.

Hall AH and Rumack BH (1986) Clinical toxicology of cyanide. *Annals of Emergency Medicne* 15: 1067–1072.

Patnaik P (1999) *A Comprehensive Guide to the Hazardous Properties of Chemical Substances*, 2nd edn. New York: Wiley.

Sauer SW and Keim ME (2001) Hydroxocobalamin: Improved public health readiness for cyanide disasters. *Annals of Emergency Medicine* 37: 635–641.

Cyanogen Chloride

Leonard I Sweet

- Chemical Abstracts Service Registry Number: CAS 506-77-4
- Synonyms: Chlorcyan; Chlorine cyanide; Chlorocyan; Chlorocyanide; Chlorocyanogen; Mauguinite
- Chemical/Pharmaceutical/Other Class: Cyanogens
- Chemical Formula: CNCl
- Chemical Structure: N≡C – Cl

Uses

Cyanogen chloride is used as a military poison gas, a warning agent in fumigant gases, tear gas, as an insecticide, metal cleaner, in ore refining, in production of synthetic rubber, and in a variety of chemical syntheses.

Exposure Routes and Pathways

Exposure to cyanogen chloride can occur via inhalation, skin absorption, ingestion, and with skin or eye contact. Cyanogen chloride is particularly hazardous when inhaled due to the strong potential for absorption in toxic amounts through bronchial mucosa and alveoli.

Toxicokinetics

Cyanogen chloride is converted to cyanide in the body by a reaction with hemoglobin and glutathione. Rapid absorption of the cyanide ion is anticipated from all tissues, and it is expected to distribute to organs and tissues via the blood; where it has the potential to concentrate in red blood cells, possibly due to binding to methemoglobin. Absorbed cyanide is excreted unchanged in the lungs, sweat, and urine, whereas greater amounts are converted by sulfurtransferase/rhodanase enzymes to thiocyanate. The estimated half-life in humans for the conversion of cyanide to thiocyanate from a nonlethal dose ranges from 20 to 60 min. The toxicity of cyanogen chloride rests largely on its ability to yield hydrocyanic acid *in vivo*.

Mechanism of Toxicity

Cyanogen chloride is similar in toxicity and mode of action to hydrogen cyanide, but is a much more potent irritant partly due to its greater volatility and chlorine moiety. Target organs include the eyes, skin, respiratory system, central nervous system, and cardiovascular system. Cyanogen chloride can cause marked irritation of the respiratory tract with hemorrhagic exudate of the bronchi and trachea, and pulmonary edema. In addition to severe potential for local irritation, systemic toxicity occurs by liberating cyanide molecules that target and bind to cells, interrupting electron transport and metabolism. Cyanides interfere with cellular oxygen uptake and transport by inhibition of cytochrome oxidase enzymes. The most oxygen-dependent organ systems are typically most affected, including the heart and brain.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cyanogen chloride causes severe irritation to the eyes, skin, mucous membranes, and respiratory system, as well as dizziness, congestion of the lungs, interference with cellular metabolism, and loss of appetite. The adverse effects of overexposure to cyanogen chloride, such as pulmonary toxicity, may be delayed. The oral LD_{50} for cats is 6 mg kg^{-1}.

The short-term inhalation LC_{50} values for laboratory animals range from 3800 to 6000 mg m^{-3}. Acute poisoning with cyanogen chloride results in signs of cyanide poisoning and pulmonary edema including difficulty in breathing, bloody nasal exudates, cyanosis, and possibly death.

Human

Cyanogen chloride is a rapidly acting and severe eye, skin, mucous membrane, and respiratory tract irritant. Effects of overexposure are similar to those for cyanide and other cyanogenic compounds. Overexposure may cause tearing, cellular hypoxia, burning of the eyes, lacrimation, rapid respiration, flushing, irregular heartbeat, vomiting, hemorrhagic changes, drowsiness, pulmonary edema, convulsions, and possibly death by asphyxia. The adverse effects of overexposure may be delayed for several hours. Irritant concentrations can occur as low as 1 ppm can cause severe eye and nasal irritation. Skin contact with liquid may cause frostbite injury. The lowest published toxic concentrations for humans via inhalation are 10 mg m^{-3} for eye effects, and 2 g m^{-3} for skin. Inhalation overexposure to cyanogen chloride concentrations of 48 ppm (for 30 min) or 159 ppm (for 10 min) has caused death in humans. The estimated lethal dose by ingestion of cyanogen chloride is approximately 13 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

The chronic effects of exposure to cyanogen chloride include hoarseness, conjunctivitis, and edema of the eyelid. Short-term fatal concentrations in animal models range from 48 to 500 ppm. The carcinogenic or mutagenic potential of cyanogen chloride is not well characterized.

Human

Repeated inhalation exposure of low concentrations may cause dizziness, weakness, congestion of the lungs, conjunctivitis, hoarseness, loss of appetite, mental deterioration, weight loss, and possibly enlarged thyroid glands.

Clinical Management

Management of individuals overexposed to cyanogen chloride begins with rapid action to remove those individuals from the source of exposure, flushing eyes and skin with copious amounts of water, and removing contaminated clothing. Skin contamination should be removed by washing with soap and water. If frostbite injury has occurred, the area should not be rubbed or flushed with water, and attempt to remove frozen clothing from frostbitten areas should not be made. A cyanide antidote kit should be kept in immediate work areas.

Triage should be conducted, and asymptomatic victims should be monitored and histories taken. Where exposure has resulted in acute signs and symptoms, oxygen and antidotes should be administered immediately. Antidotes include amyl nitrite, hydroxocobalamin, hyperbaric oxygen, sodium nitrite, and sodium thiosulfate. For irritation of the eyes, washing with a weak solution of boric acid may prove beneficial. Dermal irritation should be treated with soothing lotions, such as calamine. If inhaled and if breathing is difficult, respiration should be supported and 100% oxygen administered. If typical nitrile effect is observed, amyl nitrite should be administered. Lung function and electrocardiogram should be monitored. Additional diagnostic procedures include blood anion gap, arterial blood gases, blood cyanide levels, blood electrolytes, blood methemoglobin, and blood or urine thiocyanate. Medical observation is recommended for days after breathing overexposure, as pulmonary edema may be delayed. As first aid for pulmonary edema, administration of corticosteroid spray may prove therapeutic. If ingested, water or milk should be consumed. Gastrointestinal decontamination should be considered, with lavage or activated charcoal with cathartic. Emesis is contraindicated due to rapid course of the neurologic symptoms. For acidosis, sodium bicarbonate should be given. Hemodialysis, charcoal hemoperfusion, and chelation may prove useful in enhancing elimination.

Environmental Fate

If released to the environment, cyanogen chloride is expected to preferentially partition to the air, soil, and water. It is expected to slowly convert to cyanides, and will react slowly with water or water vapor to form hydrogen chloride. Photolysis may also be an important abiotic removal process. Bioconcentration and bioaccumulation potential is expected to be low. Volatilization is expected to be an important fate and transport process based on the vapor pressure. Cyanogen chloride is expected to persist in air if released.

Ecotoxicology

If released to the environment, cyanogen chloride and its decomposition products are expected to have high potential for aquatic toxicity to invertebrates

and fish (with effective/lethal concentrations for 50% of the organisms tested $< 1\,mg\,l^{-1}$).

Other Hazards

Flammable when exposed to heat or flame. When heated to decomposition or on contact with water or steam, it will react to produce highly toxic and corrosive fumes of hydrogen cyanide, hydrochloric acid, and nitrogen oxides. Contact with alcohols, acids, acid salts, amines, strong alkalis, olefins, and strong oxidizers may cause fire and explosion. Cyanogen chloride may polymerize violently if contaminated with chlorine.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for cyanogen chloride include the following:

- American Conference of Governmental Industrial Hygienists (0.3 ppm ceiling);
- Australia (0.3 ppm peak);
- Belgium (0.3 ppm short-term exposure level, STEL);
- Canada (0.3 ppm ceiling);
- China ($0.75\,mg\,m^{-3}$ ceiling);
- Denmark (0.1 ppm time-weighted average, TWA);
- France (0.3 ppm STEL);
- Germany ($0.75\,mg\,m^{-3}$ TWA);
- Sweden (0.1 ppm threshold limit value);
- United Kingdom (0.3 ppm STEL); and
- US Occupational Safety and Health Administration vacated permissible exposure limit (0.3 ppm ceiling).

Miscellaneous

Cyanogen chloride is a colorless liquid or gas, with a pungent acrid, bitter almond like, or choking odor that is generally detected at concentrations ~ 1 ppm. It is expected to be soluble in water, alcohol, ether, and most organic solvents.

See also: Cyanide.

Further Reading

World Health Organization; WHOTAC, Technical Report Series, 1211 Geneva, 27, Switzerland.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Cyanogen Chloride.

http://www.inchem.org – Cyanogen Chloride (International Chemical Safety Card from the International Programme on Chemical Safety).

http://www.state.nj.us – Cyanogen Chloride (Hazardous Substance Fact Sheet from the New Jersey Department of Health and Senior Services).

Cyclodienes

Benny L Blaylock

Cyclodienes are chlorinated hydrocarbon insecticides with a polycyclic structure and, as the name implies, two unsaturated bonds. Not all of the insecticides in this class meet these criteria. Chlordane, for example, contains only one double bond in its polycyclic structure. Endrin and dieldrin are epoxides of the cyclodienes isodrin and aldrin, respectively.

Cyclodienes appear to act more in the central nervous system than in the peripheral nervous system. One major mode of action is the inhibition of γ-aminobutyric acid-regulated Cl^- ion flux in neurons. Cyclodienes also exert effects on membrane-bound adenosine triphosphatases (ATPases), altering Na^+, K^+, and Ca^{2+} ion transport. The result is a partial depolarization of neurons rather than repolarization after activation. The accumulation of Ca^{2+} ions intracellularly in the terminal ends of neurons promotes the release of neurotransmitters from storage vesicles and the depolarization of adjacent neurons.

Symptomatology is essentially the same as that described for organochlorine insecticides. In many cases, convulsions are the first sign of toxicity without the progression of nerve hyperactivity seen in other classes of organochlorine insecticides. More recently, evidence of endocrine disruption in both mammalian and other species has been accumulating for several cyclodiene pesticides including chlordane, aldrin, dieldrin, lindane, and endosulfan.

Clinical management is symptomatic, as described for organochlorine insecticides.

See also: Aldrin; Chlordane; Dieldrin; Endosulfan; Endrin; Lindane.

Further Reading

Colborn T, vom Saal FS, and Soto AM (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspectives* 101: 378–384.

Narahashi T (2001) Neurophysiological effects of insecticides. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 335–351. San Diego, CA: Academic Press.

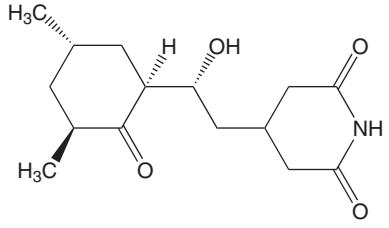
Relevant Website

http://npic.orst.edu – National Pesticide Information Center, Oregon State University and the US Environmental Protection Agency.

Cyclohexamide

Midhun C Korrapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 66-81-9
- Synonyms: 1*S*-(1α(*S*),3α,5*β*)-4-(2-(3,5-Dimethyl-2-oxo-cyclohexyl))-2-hydroxyethyl-2,6-piperidinedione; Naramycin
- Chemical/Pharmaceutical/Other Class: Bactericidal, antifungal, antibiotic, and antipsoriatic
- Chemical Formula: $C_{15}H_{20}O_4N$
- Chemical Structure:



Uses

Cyclohexamide is used as a fungicide, plant growth regulator, and as protein synthesis inhibitor. This is also used in laboratory media as a selective agent to permit isolation of pathogenic and nonpathogenic fungi.

Exposure Routes and Pathways

Cyclohexamide is produced as a by-product during the chemical synthesis of streptomycin, an antibiotic. Therefore, waste releases to the environment from streptomycin production may contain cyclohexamide. Occupational exposure to cyclohexamide may occur through dermal contact with this compound found in the waste stream at workplaces where streptomycin is produced.

Mechanism of Toxicity

Cyclohexamide is a potent inhibitor of protein synthesis in animals. It causes an increase in adrenal RNA and increased production of glucocorticoids.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals given toxic doses exhibit salivation, bloody diarrhea, tremors, and excitement, leading to coma and death due to cardiovascular collapse. A single dose of cyclohexamide ($2\,mg\,kg^{-1}$, ip) produced progressive decrease in bile flow in rats. The oral LD_{50} in monkeys and dogs is $\sim 500\,mg\,kg^{-1}$ and that for rats is only $2\,mg\,kg^{-1}$. In all three species, toxic symptoms include excessive salivation and diarrhea. Bloodstained feces may arise from vascular lesions of colon (monkey) or stomach and small intestines (dogs). Rats and dogs show transient central nervous system (CNS) excitement with tremors and in the dog perhaps meningeal irritation. Death is due to cardiovascular collapse and is preceded by coma in all species. Autopsies on rat revealed enlarged adrenals, stomach hemorrhage, liver congestion, and kidney damage.

Human

Cyclohexamide is a potent irritant. When ingested gastrointestinal symptoms of nausea, vomiting, diarrhea, and excessive salivation have been reported. Other signs of poisoning are transient CNS excitement and tremors.

Chronic Toxicity (or Exposure)

Cyclohexamide has been shown to be mutagenic in both animals and humans.

Clinical Management

Intragastric administration of charcoal as a slurry (240 ml water/30 g charcoal) should be undertaken

to minimize absorption of life-threatening levels of cyclohexamide by ingestion.

Environmental Fate

Cyclohexamide is expected to have very high mobility in soil. Volatilization from moist soil surfaces is not expected to be an important disbursement process. It is not expected to adsorb to suspended solids and sediment. Volatilization from water surfaces is not expected. It is expected to exist solely in the particulate phase in the ambient atmosphere.

Ecotoxicology

The cyclohexamide LD_{50} in male mallard duck and female pheasant by oral administration is 82.5 and 9.38 mg kg^{-1}, respectively.

Exposure Standards and Guidelines

Extremely hazardous substances that are solids are subject to either of two threshold planning quantities. The lower quantity applies only if the solid exists in powdered form and has a particle size less than 100 μm, or is handled in solution or in molten form. If the solid does not meet any of these criteria, it is subject to the upper threshold planning quantity. Cyclohexamide is an extremely hazardous substance that is subject to reporting when stored in amounts in excess of its threshold planning quantity of 100 or 10 000 lbs.

See also: Charcoal; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Occupational Exposure Limits.

Further Reading

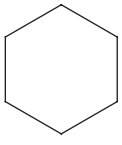
Parry EW (1981) Cycloheximide treatment modifies the pattern of 'metastasis' following intravenous injection of ehrlich ascites tumour cells. *Gann* 72: 464–467.

Satav JG, Katyare SS, Fatterparker P, and Sreenivasan A (1997) Study of protein synthesis in rat liver mitochondria use of cycloheximide. *European Journal of Biochemistry* 73: 287–296.

Cyclohexane

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 110-82-7
- Synonyms: Cicloesano; Cyclohexaan; Cyclohexan; Exahydrobenzene; Hexahydro-benzene; Hexamethylene; Hexanaphthene
- Chemical/Pharmaceutical/Other Class: Saturated alicyclic hydrocarbon
- Chemical Formula: C_6H_{12}
- Chemical Structure:



Uses

Cyclohexane is used as a solvent for lacquers, resins, fats, oils, and waxes, in paint and varnish remover, in the manufacture of nylon, in the extraction of essential oils, and in analytical chemistry for molecular weight determination. In addition, it is used in the manufacture of adipic acid, benzene, cyclohexanone, cyclohexanol, cyclohexyl chloride, nitrocyclohexane, and solid fuel for camp stoves. Further, it is used in industrial recrystallization of steroids and in fungicidal formulations (it has a slight fungicidal action).

Background Information

Cyclohexane is obtained in the distillation of petroleum or by hydrogenation of benzene. It constitutes 0.5–1.0% of petroleum.

Exposure Routes and Pathways

Cyclohexane was a predominant pollutant in shoe and leather factories in Italy, associated with the use of glue. Occupational exposure to cyclohexane may occur through inhalation and dermal contact with this compound where cyclohexane is produced or used. The general population may be exposed to cyclohexane via inhalation of ambient air, ingestion of drinking water, and dermal contact with products containing cyclohexane. The general population may also be exposed to cyclohexane due to its presence in gasoline. It has been found in mother's milk and has been detected in studies of the air in various cities.

Toxicokinetics

Cyclohexane is readily absorbed via inhalation and the oral routes of exposure. Animal studies indicate dermal absorption to be high, probably due to the defatting action of the compound. Cyclohexane absorption into the lungs is rapid, with the concentration in the lungs reaching 42–62% of the air concentration. No information is available on the rate of absorption through the gastrointestinal tract. Cyclohexane is metabolized by cytochrome P-450 enzymes in the liver and other tissues. Several metabolites have been identified including cyclohexanol and *trans*-cyclohexane-1,2-diol. These compounds have been identified in the urine of human subjects and experimental animals within 48 h of exposure.

Mechanism of Toxicity

The precise mechanism of toxicity of cyclohexane has not been identified, but is likely similar to other central nervous system (CNS) depressants and general anesthetics. These compounds are believed to exert their effects through a general interaction with the CNS, and interference with neuronal membrane functions has been postulated as a mechanism of action. Disruption of membrane enzymes and the corresponding alterations in cell functions may account for the behavioral and anesthetic effects observed following exposure to various solvents.

Acute and Short-Term Toxicity (or Exposure)

Animal

The reported oral LD_{50} in rabbits is 5.5–6.0 mg kg^{-1} indicating the relatively low oral acute toxicity of cyclohexane. Vapor concentrations of 92 000 mg m^{-3} produced rapid narcosis and death in rabbits. In mice, concentrations of 51 000 mg m^{-3} caused narcosis and death occurred at 61 200–71 400 mg m^{-3}. The oral LD_{50} was reported as 12 705 mg kg^{-1} for rats and 813 mg kg^{-1} for mice.

Human

Cyclohexane is a CNS depressant and may produce mild anesthetic effects. Inhalation exposure can cause headache, nausea, dizziness, drowsiness, and confusion. Very high concentrations may cause unconsciousness, convulsions, and death. Vapors may be irritating to the nose and throat. Severe lung irritation, damage to lung tissues, or death may result from aspiration into the lungs. Direct dermal contact with liquid may cause mild irritation, which may become more severe if exposure is prolonged. Eyes may become irritated upon exposure to vapors or liquid; however, the effect is generally mild and temporary unless exposure is prolonged. Ingestion of cyclohexane may cause sore throat, nausea, diarrhea, or vomiting.

Chronic Toxicity (or Exposure)

Animal

Lower doses (1.0–5.5 mg kg^{-1}) produced mild to extensive hepatocellular degeneration and glomerulonephritis. Microscopic changes in the liver and kidneys were observed in rabbits exposed to 2700 mg m^{-3} for 50 exposures. No changes were noted at 1490 mg m^{-3}. In subchronic inhalation studies in rats and mice, the no-observed-effect level (NOEL) in rats for acute, transient effects was 500 ppm based on a diminished/absent response to an auditory alerting stimulus at 2000 ppm and above. The NOEL for subchronic toxicity in rats was 7000 ppm based on the lack of adverse effects on body weight, clinical chemistry, tissue morphology, and neurobehavioral parameters. In mice, the NOEL for acute, transient effects was 500 ppm based on behavioral changes during exposure at 2000 ppm and above. The NOEL for subchronic toxicity in mice was 2000 ppm based on hematological changes at 7000 ppm.

Human

Prolonged exposure may produce liver and kidney damage. Cyclohexane is not a carcinogen or a developmental toxicant.

In Vitro Toxicity Data

Negative results were obtained in Ames and sister chromatid exchange, mouse lymphoma, and unscheduled DNA synthesis assays.

Clinical Management

If inhalation exposure occurs, the source of contamination should be removed or the victim should be moved to fresh air. Artificial respiration should be administered or, if the heart has stopped, cardiopulmonary resuscitation provided. If dermal contact has occurred, contaminated clothing should be removed and the affected area should be washed with water and soap for at least 5 min or until the chemical is removed. Contaminated eyes should be flushed with lukewarm, gently flowing water for 5 min or until the chemical is removed. If ingestion

occurs, vomiting should not be induced. Water should be given to dilute the compound. If vomiting occurs naturally, the victim should lean forward to reduce risk of aspiration. Aspiration of the compound into the lungs may produce chemical pneumonitis requiring antibiotic treatment and administration of oxygen and expiratory pressure.

Environmental Fate

If released to air, cyclohexane will exist solely as a vapor in the ambient atmosphere, and will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals (the half-life for this reaction in air is estimated to be 45 h). Little mineralization was detected in a soil biodegradation test and aqueous screening biodegradation tests. Cyclohexane is highly resistant to biodegradation and is catabolized chiefly by cooxidation (use of other organic matter as a carbon and energy source). A bacterium that grows aerobically on cyclohexane was isolated from the wastewater plant of a petroleum refinery. An estimated bioconcentration factor of 89 suggests the potential for bioconcentration in aquatic organisms is moderate. Hydrolysis is not expected to occur due to the lack of hydrolyzable functional groups.

Ecotoxicology

LC_{50} for fathead minnow is 95 mg l^{-1} (static).

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average, is 100 ppm.

See also: Cyclohexene; Hexane.

Further Reading

Baxter CS (2001) Alicyclic hydrocarbons. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 4, pp. 161–165. New York: Wiley.

Kreckmann KH, Baldwin JK, Roberts LG, *et al.* (2000) Inhalation developmental toxicity and reproduction studies with cyclohexane. *Drug and Chemical Toxicology* 23: 555–573.

Lewis RJ, Sr. (ed.) (2000) Cyclohexane. In: *Sax's Dangerous Properties of Industrial Materials*, vol. 2, pp. 1037–1038. New York: Wiley.

Malley LA, Bamberger JR, Stadler JC, *et al.* (2000) Subchronic toxicity of cyclohexane in rats and mice by inhalation exposure. *Drug and Chemical Toxicology* 23: 513–537.

Rouvière PE and Chen MW (2003) Isolation of *Brachymonas petroleovorans* CHX, a novel cyclohexane-degrading proteobacterium. *FEMS Microbiology Letters* 227: 101–106.

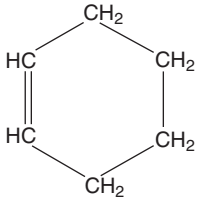
Relevant Website

http://ecb.jrc.it – European Commission (2004) Cyclohexane. European Risk Assessment Report Vol. 41.

Cyclohexene

Patricia J Beattie

- Chemical Abstracts Service Registry Number: CAS 110-83-8
- Synonyms: 1,2,3,4-Tetrahydrobenzene; Tetrahydrobenzene
- Chemical/Pharmaceutical/Other Class: Cycloalkene
- Chemical Formula: C_6H_{10}
- Chemical Structure:



Uses

Cyclohexene is used in oil extraction and in the manufacture of adipic, maleic, and hexahydrobenzoic acids and aldehydes. It is also used as a stabilizer for high-octane gasoline and as a catalyst solvent.

Exposure Routes and Pathways

Exposure occurs most commonly through either inhalation or skin contact.

Toxicokinetics

Cyclohexene is readily hydroxylated by microsomal oxidases to the corresponding dihydroxy derivatives. These are then further conjugated and eliminated in urine.

Mechanism of Toxicity

Cyclohexene is an irritant and defats skin on direct contact. It is also an anesthetic and central nervous system (CNS) depressant on inhalation exposure. The mechanism for this toxicity is unknown.

Acute and Short-Term Toxicity (or Exposure)

Animal

In general, cyclohexene appears to be only mildly toxic. Mice exposed acutely by inhalation to 8830 ppm exhibited a loss of righting reflexes; at 13 400–14 900 ppm death occurred. Dogs inhaling cyclohexene at unknown concentrations exhibited symptoms characterized by muscular quivering and incoordination.

Human

No acute effects have been reported in humans. By analogy to effects reported with structurally similar compounds and in animals, cyclohexene is regarded as a mild respiratory irritant and CNS depressant. When ingested, it represents a low to moderate pulmonary aspiration hazard.

Chronic Toxicity (or Exposure)

Animal

Rats, guinea pigs, and rabbits were exposed to cyclohexene vapors at 75, 150, 300, and 600 ppm for 6 h day^{-1}, 5 days $week^{-1}$ for 6 months. At low doses, an increase in alkaline phosphatase was reported. At 600 ppm, in rats, the same increase in alkaline phosphatase was observed along with a decrease in weight gain. Other blood and biochemical measures were within normal limits.

Human

No chronic health effects have been reported in humans.

Clinical Management

Overexposure to vapors of cyclohexene should be treated by removing the patient to fresh air. If skin or eye contact occurs, the affected areas should be flushed with water for at least 15 min to remove residual solvent. If ingestion of cyclohexene occurs, vomiting should not be induced. This could result in aspiration of solvent into the lungs leading to chemical pneumonitis and pulmonary edema, which can be fatal.

Environmental Fate

Cyclohexene biodegrades in aerobic soils, has a low to moderate mobility, and rapidly volitalizes to the atmosphere. In the atmosphere, it then undergoes a rapid, aerosol-forming reaction with ozone with an estimated half-life of 8.3 h. In water, cyclohexene degrades under aerobic conditions. Based on its water solubility and log octanol/water partition coefficient, cyclohexene does not bioconcentrate in fish or aquatic organisms.

Exposure Standards and Guidelines

- OSHA permissible exposure limit is such that the 8 h time-weighted average is 300 ppm (1015 mg m^{-3}).
- The American Conference of Governmental Industrial Hygienists guideline is such that the 8 h time-weighted average is 300 ppm.
- The NIOSH recommended exposure limit is such that the 10 h time-weighted average is 300 ppm.
- The NIOSH immediately dangerous to life or health at 2000 ppm.

See also: Gasoline.

Further Reading

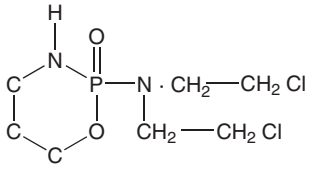
Health Council of the Netherlands: Committee on Updating of Occupational Exposure Limits (2004) *Cyclohexene: Health-Based Reassessment of Administrative Occupational Exposure Limits*. The Hague: Health Council of the Netherlands.

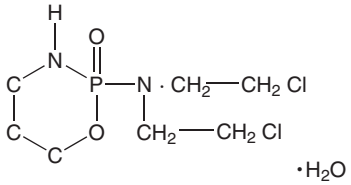
Cyclopeptide *See* Mushrooms, Cyclopeptide.

Cyclophosphamide

Greene Shepherd

- Representative Chemicals: Cyclophosphamide; Cyclophosphamide monohydrate
- Chemical Abstracts Service Registry Numbers: CAS 50-18-0; CAS 6055-19-2 (monohydrate)
- Synonyms: 2*H*-1,3,2-Oxazaphosphorin-2-amine, *N*,*N*-bis(2-chloroethyl) tetrahydro-, 2-oxide; 2*H*-1,3,2-Oxazaphosphorine; 2-chloroethyl)amino]tetrahydro-, 2-oxide; Asta B 518; B 518; Bis(2-chloroethyl)phosphoramide cyclic propanolamide ester; Clafen; Claphene; Cyclophosphamid; Cyclophosphan; Cyclophosphane; Cytophosphan; Cytoxan; Endoxan; Genoxal; *N*,*N*-bis-*O*-Trimethylenephosphoric acid ester diamide; *N*,*N*-Bis(2-chloroethyl)-*N*′,*O*-propylenephosphoric acid ester diamide; NSC 26271; Procytox; Sendoxan; Cyclophosphamide monohydrate; Cyclophosphamide hydrate; Endoxan monohydrate
- Chemical/Pharmaceutical/Other Class: Nitrogen mustard
- Chemical Formula: $C_7H_{15}Cl_2N_2O_2P$
- Chemical Structures:





Anhydrous form

Uses

Cyclophosphamide is used in human medicine as an antineoplastic (anticancer) agent in a variety of applications. Cyclophosphamide is a potent immunosuppressive agent and is used to prevent rejection episodes following renal, hepatic, and cardiac transplantation; and in non-neoplastic disorders in which there is altered immune activity, such as Wegener's granuloma, rheumatoid arthritis, the nephrotic syndrome in children, or autoimmune ocular diseases. Cyclophosphamide has also been used in veterinary practice for defleecing sheep, and it has been tested as an insect chemosterilant.

Exposure Routes and Pathways

Exposure to this odorless, white, crystalline powder may occur during its manufacture, formulation, or distribution as an antineoplastic drug. During manufacture and experimental use, exposure may be by inhalation or skin absorption. Therapeutically, patient exposure is by the oral, intramuscular, intraperitoneal, intravenous, or intrapleural route.

Toxicokinetics

In most species, cyclophosphamide is rapidly absorbed, metabolized, and excreted. In patients, cyclophosphamide was distributed rapidly to all tissues and exhibited a half-life of 6.5–7 h. The majority of an administered dose (50–68%) was excreted in the urine and no parent compound or metabolite was detected in expired air or feces. Carboxyphosphamide and phosphoramide mustard were detected in the urine. Cyclophosphamide is a racemer, and stereoselective metabolism by cytochrome P-450 of the enantiomers has been demonstrated in mice, rats, and rabbits. The primary metabolite is the 4-hydroxy derivative, and it exists in equilibrium with aldophosphamide, its ring-opened tautomer. Either metabolite can be converted by mammalian enzymes to 4-ketocyclophosphamide or to the propionic derivative. Both metabolites are relatively nontoxic and represent major urinary metabolites.

Mechanism of Toxicity

Cyclophosphamide works by interrupting the cell cycle in a nonphase specific manner. It prevents cell division by forming cross-linkages in DNA. An extensive study of cyclophosphamide analogs, some of which release acrolein but no phosphoramide mustard and others that cannot undergo complete metabolism, has provided strong evidence that (1) the phosphoramide mustard metabolite is responsible for the drug's antitumor activity, (2) the toxic side effects are probably due to the phosphoramide mustard and acrolein, and (3) nor-nitrogen mustard is responsible for the renal damage that occurs in some cases.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD in mice ranges from 370 mg kg^{-1} (subcutaneous) to 310 mg kg^{-1} (intravenous). The LD in rats was 160 mg kg^{-1} (intravenous), 180 mg kg^{-1} (oral), and 400 mg kg^{-1} (intraperitoneal in rats bearing tumors). The intravenous LD was 400 mg kg^{-1} in guinea pigs and 40 mg kg^{-1} in dogs. In mice, rats, and dogs, the predominant hematological effect of cyclophosphamide is leucopenia; some depression of thrombocytes was also noted. Prolonged treatment of rodents with cyclophosphamide has produced pathological structural changes in a variety of organs including lung, gut, pancreas, and liver. In rats, cyclophosphamide given orally decreases mitosis in crypts, decreases the height of villi, and causes degeneration of the intestinal mucosa. A single intraperitoneal dose of cyclophosphamide caused marked necrosis of the bladder and necrosis of the renal tubular and renal pelvic epithelium in mice, rats, and dogs. Cyclophosphamide is teratogenic in the rhesus monkey when given intramuscularly for various periods between 25 and 43 days of pregnancy at doses ranging from 2.5 to 20 mg kg^{-1} body weight. Placental transfer of cyclophosphamide has been demonstrated in mice, and a positive correlation between alkylation of embryonic DNA and the production of congenital abnormalities has been reported in mice.

Human

Patients treated with cyclophosphamide have been reported to exhibit various side effects such as flushing of the face, swollen lips, cardiotoxicity, pneumonitis or interstitial fibrosis, agitation, dizziness, tiredness, weakness, headache, nausea, vomiting, diarrhea, stomatitis, hemorrhagic colitis, hepatitis, hemorrhagic cystitis, fever, chills, sore throat, sweating, pancytopenia, leukopenia, alopecia, changes in the nucleoli of lymphocytes, water and sodium retention, pulmonary fibrosis, and visual blurring. Birth defects, such as limb reductions or pigmentation of the fingernails and skin, were also noted. Cystitis, hemorrhagic cystitis, and fibrosis of the bladder wall have been reported in patients treated for cystitis, rheumatoid arthritis, lupus erythematosus, and neoplasia, respectively. Fatal cardiomyopathy may result when very large doses of cyclophosphamide are given as conditioning for bone marrow transplantation. Cyclophosphamide has teratogenic and mutagenic potential and can cause sterility of either sex. It can damage germ cells in prepubertal, pubertal, and adult males and cause premature ovarian failure in females. It is most toxic to the human fetus during the first 3 months and congenital abnormalities have been detected after intravenous injections of large doses to pregnant women during this period of pregnancy. Mothers taking cyclophosphamide should avoid breast-feeding.

Chronic Toxicity (or Exposure)

Animal

Mice dosed at 7% of the LD_{50} cyclophosphamide per week subcutaneously for 1 year had higher rates of leukemias, mammary carcinomas, ovarian carcinomas, and lung tumors compared to controls.

Human

Chronic overmedication with cyclophosphamide would be expected to produce bone marrow suppression. Decreased ability to fight infection, inability for blood to clot with subsequent bleeding, hair loss, and other toxic effects may develop in patients.

In Vitro Toxicity Data

Studies of the teratorgenic potential of cyclophosphamide have demonstrated that cyclophosphamide must be metabolized in order to become teratogenic.

Clinical Management

Treatment is largely supportive. Cyclophosphamide is adsorbed to activated charcoal and charcoal should be used for substantial, recent ingestions. Patients may require aggressive fluid support. Standard supportive therapies, such as vasopressors, should be utilized as clinically indicated. Patients may require prolonged observation due to the delay in the development of adverse effects. Antibiotics may be needed due to development of immunosuppression. MESNA has been used for management of cyclophosphamide-induced hemorrhagic cystitis.

Environmental Fate

Cyclophosphamide may be released into the atmosphere secondary to production or through waste streams. If released into the air, it is expected to be present in both vapor and particulate phases at ambient temperature and pressure. Vapor-phase degradation by hydroxyl radicals in general produces a half-life of 5.5 h. Particulate cyclophosphamide will

be broken down by both wet and dry decompositions as well as through photodegradation.

See also: Acrolein.

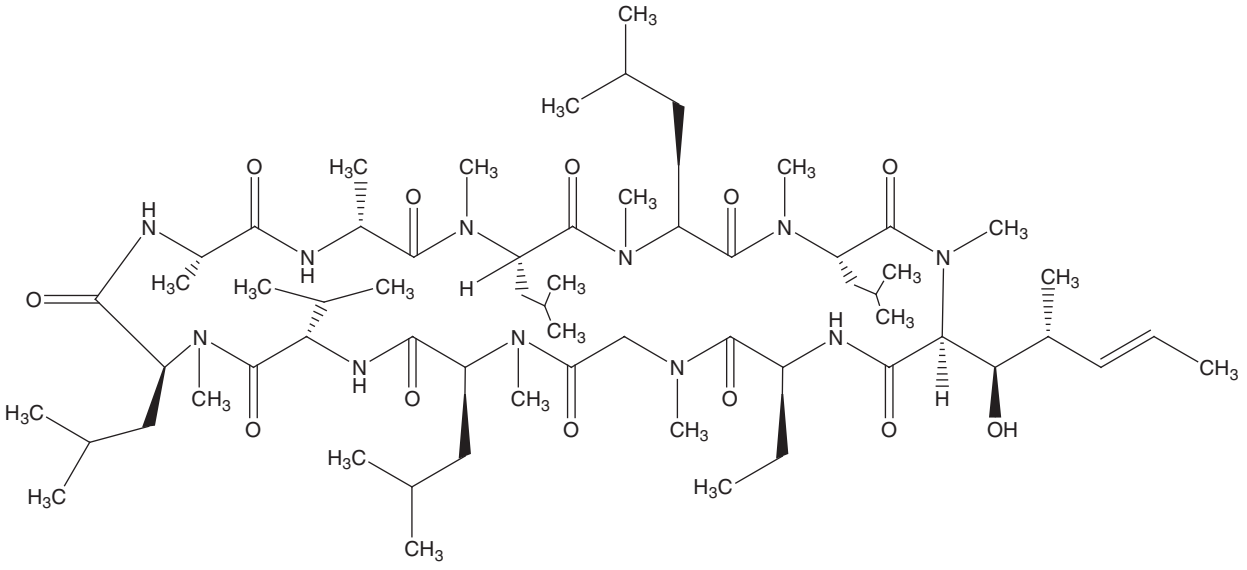
Further Reading

Braverman AC, Antin JH, and Plappert MT (1991) Cyclophosphamide cardiotoxicity in bone marrow transplantation. *Journal of Clinical Oncology* 9: 1215–1223.

Cyclosporine

Teresa Dodd-Butera and Molly Broderick

- Chemical Abstracts Service Registry Number: CAS 59865-13-3
- Synonyms: Cyclosporin A; Neoral; Sandimmune (brand names)
- Chemical/Pharmaceutical/Other Class: Immunosuppressive agent
- Chemical Structure:



Uses

Cyclosporine was approved for transplant immunosuppression in 1983. It is administered to prevent organ rejection after transplant of kidney, liver, lung, heart, or bone marrow. In addition, it has been given to patients with nephrotic syndrome, rheumatoid arthritis, psoriasis, severe Crohn's disease, and for other medical conditions.

Background Information

Cyclosporine is a macrolide antibiotic cyclopeptide with 11 amino acids. It inhibits cytokines (primarily interleukin-2) produced by T-cells in response to antigen exposure. Cyclosporin A can be biosynthesized from soil fungus *Tolypocladium inflatum* or synthetically manufactured. Hazardous combustion and decomposition products of this process include carbon monoxide, carbon dioxide, nitrogen oxide, hydrogen chloride gas, and phosgene.

Exposure Routes and Pathways

Cyclosporine is available in liquid, pill, capsule, opthalmic drops, and injectable forms. Oral and intravenous routes are the most common pathways of exposure.

Mechanism of Toxicity

The mechanism of cholestasis from cyclosporine may result due to interference with uptake and transport of bile salts. Cyclosporine also causes prerenal vasoconstriction and decreased glomerular perfusion, which is sometimes dose-dependent. Cyclosporine induces enzymes in porphyrin production, which can exacerbate the symptoms of porphyria.

Toxicokinetics

Cyclosporine is biotransformed primarily in the liver by CYP3A4; however, alternate metabolic pathways may yield toxic metabolites. Most of the cyclosporin dose is excreted in bile as an active metabolite. Less than 1% is excreted as unchanged drug. Inducers of CYP3A increase cyclosporine metabolism, whereas competitors may increase cyclosporine to potentially toxic levels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal studies show immunosuppression, renal and cardiovascular, and neurotoxicity. Studies in rats and rabbits have shown that large doses may cause death to the fetus or birth defects, depending on timing of exposure in gestation.

Human

Symptoms may include nausea, high blood pressure, bleeding, headache, disorientation, and diminished renal and hepatic functions. Some of these may occur as side effects of therapeutic doses. Allergic reactions may occur with parenteral administration of the drug and in persons allergic to polyoxyl 40 hydrogenated castor oil (present in capsules and solution).

Chronic Toxicity (or Exposure)

Animal

There was an increased incidence of lymphoma in grafted macaques and male mice also receiving cyclosporine. Rats exposed to cyclosporine A developed renal and hepatocellular tumors.

Human

There is an increased risk of getting infections; however, use of this drug demonstrated lower rates of infection than some other regimens given for immunosuppression and autoimmune diseases. Additionally, cyclosporine A is considered a human carcinogen, due to the increased risk of lymphoma and skin cancer in patients taking the drug. In some cases, tumor regression occurred after discontinuing cyclosporine. Hyperkalemia, loss of renal function, gum sensitivity, noncardiogenic pulmonary edema, and, rarely, myopathies may occur in patients on cyclosporine therapy.

Women who take cyclosporine during pregnancy may be at increased risk for delivering prematurely, though controlled scientific studies are necessary. In addition, cyclosporine passes into breast milk. Due to the potential risk to the infant for immunosuppression, impact on growth, and carcinogenicity, breastfeeding is not recommended with cyclosporine therapy.

In Vitro Toxicity Data

Most *in vitro* tests were negative for genetic damage; however, there was a slight increase in sister chromatid exchange in human lymphocytes exposed *in vitro*.

Clinical Management

Multiple-dose activated charcoal has been used to increase elimination of cyclosporine. Dose reduction when needed and close monitoring of the patient for symptoms of toxicity are required. However, many of the issues of clinical management are related to adverse effects and interactions with other substances. The following precautions are recommended for patients taking cyclosporine. Medication should not be taken with grapefruit or grapefruit juice, as this interferes with biotransformation and elimination of the drug. Patients should be advised to avoid alcohol, cough and cold remedies, diet pills, stimulants, ibuprofen, and certain herbs and supplements. Discuss any medications with a healthcare professional before taking. Similar brands are not necessarily interchangeable. No double dosing for missed doses. Patients using cyclosporine should avoid others with diseases and infections, and live vaccinations. Vaccinations received by patients on cyclosporine may not be as effective.

See also: Charcoal; Sister Chromatid Exchanges.

Further Reading

Goldfrank L, Flomenbaum N, Lewin N, *et al.* (eds.) (2002) *Goldfrank's Toxicologic Emergencies*, 7th edn. New York: McGraw-Hill Medical Publishing.

Klaassen C (ed.) (2001) *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.
Rezzani R (2004) Cyclosporine A and adverse effects on organs: histochemical studies. *ProgHistochemCytochem* 39(2) 85–128.

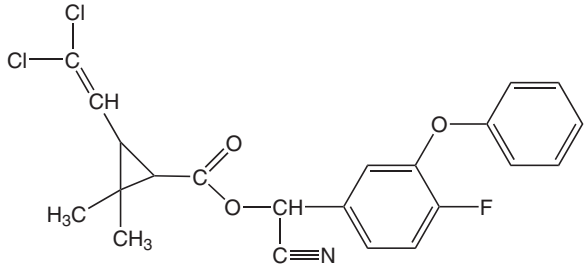
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Cyclosporine.

Cyfluthrin

Subramanya Karanth

- Chemical Abstracts Service Registry Number: CAS 68359-37-5
- Synonyms: Attatox; Baythroid; Contur; Solfac
- Chemical/Pharmaceutical/Other Class: Pyrethroid insecticide. Technical-grade cyfluthrin consists of a mixture of eight stereoisomers, consisting of two *cis* and two *trans* isomeric pairs
- Chemical Formula: $C_{22}H_{18}Cl_2FNO_3$
- Chemical Structure:



Uses

Cyfluthrin is a broad-spectrum nonsystemic insecticide used to control cockroaches, ants, termites, mosquitoes, flies, tobacco budworms, and common chewing and sucking insects of cotton, cereal, potatoes, and peanuts. It is also used effectively in the control of public health pests.

Exposure Routes and Pathways

Common routes of cyfluthrin exposure include dermal, ingestion, and inhalation.

Toxicokinetics

Cyfluthrin is excreted mainly as urinary metabolites but a portion of it is also excreted unchanged in feces. Toxicokinetic studies with ^{14}C-cyfluthrin in rats have shown that the initial step of biotransformation includes ester hydrolysis resulting in 3-phenoxy-4-fluorobenzyl alcohol intermediate and permethric acid. 3-Phenoxy-4-fluorobenzyl alcohol is further oxidized to 3-phenoxy-4-fluorobenzoic acid, which is either hydroxylated to 4′-hydroxy-3-phenoxy-4-fluorobenzoic or conjugated with glycine to 4′-hydroxy-3-phenoxy-4-fluorobenzoic acid.

Mechanism of Toxicity

Cyfluthrin elicits toxicity by modifying the voltage-sensitive sodium channels in neuronal membranes. It binds to a receptor site on the alpha subunit of the sodium channel, which results in prolonged opening of sodium channels. This delay in the closure of sodium channels leads to a protracted sodium influx causing repetitive firing of sensory nerve endings and hyperexcitation. High doses may result in complete depolarization of the nerve membrane and blockade of nerve conduction.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity studies in laboratory animals have shown that cyfluthrin is moderately toxic to mammals with an oral LD_{50} of 850–1200 mg kg^{-1} in rats.

Human

Cyfluthrin is slightly irritating to skin and eyes in humans. One of the common symptoms of cyfluthrin poisoning is paresthesia (a stinging, burning, and itching skin particularly on the face), progressing to numbness. Dermal irritation may worsen if exposed to sun or heat. Large doses of cyfluthrin may cause excessive salivation, irritability, tremors, convulsions, and death. Inhalation exposure may result in labored breathing and nasal discharge.

Chronic Toxicity (or Exposure)

Animal

Long-term exposure to cyfluthrin has been reported to cause diarrhea, reduced body temperature, and weight loss in laboratory animals. A 24 month chronic

feeding study in rats demonstrated a no-observed-adverse-effect level (NOAEL) of 2.6 mg kg^{-1} day^{-1}. No organ-specific toxicities were observed in laboratory animals following long-term dietary exposure. Mutagenicity and carcinogenicity studies have shown no evidence of potential effects in rats and mice.

Human

Little is known regarding chronic effects of cyfluthrin in humans. As cyfluthrin is commonly used on food crops, risks of dietary exposure and through water and air are relatively high. Exposure may also occur through inhalation and contact from indoor and outdoor uses.

Clinical Management

In the case of dermal exposure, the contaminated area must be washed with plenty of water and soap. Topical application of vitamin E preparations may help to reduce the severity of skin reactions. The affected eye must be irrigated with lukewarm water for at least 10 min. The contaminated clothing is removed and the airway cleared. In the case of ingestion, gastric lavage is avoided as solvents present in cyfluthrin formulations may increase the risk of aspiration pneumonia. Atropine (adults and children >12 years: 0.6–1.2 mg kg^{-1}; children <12 years: 0.02 mg kg^{-1} by IV infusion) may be useful to control excessive salivation but care should be taken to avoid excess administration. If prolonged and frequent seizures appear, diazepam should be used for treatment (5–10 mg kg^{-1}, IV).

Environmental Fate

Cyfluthrin is a photosensitive compound and following exposure to sunlight, it readily breaks down. It is highly immobile in soil and unstable in water. On soil surfaces its half-life is 2–3 days. Under anaerobic conditions, its half-life in soils is $\sim$2 months. Cyfluthrin does not move through soils and is not a groundwater contaminant. It rapidly breaks down in surface waters as it floats on the surface where it is subject to photodegradation.

Ecotoxicology

Cyfluthrin is least toxic to birds while acute toxicity studies have shown that it is highly toxic to marine and freshwater organisms. It is extremely toxic to honey bees and other beneficial insects.

Exposure Standards and Guidelines

The reference dose for cyfluthrin is 0.008 mg kg^{-1} day^{-1}, while the acceptable daily intake is 0.02 mg kg^{-1} day^{-1}.

See also: Neurotoxicity; Pyrethrins/Pyrethroids.

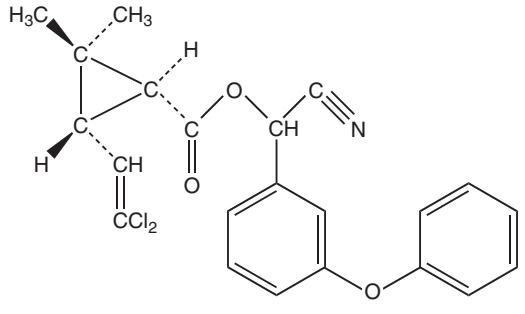
Relevant Websites

http://pmep.cce.cornell.edu – Cornell University.
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Cypermethrin

Paul R Harp

- Chemical Abstracts Service Registry Numbers: CAS 52315-07-8; CAS 69865-47-0; CAS 86752-99-0; CAS 86753-92-6; CAS 88161-75-5; CAS 97955-44-7
- Synonyms: (*R*,*S*)-α-cyano-3-phenoxybenzyl-2,2-dimethyl (1*R*,1*S*)-*cis*,*trans*-3-(2,2-dichlorovinyl) cyclopropane carboxylate; Agrothrin; Ammo; Arrivo; Cymbush; Cymperator; Cynoff; Demon; Folcord; Prevail; Polytrin; Ripcord; Stockade; CCN 52; FMC 30980; OMS 2002; NRDC 149; PP 383; SHA 109704
- Chemical/Pharmaceutical/Other Class: Type II pyrethroid insecticide
- Chemical Structure:



Uses

Cypermethrin is a broad-spectrum insecticide used in a variety of agricultural, commercial, and residential

applications. It is available as a wettable powder, an emulsifiable concentrate, or a concentrate for ULV application. Technical cypermethrin is a mixture of eight isomers.

Exposure Routes and Pathways

Dermal contact is probably the most common route of exposure but cases of ingestion and inhalation have also been reported.

Toxicokinetics

Pyrethroids are poorly absorbed through the skin and are only moderately absorbed in the gastrointestinal tract. In one case of dermal exposure, absorption was estimated to be ~3%. Metabolism of cypermethrin occurs rapidly through ester cleavage and hydroxylation. Adipose tissue acts as a storage depot and has varying affinities for the different isomeric forms; elimination half-lives of 3.4 and 18 days have been reported in rats for *trans* and *cis* isomers, respectively. Urinary excretion is a primary route of elimination but fecal excretion may also be significant depending on species of animal and isomeric configuration.

Mechanism of Toxicity

Several mechanisms of action have been identified for the pyrethroids with the primary mechanism related to a selective high affinity for membrane sodium channels. Closing of the channel, which ends the action potential, is slowed resulting in a prolonged 'tail' current and repetitive firing of presynaptic and accompanying postsynaptic cells following a single action potential. High enough doses can cause complete depolarization and blockade of nerve conduction. Cypermethrin also inhibits Ca^{2+},Mg^{2+}-ATPase and type II pyrethroids such as cypermethrin have been shown to act on γ-aminobutyric acid-mediated chloride ionophores and voltage-sensitive calcium-independent chloride channels.

Acute and Short-Term Toxicity (or Exposure)

Animal

In mammals, cypermethrin produces type II motor symptoms characterized by hyperactivity, incoordination, choreoathetosis, and convulsions.

Human

In humans, extensive dermal exposure to cypermethrin may cause temporary effects of paresthesia (stinging, burning, tingling) and numbness. Symptoms following ingestion include nausea, vomiting, tenesmus, diarrhea, unconsciousness, and death due to respiratory failure.

Chronic Toxicity (or Exposure)

Animal and Human

Chronic effects in humans following cypermethrin exposure have not been reported. Studies have demonstrated possible genotoxicity in mouse spleen and bone marrow. US Environment Protection Agency has classified cypermethrin as a possible human carcinogen based on findings of benign lung adenomas in mice.

Clinical Management

Exposed skin should be washed promptly with soap and water. Dermal application of vitamin E oil preparations may be used for both prophylaxis and treatment of paresthesia. For contact with eyes, flush immediately and for an extended period with generous amounts of clean water or saline. Gastric lavage is indicated if patient has ingested a large amount of pyrethroid and can be treated soon after exposure. For ingestion of smaller amounts or if treatment has been delayed, activated charcoal and catharsis are indicated. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenytoin or phenobarbital may be helpful for recurrent seizures. No specific antidotes for pyrethroid-induced neurotoxic effects have been approved for use in humans. Spontaneous recovery usually occurs with mild or moderate intoxication.

Environmental Fate

Cypermethrin is moderately persistent in soils. Cypermethrin degrades more rapidly in sandy compared to clay soils, and in soils with low organic content. Under aerobic conditions, the half-life is 0.5–8 weeks. Cypermethrin is more persistent under anaerobic conditions. Cypermethrin is subject to photodegradation and microbial degradation under aerobic conditions. Cypermethrin binds strongly to soil particles and poses minimal leaching concerns.

Cypermethrin hydrolyzes slowly under acidic or neutral conditions but more rapidly under alkaline conditions. Concentrations decrease rapidly due by adsorption to sediment, particles, and plants.

Ecotoxicology

Cypermethrin is practically nontoxic to birds but is highly toxic to bees. Fish and crustaceans are extremely sensitive to cypermethrin and pyrethroid compounds in laboratory settings. However, various factors (e.g., sediment binding) may reduce pyrethroid toxicity to these nontarget organisms in a natural environment.

Exposure Standards and Guidelines

The reference dose for cypermethrin is 0.01 mg kg^{-1} day^{-1}. The acceptable daily intake is 0.05 mg kg^{-1} day^{-1}.

See also: Neurotoxicity; Pyrethrins/Pyrethroids.

Further Reading

Vijverberg HPM (2000) Pyrethroids. In: Spencer PS and Schaumburg HH (eds.) *Experimental and Clinical Neurotoxicology*, 2nd edn. New York: Oxford University Press.

Relevant Websites

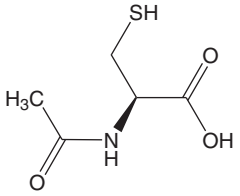
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Cysteine, *N*-Acetyl-L

Stephen R Clough

This article is a revision of the previous print edition article by David M Krentz and Linda A Malley, volume 1, pp. 392–393, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 616-91-1
- Synonyms: L-α-Acetamido-β-mercaptopropionic acid; Acetylcysteine; Airbron; Broncholysin; Brunac; Fabrol; Fluatox; Fluimucetin; Fluimucil; Fluprowit; Inspir; L -α-Acetamido-β-mercaptopropionic acid; Mercapturic acid; Mucocedyl; Mucolator; Mucolyticum; Mucomyst; Muco Sanigen; Mucosil; Mucosol; Mucosolvin; Mucret; *N*-Acetyl-L-cysteine (NAC); *N*-Acetyl-L-(+)-cysteine; *N*-Acetyl-3-mercaptoalanine (IUPAC); Neo-Fluimucil; Parvolex; Respaire; Tixair
- Chemical Formula: $C_5H_9NO_3S$
- Chemical Structure:



Uses

N-Acetyl-L-cysteine (NAC) is a natural sulfur-containing compound that is produced in living organisms from the amino acid cysteine. It is involved in the intracellular synthesis of a chemical called glutathione (or GSH). Cells (particularly liver cells) use glutathione to detoxify chemicals by making them more water soluble and thus easier to excrete from the body. NAC is also a powerful antioxidant.

NAC is primarily marketed and used as a mucolytic agent to break up mucus (by reducing disulfide bonds in mucoproteins) in persons having bronchopulmonary diseases including chronic bronchitis, cystic fibrosis, asthma, sinusitis, and pneumonia. It is also used extensively as an antidote for acetaminophen (paracetamol) overdose or toxicity. Because it is a precursor of glutathione, it has been proven useful in replenishing depleted glutathione levels in the liver. Other studies have shown it can be used as a chelating agent for the treatment of heavy metal (mercury, lead, cadmium) poisoning. Other reports (primarily animal studies) have suggested that NAC can find use as a detoxifying agent for a number of toxicants, such as paraquat, urethane, aflatoxin, *Escherichia coli*, carbon tetrachloride, chloroform, and carbon monoxide.

Exposure Routes and Pathways

The most common route of exposure to NAC is (voluntary) inhalation through the respiratory tract. Although not approved by the US Food and Drug Administration, it may be given intravenously in emergency situations. According to a National Institute for Occupational Safety and Health survey conducted between 1981 and 1983, over 30 000 workers in the United States are exposed to NAC on a daily basis. Over two-thirds of those people are inhalation therapists and clinical laboratory technicians, with the remaining majority in some type of medical profession.

Toxicokinetics

NAC is rapidly absorbed after oral administration, with peak plasma levels occurring in 2 or 3 h. With intravenous administration, peak plasma levels occur immediately. Orally administered NAC appears to distribute primarily to the kidneys, liver, and lungs. It is detectable in pulmonary secretions for at least 5 h after the dose. NAC is rapidly absorbed and exists as the free species in plasma with a concomitant increase both in plasma L-acetylcysteine levels and in protein and nonprotein –SH concentrations. Protein binding is more than 50%. The volume of distribution in humans is 0.337–0.471 kg^{-1}.

Thirty percent of intravenously administered NAC is renally cleared. Acetylcysteine elimination is not impaired in patients with severe liver damage. The terminal half-life of NAC is 2–6 h. This may be increased to 13 h after an intravenous injection.

Mechanism of Toxicity

Fatalities from normal doses and overdoses of intravenous NAC have not been reported. This is most probably due to the fact that the body produces this compound naturally and can rapidly metabolize it in the liver. Toxicity is usually limited to anaphylactoid reactions and nausea/vomiting. The average time for the onset of adverse effects following commencement of the infusion of NAC was 30 min (range, 5–70 min). *In vivo* and *in vitro* tests indicate that NAC is an inhibitor of allergen tachyphylaxis by inhibition of prostaglandin E synthesis. Adverse reactions are anaphylactoid in type and have been attributed to cause histamine release.

Acute and Short-Term Toxicity (or Exposure)

N-Acetylcysteine is used primarily in the treatment of acetaminophen overdose and/or toxicity. It is also nebulized for mucolytic effects and less often used to treat corneal ulcers. It has a very low potential to cause acute toxicity in either animals or humans.

Animal

Oral formulations of *N*-acetylcysteine are used intravenously in the clinical treatment of animals, although it has not been approved for this use. The Registry of Toxic Effects of Chemical Substances (RTECS) lists an acute oral, intravenous, and intraperitoneal LD_{50} in dogs of 1.0, 0.7, and 0.7 g kg^{-1} body weight, respectively. For mice, RTECS lists an oral, intravenous, and intraperitoneal LD_{50} of 4.4, 3.8, and 0.4 g kg^{-1} body weight, respectively. For rats, RTECS lists an oral and intravenous LD_{50} of 5.05 and 1.14 g kg^{-1} body weight, respectively. Acute effects cited for mice include central nervous system depression and somnolence; rats showed gastrointestinal changes.

Human

The primary toxicity of NAC consists of nausea/vomiting, particularly after oral therapy, and an anaphylactoid reaction that may be life-threatening. Many cases of anaphylactic reactions have been reported with symptoms primarily consisting of rash, nausea, hypotension, bronchospasm, angioedema, tachycardia, and respiratory distress. NAC may also have some neurological toxicity that includes dizziness, intracranial hypertension, hypoactivity, ataxia, and seizures. There have been reports of mucosal damage with full strength (20%) NAC, which causes hyperemia and hemorrhages of bowel mucosa. During inhalation therapy, irritation or soreness of the mouth may occur. The RTECS cites a 'lowest published toxic dose' reported for a child of 8.48 g kg^{-1} over a 3 day period. This is a very large dose and places this substance in the acute category of 'practically nontoxic'.

Chronic Toxicity (or Exposure)

Animal

N-Acetylcysteine has not been shown to be teratogenic in rats or rabbits. When administered to rabbits during the critical phase of embryogenesis, no malformation resulted.

Human

Experience in 59 pregnant patients suggested that use of *N*-acetylcysteine in pregnancy did not result in toxic effects on the fetus. In practice, the risk to the mother and baby of paracetamol-induced liver damage probably far outweighs any potential risk of *N*-acetylcysteine, and pregnancy should not be considered a contraindication to the use of this agent.

In Vitro Toxicity Data

N-Acetylcysteine is negative in the Ames mutagenicity test and also reduces the mutagenic affect of chemical carcinogens in the same assay.

Clinical Management

Since 1974, it has been known, and generally accepted, that NAC is hepatoprotective, especially for treating overdoses of acetamenophen. Basic and advanced life-support measures should be utilized as

necessary. For acetaminophen overdose, a $140\,mg\,kg^{-1}$ dose followed by $70\,mg\,kg^{-1}$ every 4 h for an additional 17 doses should be administered. Since NAC has not been approved for intravenous administration, assistance is available through the Rocky Mountain Poison Center.

Environmental Fate

Because NAC is a natural compound that contains no halogen atoms or substitutions, it would be expected to be easily metabolized by microorganisms in the environment and thus not present a risk from the standpoint of persistence or bioaccumulation.

Ecotoxicology

NAC is produced naturally in the body and is therefore not anticipated to be a hazard to ecological receptors.

Exposure Standards and Guidelines

There are no regulatory exposure standards or guidelines for NAC. Acute doses of $140\,mg\,kg^{-1}$ are recommended for the initial 'loading' dose in humans (i.e., for paracetamol poisoning) and $1330\,mg\,kg^{-1}$ can be tolerated by humans over a 72 h period.

Further Reading

Meredith TJ, Jacobsen T, Haines JA, and Berger J-C (eds.) (1995) *IPCS/CES Evaluation of Antidotes Series, vol. 3: Antidotes for Poisoning by Paracetamol*. Published by Cambridge University Press on behalf of the World Health Organization and of the Commission of the European Communities.

Relevant Website

http://www.intox.org – IPCS INTOX Data Bank.

Cytochrome P-450

Kartik Shankar and Harihara M Mehendale

Evolution of P-450s

Cytochrome P-450 proteins are one of the largest superfamily of enzyme proteins. Genes encoding cytochrome P-450 (called CYP) are found in virtually all genomes. Sequence comparisons indicate that the diverse superfamily originated from a common ancestral gene some three billion years ago. The origin of the P-450 superfamily lies in prokaryotes, before the advent of eukaryotes and before the accumulation of molecular oxygen in the atmosphere. As a comparison, *Mycobacterium tuberculosis* has 20 P-450 genes, baker's yeast has three, and the fruit fly *Drosophila melanogaster* has 83 genes. Humans have 57 different active P-450 genes and a similar number (58) of pseudogenes. There is particularly the large number of P-450 genes in the plants with 323 genes in the rice and 249 genes in the thale cress genomes. In the human genome, the P-450 genes are arranged into 18 families and 42 subfamilies.

Biochemistry of P-450 Enzymes

In vertebrates, the liver is the richest source of P-450 and is also the most active organ in the oxidation of xenobiotics. P-450 enzymes are expressed in the microsomal fraction (smooth endoplasmic reticulum) of the cell where they are anchored in the lipid bilayer. In addition to the liver, P-450s are also ubiquitously expressed in the lung, kidney, skin, nasal mucosal, gastrointestinal tract, placenta, bladder, nervous system, and blood platelets among other tissues. Although they are expressed in a variety of tissues, the function of P-450s seems to differ in each case. The liver, lung, and small intestine carry out mainly xenobiotic biotransformation. Placental P-450s are devoid of the ability to metabolize any appreciable xenobiotics but function mainly as a steroid hormone metabolizing system. Kidney P-450s are involved in some metabolism of xenobiotics, but are involved in cholecalciferol and salt balance regulation. Cytochrome P-450s are hemoproteins (iron-containing) of the *b* cytochrome type and derive the name P-450 from the wavelength (450 nm) at which the carbon monoxide derivative of the reduced cytochrome has an absorption maximum. Cytochrome P-450s, like other monooxygenases, carry out oxidation reactions in which one atom of molecular oxygen is reduced to water while another is incorporated into the substrate. Reducing equivalents are transferred from NADPH to P-450 by a flavoprotein enzyme called the NADPH–cytochrome P-450 reductase (P-450 reductase).

Reactions Catalyzed by P-450s

P-450s catalyze a large number of substrates that may be exogenous or endogenous compounds.

P-450s carry out aliphatic and aromatic hydroxylations, aromatic epoxidations (leading to stable epoxides like dieldrin from aldrin, or arene oxides), O-, N-, and S-dealkylations and oxidations, oxidative deaminations, and desulfurations among other reactions. Although the primary evolutionary role of the P-450 enzymes is to convert hydrophobic xenobiotics into more hydrophilic compounds and enhance their removal from the body, P-450s also catalyze reactions that lead to more reactive (and hence toxic) compounds. Several xenobiotics are converted into potential carcinogens via the cytochrome P-450 system.

Major CYP Families

Human cytochrome P-450s that metabolize xenobiotic compounds are almost exclusively in the *CYP1*, *CYP2*, *CYP3* and, to a small degree, *CYP4* families. The CYP1 family consists of three genes and two subfamilies. Genes in this family are controlled by the aryl hydrocarbon (Ah receptor), which is activated most notably by components of incineration products and cigarette smoke. CYP1A1 and 1B1 are expressed in varying amounts in different tissues and are most efficient in metabolizing polycyclic aromatic hydrocarbons (PAHs), while CYP1A2 preferentially metabolizes arylamines and *N*-heterocyclics. In addition, CYP1A2 metabolizes ~10–20 drugs, whereas CYP1B1 and 1A1 do not seem to act mainly as drugs. The *CYP2* family is the largest P-450 family in humans containing 16 individual isozymes. Human CYP2C8, CYP2C9, CYP2C18, and CYP2C19 together metabolize to varying amounts greater than half of all frequently prescribed drugs. Results from *in vitro* assays show that CYP2D6 metabolizes more than 75 drugs. CYP2E1, a toxicologically important isozyme, bioactivates several compounds, including acetaminophen, benzene, chloroform, carbon tetrachloride, butadiene, and vinyl chloride. The *CYP3* family has four members and has the most abundantly expressed P-450s in the liver. CYP3A4 and 3A5 are known to metabolize more than 120 frequently prescribed drugs. The CYP3A family is regulated via the pregnane-X-receptor, a nuclear receptor that can be induced by pregnenolone-related compounds.

Endogenous Functions of P-450s

Following the sequencing of the human genome, all the human P-450s have been identified. However, the endogenous physiological role of the majority of P-450s remains unknown. The critical roles of several individual isozymes are now becoming clear with the use of gene knockout and transgenic animals. Three important biological systems that are intrinsically regulated by several P-450 enzymes require special mention:

1. *Cholesterol metabolism and bile acid biosynthesis*. At least seven cytochrome P-450 enzymes play critical roles in the conversion of acetate into sterols and bile acids. Key among these are CYP51A1, CYP7A1, CYP7B1, and CYP39A1. The roles of each of these enzymes are beyond the scope of this article, but some excellent reviews and texts are available on the topic.
2. *Steroid synthesis and metabolism*. Six P-450s participate in steroid synthesis. CYP11A1 catalyzes the synthesis of pregnenolone. CYP17A1 is required for the biosynthesis of cortisol, testosterone, and estrogen. CYP19A1 converts androgenic steroids to estrogens. CYP11B2, 21A1, and 21A2 are also involved in the intermediary steps in the formation of corticosterone and aldosterone.
3. *Vitamin D_3 biosynthesis and metabolism*. The vitamin D_3 system, which acutely controls calcium status in addition to a host of other physiological functions, is a classical example of P-450s in multiple tissues being involved in the biosynthesis of a biologically active metabolite. Cholecalciferol is hydroxylated at the 25-position by either CYP27A1 or CYP2D25, both of which are expressed in the liver. The 25-hydroxycholecalciferol undergoes another P-450-mediated hydroxylation at the 1-α position by the renal CYP27B1 to the active 1,25-dihydroxycholecalciferol (vitamin D_3). Most of the biological function is attributed to this metabolite, although recent studies suggest that even the 25-hydroxy metabolite may be exerting certain biological effects. The degradation of the active 1,25-vitamin D_3 is catalyzed by the renal CYP24A1 enzyme, which catalyzes a third 24-hydroxylation leading to 1,24,25-vitamin D_3 metabolite.

See also: Biotransformation; Genomics, Toxicogenomics; Mechanisms of Toxicity; Vitamin D; Xenobiotics.

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Nebert DW and Russell DW (2002) Clinical importance of the cytochromes P450. *Lancet* 360: 1155–1162.

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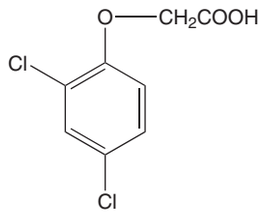
D

2,4-D (2,4-Dichlorophenoxy Acetic Acid)

Raja S Mangipudy and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 94-75-7
- Synonyms: 2,4-Dichlorophenoxyacetic acid; Dichlorophenoxyacetic acid
- Chemical/Pharmaceutical/Other Class: Chlorinated phenoxyacetic acids. A closely related compound is 2,4,5-T
- Chemical Structure:



Uses

2,4-D free acids, esters, and salts are formulated in water suspensions or solutions, or in various organic solvents, for application as systemic herbicides. Some esters are fairly volatile, whereas salts are not. The acid is corrosive. There are many commercial formulations available for weed and brush control, for certain agricultural uses, and for lawn and garden weed control.

Exposure Routes and Pathways

2,4-D may be encountered as a vapor, liquid, or as a component of mixtures. It may cause damage at the point of contact (skin, eyes, lungs, and gastrointestinal tract). Occupational exposure may occur through inhalation and dermal contact when 2,4-D is produced or used.

Toxicokinetics

Rapid and complete absorption of chlorphenoxy compounds from the gastrointestinal tract has been reported. Nearly complete absorption of 2,4-D occurs within 24 h in humans. 2,4-D is primarily metabolized by acid hydrolysis, and a minor amount is conjugated. It is highly protein bound and widely distributed. The chief organs of deposition are kidneys, liver, and the central and peripheral nervous systems.

2,4-D is primarily excreted unchanged (90%) in the kidneys via the renal organic anion secretory system. It may be conjugated to glycine or taurine. A minor fraction of 2,4-D is filtered by the glomerulus. The estimated half-life of 2,4-D is $\sim$18 h.

Mechanism of Toxicity

2,4-D is a plant-growth regulator that stimulates nucleic acid and protein synthesis and affects enzyme activity, respiration, and cell division. It is absorbed by plant leaves, stems, and roots and moves throughout the plant. It accumulates in growing tips.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in mice is 368 mg kg^{-1}. The oral LD_{50} in rats is 375 mg kg^{-1}.

2,4-D and all of its derivatives have been either embryotoxic, fetotoxic, or teratogenic in animals. Rats treated orally with 100 mg kg^{-1} day^{-1} 2,4-D from birth to 25 days of age had lower levels of acetylcholinesterase in the olfactory bulb and hippocampus regions of the brain. Monoamine neurotransmitters were also reduced. These results may account for slower learning in rats that were exposed to 2,4-D during postnatal development of the brain.

Human

Acute ingestion can cause miosis, coma, fever, hypotension, emesis, tachycardia, muscle rigidity, possible respiratory failure, pulmonary edema, and rhabdomyolysis. Alteration in liver functions such as elevated lactate dehydrogenase and aspartate aminotransferase has also been reported. In humans, the

causal relationship between these effects and chlorphenoxy herbicides such as 2,4-D remains controversial and not yet proven.

An extensive review of the published literature concluded that there is 'reasonable evidence' that occupational exposure to phenoxy herbicides is associated with increased risk for non-Hodgkin's lymphoma. Evidence is weaker or conflicting for soft-tissue sarcomas. In another review, however, case–control epidemiological studies were called inconclusive; occupational exposure studies have generally not shown associations between exposure to 2,4-D and cancer.

There appears to be sufficient evidence to regard 2,4-D as a suspect human carcinogen for non-Hodgkin's lymphoma and, possibly, for soft-tissue sarcomas and Hodgkin's disease as well. The possibility that 2,4-D may be a human carcinogen needs further study.

Chronic Toxicity (or Exposure)

Animal

In 2-year dietary tests in mice and rats, 2,4-D was not oncogenic (tumor causing). Toxic effects in the animals' kidneys were seen at low dosages in these tests.

Human

Long-term exposure to 2,4-D has been reported to cause liver, kidney, digestive, muscular, or nervous system damage. Symptoms may include weakness, fatigue, headache, dizziness, loss of appetite, nausea, eye and nasal irritation, skin irritation, hypertension, and slowed heart rate.

In Vitro Toxicity Data

2,4-D has been active in many different short-term genetic assays, including DNA damage and repair, mutations in yeast and human cells, sex-linked recessive mutations in fruit flies, and chromosome aberrations *in vitro*.

Clinical Management

No specific antidote is available. The patient must be monitored for seizures; gastrointestinal irritation; possible liver, kidney, or muscle damage; arrhythmias; acidosis; dyspnea; headache; coma; hyperthermia; and hypotension. Gastric lavage and activated charcoal/cathartic are probably more useful decontamination methods.

Environmental Fate

2,4-D has a relatively short half-life and is rather immobile in the soil. In 35 recent studies across the United States, the average lowest depth detected ranged from 6–12 in. in soils of the southern United States to 16–24 in. in low organic soils. Soils were sampled to a depth of 48 in. Its average half-life in soils ranged from 6.4 days in southern soils to 8.3 days in high organic matter soils. The average half-life was 6.1 days in grass and 6.9 days in thatch. The half-life in natural water was 2–4 weeks, although in areas such as a treated rice paddy, the half-life was as short as 1 day. The acid form of 2,4-D, as well as the amine and ester chemical groups, metabolized to compounds of nontoxicological significance and ultimately to forms of carbon. Thus, 2,4-D is considered a biodegradable compound. Under normal conditions, 2,4-D residues are not persistent in soil, water, or vegetation.

Other Hazards

To keep residues of 2,4-D out of meat or milk, dairy cattle should not be grazed on treated areas for 7 days after application. Also, hay should not be cut for 30 days and meat animals should not be slaughtered for 3 days. Contact with dried residues on vegetation is not expected to be hazardous. Inert ingredients found in 2,4-D products may include ethylene glycol, methanol, sequestering agents, petroleum hydrocarbons, and surfactants. Ethylene glycol is moderately toxic to humans; it may cause tearing, anesthesia, headache, cough, respiratory stimulation, nausea or vomiting, pulmonary, kidney, and liver changes. Methanol is moderately toxic to humans; it may cause damage to the optic nerve, tearing, headache, cough, difficult breathing, other respiratory effects, nausea, or vomiting. Some commercially formulated 2,4-D products have LD_{50} values that are much higher than the 2,4-D acid. This indicates that these formulations may have considerably less acute toxicity than the acid form. However, exposure to these formulated products may have other health effects similar to those reported for 2,4-D alone or for inert ingredients in commercial formulations. Some 2,4-D formulations may be contaminated with halogenated dibenzo-*p*-dioxins (but not TCDD), dibenzofurans, or *N*-nitrosamines. Dibenzodioxins and dibenzofurans may cause disorders of the skin, blood, and gastrointestinal tract; they may also cause headaches, numbness, birth defects, or fetal toxicity. Nitrosamines are carcinogenic.

The Material Safety Data Sheet (MSDS) should always be referred to for detailed information on handling and disposal.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.3\,mg\,kg^{-1}\,day^{-1}$.
- Maximum contaminant level is $0.07\,mg\,l^{-1}$.
- Reference dose is $0.01\,mg\,kg^{-1}\,day^{-1}$.
- Permissible exposure limit is $10\,mg\,m^{-3}$ (8 h).

See also: Pesticides; 2,4,5-T.

Further Reading

Charles JM, Bond DM, Jeffries TK *et al.* (1996) Chronic dietary toxicity/oncogenicity studies on 2,4-dichlorophenoxyacetic acid in rodents. *Fundamental and Applied Toxicology* 33: 166–172.

Charles JM, Cunny HC, Wilson RD, and Bus JS (1996) Comparative subchronic studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in rats. *Fundamental and Applied Toxicology* 33: 161–165.

Charles JM, Dalgard DW, Cunny HC, Wilson RD, and Bus JS (1996) Comparative subchronic and chronic dietary toxicity studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in the dog. *Fundamental and Applied Toxicology* 29(1): 78–85.

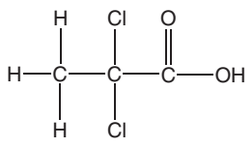
EPA, Health Effects Division (1997) Carcinogenicity Peer Review Committee Report on 2,4-D, January 29, 1997.

Rowland J (1996) 2,4-Dichlorophenoxyacetic Acid: Review of a Chronic Toxicity/Carcinogenicity Study in Rats: A Carcinogenicity Study in Mice, and a Re-review of a Developmental Toxicity Study in Rats, EPA Memorandum.

Dalapon

Priya Raman

- Chemical Abstracts Service Registry Number: CAS 75-99-0
- Preferred Name: Dalapon sodium salt
- Synonyms: Basfapon; Basfapon B; Basfapon/Basfapon N; BH Dalapon; Basinex; Crisapon; Dalapon 85; Ded-Weed; Devipon; 2,2-Dicloropropionic acid; α-Dichloropropionic acid; α,α-Dichloropropionic acid; Dowpon; Dowpon M; Gramevin; Kenapon; Kyselina; Liropon; Proprop; Radapon; Revenge; Unipon
- Chemical/Pharmaceutical/Other Class: Chlorinated aliphatic herbicide
- Chemical Structure:



Uses

Dalapon is used as a herbicide primarily to control annual and perennial grasses including Bermuda grass and Johnson grass. Use of dalapon on food crops is primarily with sugarcane and sugar beets. Dalapon is also used on fruits, potatoes, carrots, asparagus, alfalfa, and flax, and in forestry, home gardening, and to control reed and sedge growth in aquatic environments.

Exposure Routes and Pathways

Dermal inhalation and ocular exposures of liquid or vapor are the most common routes of exposure. If the chemical leaches into groundwater there is the possibility of ingestion.

Toxicokinetics

Dalapon is a polar compound that is not readily absorbed by tissues. It causes irritation to the tissue with which it comes into contact. If absorbed in the gastrointestinal tract, it is principally eliminated as the parent compound in the urine.

Mechanism of Toxicity

The mechanism of action of dalapon is the same as for most acids. The acid denatures tissue proteins upon contact.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values were $7.5–9.3\,g\,kg^{-1}$ in male and female rats. Relatively similar high oral LD_{50} values were noted in rabbits and guinea pigs ($3.9–4.6\,g\,kg^{-1}$). Dalapon is a moderate skin and eye irritant. The sodium salt of dalapon caused irritation, severe conjunctivitis, and corneal injury in rabbits, with recovery over several days.

Human

Dalapon can cause corrosive injury to tissues. Burning and irritation are the predominant acute toxicities

seen with exposure to dalapon. Skin lesions are more likely with moistened skin. Eye exposure may cause corneal destruction and conjunctival edema accompanied by pain and tearing. Permanent eye damage can result from ocular exposure. Ingestion can lead to oral, throat, and gastrointestinal irritation. Inhalation of vapors causes irritation of the eyes, nose, and throat with destruction of mucus membranes. Severe inhalation exposure may cause respiratory distress accompanied by pulmonary edema. Some other symptoms of high acute exposure to dalapon include loss of appetite, slowed heartbeat, and gastrointestinal disturbances such as vomiting, diarrhea, tiredness, pain, and irritation of respiratory tract.

Chronic Toxicity (or Exposure)

Animal

Long-term feeding studies in dogs and rats indicated increased kidney weights at high dosages. The no-observed-adverse-effect level (NOAEL) in a 2 year feeding study in rats was $15\,mg\,kg^{-1}\,day^{-1}$. The NOAEL in a 1 year feeding study in dogs was $50\,mg\,kg^{-1}\,day^{-1}$.

Human

Long-term exposure may cause increased kidney and liver weights. Repeated or prolonged exposure to dalapon may cause irritation to the mucuous membrane linings of the mouth, nose, throat, lungs, and to the eyes. Chronic skin contact can lead to moderate irritation or even mild burns.

In Vitro Toxicity Data

Dalapon was negative in a variety of mutagenic test assays.

Clinical Management

In the event of dermal exposure to dalapon, contaminated clothing should be removed quickly and the exposed area should be flushed with copious amounts of water for 15 min. With eye exposure, the affected eye should be flushed with water for 30 min, occasionally lifting the upper and lower lids. With inhalation exposure, the victim should be moved to fresh air. If the victim is not breathing, artificial respiration should be administered. If swallowed, vomiting should not be induced. If the victim is conscious, he or she should drink plenty of water or milk.

Environmental Fate

Dalapon is somewhat persistent in soil but does not readily adsorb to soil particles. It remains active in soil for several months when applied at high rates. Dalapon has a relatively high mobility in soil, with leaching possible. Soil microorganisms are efficient at degrading dalapon, however, such that dalapon is not typically found in groundwater. High temperatures and increased moisture accelerate dalapon degradation in soil. Dalapon can also be degraded by ultraviolet light. In aquatic environments, dalapon is degraded by microorganisms (most important), hydrolysis, and photolysis. Dalapon is absorbed by both plant roots and leaves and translocates. With high applications, dalapon precipitates and leads to local corrosive effects on plants.

Ecotoxicology

The LC_{50} of dietary (5 day exposure) dalapon was more than 5000 ppm in mallards, ring-necked pheasants, and Japanese quail. The acute oral LD_{50} of dalapon in chickens was $>5\,g\,kg^{-1}$. Reproduction may be affected in birds at nonlethal exposures. LC_{50} values for dalapon in a variety of fish species was $\sim 100\,mg\,l^{-1}$. Dalapon is only slightly toxic to mollusks. Crustaceans and insects are most sensitive of aquatic invertebrates. Dalapon is relatively nontoxic to honeybees and terrestrial insects.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists Threshold Limit Value is 1 ppm; the Maximum Contaminant Level is $0.2\,mg\,l^{-1}$; and the reference dose is $0.03\,mg\,kg^{-1}\,day^{-1}$.

See also: Pesticides.

Relevant Websites

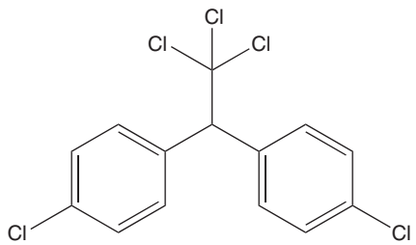
http://www.envirotools.org – Envirotools.org, Michigan State University.

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

DDT (Dichlorodiphenyltrichloroethane)

Benny L Blaylock

- Chemical Abstracts Service Registry Number: CAS 50-29-3
- Synonyms: Dichlorodiphenyltrichloroethane; *p*,*p*′-DDT; 1,1′-(2,2,2-trichloroethylidene)-bis-(4-chlorobenzene); Anofex; Cesarex; Chlorophenothane; Dedelo; Dinocide; Didimac; Digmar; ENT 1506; Genitox; Guesapon; Guesarol; Gexarex; Gyron; Hildit; Ixodex; Kopsol; Neocid; OMS 16; Micro DDT 75; Pentachlorin; Rukseam; R50; and Zerdane
- Chemical/Pharmaceutical/Other Class: Synthetic organochlorine insecticide
- Chemical Formula: $C_{14}H_9Cl_5$
- Chemical Structure:



Uses

DDT is an insecticide whose use has been banned in the United States. It is still used in some parts of the world.

Exposure Routes and Pathways

The most common route of exposure is ingestion. Data indicate that, even with relatively high doses, there is minimal absorption of DDT through skin. Therefore, exposure via dermal absorption was considered to be negligible. DDT and its metabolites are ubiquitous in the atmosphere but are present in such low concentrations that exposure via inhalation is negligible. Potential inhalation of relatively high levels of DDT should be possible only in areas of production or formulation.

Toxicokinetics

Gastrointestinal absorption of DDT is slow with symptoms delayed by several hours. DDT dissolved in solvents containing vegetable or animal fat is absorbed several times faster than the undissolved compound. Due to DDT's large particle size, absorption through the respiratory tract is less important to toxicity. Skin absorption is considered almost negligible.

Metabolism of DDT proceeds at a very slow rate. Liver microsomal P450 and other microsomal enzymes initially dechlorinate DDT to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) and reduce to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD). The conversion of DDD to bis(*p*-chlorophenyl)acetic acid (DDA) involves the formation of an acyl chloride intermediate by hydroxylation followed by hydrolysis to yield the final product.

DDT, like most other organochlorine insecticides, is highly lipophilic. It is stored in all tissues with higher levels generally found in adipose tissue. Most species store DDE more tightly than DDT.

DDA is the main form in which DDT is excreted. Most excretion takes place through bile with ~2% in urine and less than 1% in feces. Cows excrete ~10% of DDT doses in their milk; rodent females also excrete DDT in mother's milk.

Mechanism of Toxicity

The nervous system is the main site of toxicity for DDT. Effects are observed on both the central nervous system (CNS) and peripherally. There is significant alteration of neuronal membrane enzymatic and electrophysiological properties. In particular, sodium channels are altered such that once activated they close slowly, prolonging the depolarization of the nerve by interfering with the active transport of Na^+ ions out of the axon. Potassium channels are also affected. DDT specifically affects Na^+,K^+-adenosine triphosphatases (ATPases) and Ca^{2+}-ATPases, which inhibit repolarization of neurons. The membrane remains partially depolarized and is extremely sensitive to complete depolarization by very small stimuli. DDT also inhibits calmodulin that is necessary for Ca^{2+} transport essential for the subsequent release of neurotransmitters.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values in rats range from 133 to 800 mg kg^{-1} and from 150 to 300 mg kg^{-1} in mice. In animal models, the main toxic effect of DDT is neuronal hyperactivity. Alterations in the previously

mentioned ion transport channels in axon membranes produce paresthesia, hyperexcitability, irritability, fine tremors, and convulsions. Administration of 50 mg kg^{-1} as a one-time dose in rats resulted in decreased thyroid function. Monkeys dosed with 50–160 mg kg^{-1} showed increased liver enzymes.

In a recent retrospective study (US Collaborative Perinatal Project), it was suggested that DDT may be responsible for premature births and low birth weights in mothers with high DDE blood levels.

Human

Most human exposures are from ingesting very large amounts of DDT. The nervous system appears to be one of the primary target systems for DDT toxicity in humans after acute, high exposures. Symptoms range from nausea, fatigue, and vomiting to tremor and convulsions in severe poisoning. The vomiting is not due to irritation of the gastrointestinal tract and is probably central in origin. Symptoms following high oral doses include paresthesia of the tongue, lips, and face, apprehension and hypersensitivity to external stimuli, tremor with a gradual onset and usually mild effects, and, in extremely high doses, convulsions.

Liver involvement has not been a prominent symptom in DDT poisonings. Jaundice has been reported after 4–5 days following ingestion of 5000–6000 mg of DDT.

Chronic Toxicity (or Exposure)

Animal

In the liver, hepatocyte hypertrophy and centrilobular necrosis are seen. There is also an increase in liver cancers. Immune modulation has been described in both chickens and mice showing decreased antibody titers. Nervous system effects include tremors, loss of equilibrium, and changes in cellular chemistry in monkeys.

Sterility in rats and decreased fetal weights in rabbits have been reported after chronic or subchronic exposure. Gestational and lactational exposures in mice resulted in impaired learning performance. Abnormal tail development was reported in rats in a two-generational study with dosages of 10 mg kg^{-1} day^{-1}.

Carcinogenicity evidence in animals is equivocal with the production of liver and lung tumors in rats, mice, and hamsters in some studies but not in others.

Human

Almost continuous daily exposure to aerosols, sufficient to leave a white deposit of DDT on nasal vibrissae of volunteers, produced moderate irritation of nose, throat, and eyes. Except for this irritation during exposure, there were no symptoms. Anemia and thrombocytopenia have been reported after exposure in a house sprayed with the pesticide over a period of 4 months. Peripheral neuropathy has been rarely reported after chronic occupational exposure.

Neither reproductive nor teratogenic effects in humans have been reported. International Agency for Research on Cancer classifies DDT as 2B (possibly carcinogenic to humans).

Clinical Management

Diazepam may be beneficial to control convulsions. Activated charcoal is administered as a slurry. Phenobarbital is used if seizures recur after the use of diazepam. Gastric lavage may be of benefit if the patient has ingested a large amount of DDT and the procedure can be done within 1 h after ingestion. Emetics are contraindicated.

Environmental Fate

DDT is highly environmentally stable with a reported half-life of between 2 and 15 years. The insecticide is immobile in most soils and loss is the result of runoff, volatilization, photolysis, and biodegradation. Due to its extremely low solubility in water, DDT will be retained to a greater degree by soils and soil fractions. However, due to its persistence, DDT may be able to eventually leach into groundwater, especially in soils with little soil organic matter.

DDT may reach surface waters primarily by runoff, atmospheric transport, drift, or by direct application (e.g., to control mosquito-borne malaria). The reported half-life for DDT in the water environment is 56 days in lake water and $\sim$28 days in river water. The main pathways for loss are volatilization, photodegradation, adsorption to waterborne particulates, and sedimentation. Aquatic organisms, as noted above, also readily take up and store DDT and its metabolites.

Ecotoxicology

Birds are generally only slightly affected or not affected by direct exposure to DDT. Exposure is through the food chain by consuming organisms such as fish and earthworms with significant body burdens of DDT. The major concern has been eggshell thinning and altered reproductive success. It is thought that DDE is responsible for thinning of the egg shell. This seems more pronounced in predator birds. Alterations in mating behavior have also been linked to DDT exposure.

DDT is highly toxic to aquatic invertebrates. Early developmental stages are more susceptible than are the

adults. The same level of toxicity is also seen in a large number of fish including coho salmon, rainbow trout, northern pike, black bullhead, bluegill sunfish, largemouth bass, and walleye. In addition, DDT may bioaccumulate in fish, leading to long-term exposure as well as to exposure of other species that prey on fish.

Nontarget species such as bees and earthworms are not affected.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.02\,mg\,kg^{-1}$.
- Reference dose is $0.0005\,mg\,kg^{-1}\,day^{-1}$.
- Permissible exposure limit is $1.0\,mg\,m^{-3}$ (8 h).

Further Reading

Jaga K and Dharmani C (2003) Global surveillance of DDT and DDE levels in human tissues. *International Journal of Occupational Medicine and Environmental Health* 16(1): 7–20.

Turusov V, Rakitsky V, and Tomatis L (2002) Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence, and risk. *Environmental Health Perspectives* 110(2): 125–128.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for DDT, DDE, DDD.

http://extoxnet.orst.edu – Extension Toxicology Network, a collaborative effort of University of California, Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho; search for DDT.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for DDT.

Decane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 124-18-5
- Synonyms: Decane; UN2247 (DOT) (also called Alkane C(10); Decyl hydride; *n*-Decane)
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon (C10)
- Chemical Formula: $C_{10}H_{22}$

Uses

Decane is a constituent in the paraffin fraction of petroleum and is also present in low concentrations as a component of gasoline. It is used as a solvent, in organic synthesis reactions, as a hydrocarbon standard, in the manufacture of petroleum products, in the rubber industry, in the paper processing industry, and as a constituent in polyolefin manufacturing wastes. Decane is a flammable liquid (at room temperature) that is lighter than water.

Exposure Routes and Pathways

Because decane can exist as a liquid and a vapor at normal temperature and pressure, exposure could occur by either dermal contact or inhalation; oral exposure would most likely be either incidental or accidental. Decane can be detected in urban air (up to 3 ppb) as a result of automobile emissions.

Mechanism of Toxicity

Decane is generally considered to be fairly nontoxic, relative to other aliphatic hydrocarbons. This is probably due to the fact that it is less volatile than octane or heptane and may not be as readily transferred across either the pulmonary alveoli or the blood–brain barrier. If it is aspirated into the lungs, however, decane will cause adverse effects similar to those seen with heptane or octane.

Using *in vitro* and/or microbial systems, decane has been shown to be metabolized to decanol and is thus thought to be readily biodegradable in the natural environment.

Human Toxicity

Adverse effects to humans would be expected to be similar to those seen in laboratory animals (see below). There is currently no industrial air standard for occupational exposure to decane.

Animal Toxicity

Decane has been shown to have narcotic effects in both mice and rats, primarily in experiments documenting acute exposure at high concentrations. One study estimated a 2 h LC_{50} of $72\,300\,mg\,m^{-3}$ in

rats. In mice, an intravenous dose of 912 mg kg^{-1} is expected to cause death in 50% of the experimental animals. Another rat study showed that a concentration of 540 ppm in air (18 h day^{-1}, 7 days week^{-1}, 8 weeks) had a significant positive effect on weight gain. This exposure also caused some slight adverse effects (e.g., decreased white blood cell count) but no significant toxic effects overall.

Clinical Management

Persons who are exposed to high concentrations of decane in air should vacate or be removed from the source and seek fresh air. Upon oral ingestion, vomiting should not be induced as pulmonary aspiration may occur, resulting in severe narcosis and/or death. In areas of expected increased concentration, extreme care must be taken to use explosion-proof apparatus and keep the areas free from ignition sources, such as sparks from static electricity.

Aquatic Toxicity

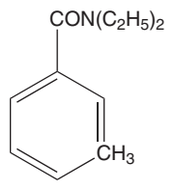
The US Environmental Protection Agency ECOTOX database has very few records for decane. An acute (48–96 h) no-observed-effect concentration of 500 mg l^{-1} was recorded for the sheepshead minnow (saltwater fish). Acute effects (48 h exposure) on the water flea (*Daphnia magna* LC_{50}) ranged from 1.3 to 29 mg l^{-1} (freshwater cladocercan).

See also: Heptane; Octane.

DEET (Diethyltoluamide)

Mark L Winter

- Chemical Abstracts Service Registry Number: CAS 134-62-3
- Synonyms: Detamide; Autan; I-Delphene; Metadelphene; Black Flag; Tabarad; Delphene; Dieltamide; Flypel; Muskol; Naugatuck Det; Off; 612 Plus; Jungle plus; Pellit; DETA; DET; *N*,*N*-Diethyl-3-methylbenzamide; *N*,*N*-Diethyl-*m*-toluamide
- Chemical/Pharmaceutical/Other Class: Methyl benzamide repellent
- Chemical Structure:



Uses

DEET is used as an insect repellent.

Exposure Routes and Pathways

DEET is available in solutions, lotions, gels, aerosol sprays, sticks, impregnated towlettes, and wristbands. Dermal and ocular exposures are the most common exposure pathways.

Toxicokinetics

Approximately 50% of each topically applied dose of DEET is absorbed within 6 h. Peak plasma levels are attained within 1 h. Ingestion of DEET may result in symptoms within 30 min, implying very rapid absorption. Oxidative enzymes in the liver metabolize DEET. Metabolites of DEET have not yet been characterized. Following movement through the skin, DEET is absorbed and distributed rather rapidly. Some studies indicate, however, that DEET and metabolites can remain in the skin and fatty tissues for 1 or 2 months after topical application. Ingestion of 50 ml of 100% DEET by adolescents or adults has resulted in severe toxicity and death. Ingestion of 25 ml of 50% DEET by a 1-year-old child resulted in severe toxicity.

Mechanism of Toxicity

DEET is primarily toxic to the central nervous system (CNS). The mechanism of toxicity is still unknown.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral LD_{50} values were reported as 1800–2700 mg kg^{-1} in male rats and 1750–1800 mg kg^{-1} in females. Rats given an LD_{50} dose showed signs of

toxicity that included lacrimation, chromodachryorrhea, depression, prostration, tremors, asphyxial convulsions, and respiratory failure. Ocular administration of DEET led to mild to moderate edema of the nictitating membrane, lacrimation, conjunctivitis, and corneal injury. These signs dissipated within 5 days. DEET is not a sensitizer in guinea pigs.

Human

A toxic syndrome consisting of ataxia, hypertonicity, tremor, and clonic jerking, and progressing to coma and seizures, may occur after dermal or oral exposure. Symptoms may occur within 30 min after an acute ingestion. Dermal exposure may cause irritation, sensitization, and erythema. A study with volunteers using 75% DEET showed that 48% had severe dermal reactions at the crease of the elbow but not at other dermal application sites.

Chronic Toxicity (or Exposure)

Animal

Dietary exposure to DEET for 200 days at 10 000 ppm led to growth retardation. Relative increases in testes and liver weights were noted in male rats, liver, and spleen in female rats, and kidneys of both sexes. No significant changes were noted at 500 ppm. Dermal application of DEET ($1\,g\,kg^{-1}\,day^{-1}$) led to reproductive toxicity in rats. No teratogenic response was noted in rabbits treated with dermal dosages as high as $5\,g\,kg^{-1}\,day^{-1}$. DEET may cause testicular and renal hypetrophy with repeated dosing.

Human

Chronic application of 70% DEET solution caused paranoid psychosis, pressurized speech, flight of ideas, and delusions after 2 weeks of daily application for the inappropriate treatment of a skin rash. Repeated application causes erythema. Extensive daily dermal application of 10–15% DEET for 2 days to 3 months has resulted in encephalopathy in children. Toxic encephalopathy has been associated with DEET in children. Signs of toxicity included agitation, weakness, disorientation, ataxia, seizures, coma, and, in three cases, death. As part of the Reregistration Eligibility Decision on DEET released in 1998, however, the US Environmental Protection Agency reviewed all available data on the toxicity of DEET and concluded that "normal use of DEET does not present a health concern to the general US population."

Clinical Management

Basic life-support measures for respiratory and cardiovascular function should be utilized. Dermal decontamination should be accomplished by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min. If eye irritation persists after irrigation, medical assistance should be sought. Ipecac-induced emesis is not recommended in cases of accidental oral exposure as coma and seizures can occur rapidly within 30 min to 1 h of ingestion. Gastric lavage should be performed cautiously with a small-bore soft nasogastric tube with small aliquots of water or saline. Activated charcoal can also be used. Initial control of seizure activity may be attempted with a benzodiazepine. Respiratory depression, hypotension, dysrhythmias, and the need for endotrachael intubation should be monitored.

Environmental Fate

Little information is available on the environmental fate of DEET. DEET is stable to hydrolysis at environmental pH levels.

Ecotoxicology

DEET is slightly toxic to birds: the acute LD_{50} in quail was $1375\,mg\,kg^{-1}$. DEET appears only slightly toxic to fish. The 24 h and 96 h LC_{50} values in trout were 125 and 172 ppm. DEET is slightly toxic to Daphnia, with a 48 h EC_{50} of 75 ppm.

See also: Benzodiazepines; Charcoal; Toxicity Testing, Dermal.

Further Reading

Sudakin DL and Trevathan WR (2003) DEET: A review and update of safety and risk in the general population. *Journal of Toxicology. Clinical Toxicology* 41: 831–839.

Relevant Websites

http://pmep.cce.cornell.edu – Cornell University.
http://www.epa.gov – US Environmental Protection Agency.

DEF (Butyl Phosphorotrithioate)

Priya Raman

- SYNONYM: Tributyl *S,S,S*-phosphorotrithioate
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organophosphorus herbicide
- CHEMICAL STRUCTURE: $(C_4H_9\text{–}S)_3P{=}O$

Uses

DEF is used as a cotton defoliant to facilitate mechanical harvesting.

Exposure Routes and Pathways

The major route of exposure to DEF is skin contact.

Toxicokinetics

DEF is readily absorbed through the skin. It is metabolized to *n*-butyl mercaptan in the gastrointestinal tract by hydrolysis. The metabolite is excreted in the urine. A urinary metabolic profiling following oral administration of DEF to a lactating goat revealed that DEF is efficiently metabolized to many metabolites. The amount of DEF in liver, kidney, and muscle represented $<1\%$ of the total residue. A major metabolite, 3-hydroxybutylmethyl sulfone was found in the tissue, milk, and urine. The hydrolytic products of DEF, *S,S*-dibutyl phosphorodithioate and *S*-butyl phosphorothiate were identified as minor components in urine, comprising 5% and 4% of the total residue, respectively.

Mechanism of Toxicity

DEF is a relatively weak inhibitor of acetylcholinesterase. The compound is hydrolyzed to a large extent in the intestine to *n*-butyl mercaptan, which is responsible for the late acute effects of DEF. The putative molecular target in neural tissue for initiation of delayed neuropathy is neurotoxic esterase or neuropathy target esterase (NTE).

Acute and Short-Term Toxicity (or Exposure)

Animal

DEF produces profound hypothermia in rats, mice, and guinea pigs by inhibition of thermogenesis. Its actions on heat conservation and motor control are, however, minimal. It is effective against both shivering and nonshivering thermogenesis and completely blocks the increase in body temperature evoked by anterior hypothalamic stimulation. The toxicologic effect of DEF, the extent and permanence of injury, and the progression or improvement of clinical signs of toxicity depended on the dose, duration, and route of exposure.

Human

Late acute poisoning from DEF is related to the release of its breakdown product, *n*-butyl mercaptan. Signs of toxicity appear within 1 hr after exposure and include general weakness, malaise, sweating, nausea, vomiting, anxiety, and drowsiness. DEF affects the lymphocyte NTE in exposed workers.

Chronic Toxicity (or Exposure)

Animal

A subchronic administration of DEF caused three toxicologic effects in hens, depending on the route of exposure: (1) an acute cholinergic effect resulting from inhibition of acetylcholinesterase, relieved by atropine, not associated with neuropathological lesions; (2) a late acute effect in chickens resulting from *n*-butyl mercaptan toxicity 4 days after oral administration of daily large doses of DEF resulting in darkening and drooping of the comb, loss of appetite and weight, weakness, emaciation, paralysis, and death, not relieved by atropine nor associated with histopathological changes in nerve tissues; and (3) delayed neurotoxicity after a delay period following topical application causing axonal and myelin degeneration resulting in ataxia, paralysis, and death.

Human

Both intensity and length of exposure play important roles in determining the extent of inhibition of NTE in lymphocytes; 50% of preexposed values of NTE activity were obtained when measured 3 or 4 weeks after the beginning of DEF exposure. However, there is no direct evidence of a correlation between a high level of lymphocyte NTE inhibition and development of neuropathy in humans. Blood acetylcholinesterase and plasma butyrylcholinesterase levels remained unchanged during the study period. There is no available weight-of-the-evidence summary assessment for DEF as a developmental or reproductive toxin.

In Vitro Toxicity Data

DEF has been reported to cause extensive alterations in morphological features of erythrocyte and nuclear membranes and affected the permeability properties of rat liver mitochondrial membrane. A reduction in the activity of cytochrome-*c*-oxidase and NAD. H-oxidase has also been observed. Content of both DNA and RNA decreased in tissues studied within 1 month of DEF intoxication and was usually restored within 3 months. Histological study showed development of necrodystrophy in liver tissue and of fibroplastic glomerulonephritis in kidney. The deteriorating effect of DEF on cellular genome functions relates not only to its cytotoxicity but also to the cancerogenic and mutagenic properties of the pesticide.

Clinical Management

Supportive and symptomatic treatment should be provided to the patient following accidental or intentional exposure to DEF.

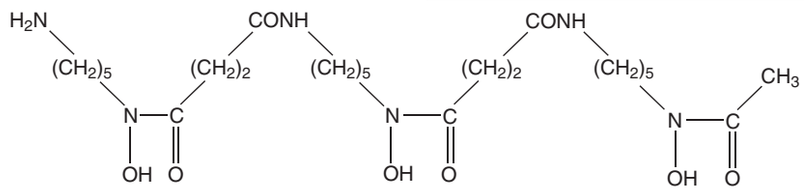
See also: Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphates; Pesticides.

Deferoxamine

Greene Shepherd

This article is a revision of the previous print edition article by Zhengwei Cai, volume 1, pp. 403–404 © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 70-51-9
- Synonyms: *N*-Benzoylferrioxamine B; Deferoxaminum; Deferrioxamine; Desferal; Desferral; Desferrin; DFO; DFOA; DFOM; Desferrioxamine B; Propionohydroxamic acid
- Chemical/Pharmaceutical/Other Class: Deferoxamine is an iron-chelating agent. It is an iron-free derivative of ferrioxamine B, which belongs to a group of siderophores, growth factors for certain microorganisms
- Chemical Formula: $C_{25}H_{48}N_6O_8$
- Chemical Structure:



Uses

Deferoxamine is widely used for treatment of both acute iron intoxication and iron-overload anemia. Recently, it has been used in trials of malaria treatment.

Exposure Routes and Pathways

In acute cases, intravenous injection and oral ingestion are the most common routes of administration. Nightly subcutaneous infusions combined with monthly intravenous infusions are used in chronic illness.

Toxicokinetics

Deferoxamine is poorly absorbed from the gastrointestinal tract. When given orally in the setting of an iron overdose, deferoxamine has been shown to bind with iron-forming ferrioxamine, which promotes absorption. The absorbed complex is cleaved during first-pass metabolism resulting in free iron capable of damaging hepatic tissue.

Deferoxamine metabolism takes place in primarily the plasma and occurs rapidly. Of the three metabolites produced, the major one is known as metabolite C. Other organs may also have some metabolizing capacity. Deferoxamine has a very high affinity and specificity for the ferric iron and chelates it in a 1:1 molar ratio. Its elimination half-life in human plasma is between 10 and 30 min. Deferoxamine mainly distributes in blood, but bile excretion is a possible elimination route. Renal clearance is the major elimination route of deferoxamine in humans. It accounts for about one-third of the total body clearance; 0.296 and $0.234\,l\,h^{-1}$ in healthy and hemochromatotic adults, respectively. Once formed in the blood ferrioxamine is rapidly excreted

unchanged in the urine. Its elimination half-life is 5.9 and 4.6 h in normal and hemochromatosis adults, respectively.

Mechanism of Toxicity

Deferoxamine has some serious side effects including infusion rate related hypotension, renal insufficiency, neurotoxicity, growth retardation, and bacterial infections. Deferoxamine may induce venous dilation leading to poor venous return, depressed cardiac output, and eventually hypotension. An acute decrease in glomerular filtration rate and renal plasma flow is the possible mechanism underlying the nephrotoxicity induced by deferoxamine. Depletion of iron, translocation of copper, and chelation of other trace elements including zinc, due to excessive deferoxamine, may interfere with critical iron-dependent enzymes and cause oxidative damage within neural tissue. These are possible mechanisms responsible for deferoxamine-induced neurotoxicity and growth retardation.

The iron–deferoxamine complex, ferrioxamine, is a growth factor for many bacteria and by providing easily utilized iron, deferoxamine has been associated with *Yersenia enterocolitica* overgrowth with prolonged therapy. *In vitro* studies have shown that deferoxamine inhibits the synthesis of prostaglandin, hemoglobin, ferritin, collagen, and DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} of deferoxamine in mice and rats is $>325\,mg\,kg^{-1}$ with intravenous administration and $>1000\,mg\,kg^{-1}$ with oral administration. Deferoxamine-induced hypotension, tachycardia, and renal insufficiency have been also reported in rats and dogs.

Human

Rapid infusion of deferoxamine over 15 min results in hypotension and tachycardia. An infusion rates of $15\,mg\,kg^{-1}\,h^{-1}$ or longer is recommended. Intravenous deferoxamine administration has been reported to cause renal insufficiency indicated by a progressive increase in serum creatinine and decrease in creatinine clearance.

Patients treated with deferoxamine chronically may develop neurotoxicity manifested as visual and hearing losses, growth retardation, and bacterial infections.

Chronic Toxicity (or Exposure)

Animal

Dogs given subcutaneous injections of high-dose deferoxamine developed lens opacities.

Human

Patients with inherited or acquired anemias that require regular blood transfusions frequently have symptoms or laboratory evidence of iron overload. Deferoxamine given subcutaneously at $40\,mg\,kg^{-1}$ over 8–12 h has been the standard of therapy for these patients. Patients receiving higher doses (e.g., $125\,mg\,kg^{-1}$) demonstrated ocular toxicity of blurriness, loss of night vision, and optic neuropathy. Auditory toxicity has been noted as well. Up to 25% of patients on chronic deferoxamine have some impairment of high-frequency hearing.

In Vitro Toxicity Data

Studies of a rat model of intracerebral hemorrhage have noted that deferoxamine treatment reduced oxidative stress from iron release. Iron chelators may have a role in preventing damage associate with strokes.

Clinical Management

For patients who develop hypotension secondary to deferoxamine, the infusion should be discontinued and restarted at a slower rate after recovery of the blood pressure. Fluids and pressors should be used to support the patient. Patients experiencing anaphylactic reactions should discontinue deferoxamine and start treatment with antihistamines and steroids. Patients who develop infections with *Yersinia* species secondary to deferoxamine use should be started on antibiotics (e.g., sulfamethoxasole–trimethoprim) while cultures and sensitivities are pending. Patients experiencing neurologic complications should have the deferoxamine discontinued and a neurologist should be consulted.

See also: Iron; Neurotoxicity.

Further Reading

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Howland MA (1996) Risks of parenteral deferoxamine for acute iron poisoning. *Journal of Toxicology: Clinical Toxicology* 34(5): 491–497.

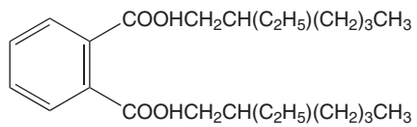
Olivieri NF, Buncic JR, and Chew E (1986) Visual and auditory neurotoxicity in patients receiving subcutaneous deferoxamine infusions. *New England Journal of Medicine* 314: 869–873.

Tenenbein M, Kowalski S, and Sienko A (1992) Pulmonary toxic effects of continuous deferoxamine administration in acute iron poisoning. *Lancet* 339: 699–701.

DEHP (Di-Ethyl Hexyl Phthalate)

Raja S Mangipudy and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 117-81-7
- Synonyms: Di(2-ethylhexyl) phthalate; Dioctyl phthalate; DOP; BEHP; Compound 889; DAF 68; Octyl phthalate; Ethylhexyl phthalate; OCTOIL
- Chemical Structure:



Uses

Di-ethyl hexyl phthalate (DEHP) is a softening agent commonly used in plastics such as polyvinyl chloride (PVC). It is found in products such as telephone cords, kidney dialysis machine tubing, medical plastic bags, shower curtains, vinyl wall coverings, and children's toys.

Exposure Routes and Pathways

DEHP is readily absorbed via ingestion; it is not absorbed significantly via dermal contact. Inhalation exposure is not likely because of the compound's low vapor pressure.

Toxicokinetics

DEHP is absorbed by the oral and parenteral routes. It is not absorbed in any significant amount through intact skin. Intravenously or orally administered DEHP is rapidly metabolized to derivatives of mono-(2-ethylhexyl) phthalate (MEHP). The MEHP metabolites are mainly excreted in the urine and the bile. The estimated half-life of DEHP in humans following intravenous administration is 28 min.

Mechanism of Toxicity

DEHP is a peroxisome proliferator, so named because it causes extensive proliferation of hepatic peroxisomes in susceptible species. As peroxisome proliferators are uniformly carcinogenic in rats and mice, there is concern as to whether this effect is rodent specific or whether it can manifest in other species, including humans via drugs used in long-term therapy or through blood transfusion bags.

Acute and Short-Term Toxicity (or Exposure)

Animal

The maternal–fetal transfer of DEHP and its major metabolite, MEHP, has been demonstrated in rats, and associated with the inhibition of brain and liver steroidogenesis in exposed offspring. In female rats, DEHP administration can suppress estradiol levels and ovulation. A possible mechanism for this effect is the inhibition of the enzymatic formation of estradiol by MEHP.

The oral administration of DEHP to pregnant mice on days 0–17 of gestation can cause exophthalmia and exencephaly, as well as malformations of the tail, major vessels, ribs, and vertebrae. Similar teratogenic effects were observed when larger doses of DEHP were fed to pregnant mice on gestational days 6, 7, 8, 9, or 10. Although in sufficient doses DEHP is fetotoxic in rats, several studies did not find DEHP to produce an increase in malformations in rats at any dose tested and a 1984 summary source found available data insufficient to conclude that DEHP increases congenital malformations in this species. A detailed analysis of the developmental toxicity of DEHP in rats, completed in the fall of 2000, reviewed many of the more recent studies and found minimal indications of congenital malformations in the absence of maternal toxicity, except for effects on genital development in males. In rats, a maternal dose of 750 mg DEHP per kilogram per day during late gestation reduced testosterone levels in male offspring and reduced anogenital distance by an average of 36%. The authors of this report did not find evidence that DEHP interferes with the binding of testosterone to the androgen receptor, but suggested that these effects were a result of the testicular toxicity of DEHP (see below).

An evaluation of the developmental effects of DEHP by the National Toxicology Program estimated that the no-observed-adverse-effect level (NOAEL) for DEHP in sensitive species to be 44 mg kg^{-1} day^{-1}, more than 500 times the estimated daily human intake. A more recent estimate found a similar wide disparity between typical oral exposures to DEHP in humans and an NOAEL in rodents of 4–14 mg kg^{-1} day^{-1}.

The testicular toxicity of DEHP and other phthalic acid esters is well documented in various animal species. The monoester (MEHP) appears to be the active toxicant and young animals appear more susceptible than older animals. Human testicular toxicity from these compounds has not been demonstrated but concern has been registered about the potential for testicular toxicity in male infants who are exposed to procedures involving PVC tubing, such as extracorporeal membrane oxygenation. Estimates for DEHP exposure in these children are imprecise, but range up to 30 mg kg^{-1} day^{-1}. The concern about this level of exposure in neonates was addressed in part by a study of neonatal male rats treated for 3 weeks with either intravenous or oral DEHP. The intravenous doses were 60, 300, and 600 mg kg^{-1} day^{-1}, and the oral doses were 300 and 600 mg kg^{-1} day^{-1}. There were no adverse effects of 60 mg kg^{-1} day^{-1} intravenously. The 300 and 600 mg kg^{-1} day^{-1} doses were associated with decreased testicular weight and associated histologic changes consistent with impaired spermatogenesis. By 90 days of age, after being permitted to recover, the animals that had been treated intravenously had no residual histologic changes, although testis weight had not completely recovered. The orally treated animals had residual decreases in testis weight and abnormal histopathology findings. There were no changes in sperm motility, epididymal and testicular sperm count, or morphology at any dose at any time.

Human

The effects of DEHP on humans have not been established.

Human data on the possible pregnancy effects of intravenous DEHP are limited to a 1981 study of 19 gravid women exposed to hemodialysis for renal failure or drug overdose. These pregnancies resulted in two stillbirths and more than half of the infants were premature and of low birth weight; however, no malformations were observed and neonatal growth was normal. There is no evidence that the adverse outcomes reported were due to DEHP rather than to the medical complications or other drug exposures that occurred during the pregnancies. This report did not specifically monitor or address the likely exposure to DEHP from dialysis equipment in the population studied. Despite an absence of defined embryotoxic effects, the restriction of exposure to DEHP during pregnancy has often been recommended. A specific focus has been placed on the use of freshly drawn blood products for transfusion during pregnancy to avoid the intravenous administration of DEHP, which may reach a concentration of 5 mg dl^{-1} in whole blood when it has been stored in PVC blood bags for 21 days.

More recently, the use of other plasticizers in blood and intravenous fluid delivery systems has obviated concerns associated with DEHP.

Chronic Toxicity (or Exposure)

Animal

DEHP has been shown to produce liver cancer in mice and rats after lifetime exposure.

Clinical Management

The potential for esophageal or gastrointestinal tract irritation following ingestion suggests that emesis should not be induced. Other measures to prevent absorption may be beneficial. Exposed skin and eyes should be copiously flushed. Liver function and blood glucose must be monitored.

See also: Peroxisome Proliferators; Polymers.

Further Reading

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Delaney Clause

Robin C Guy

This article is a revision of the previous print edition article by Shayne C Gad, volume 1, p. 405, © 1998, Elsevier Inc.

Background Information

In 1958, the US Food and Drug Administration's (FDA) Food, Drug, and Cosmetic Act (FD&C Act) was amended to include a clause that essentially banned the use of food additives and pesticides which were shown to cause cancer in humans or animals. The Delaney Clause was contained in Section 409 (348(c) (3) (A)) of the FD&C Act. Section 409 lays out requirements for the use of food additives, including pesticide residues. The Delaney Clause states "no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal."

This clause regulates pesticide residues in processed foods to mean that carcinogenicity potential is the only factor, and that any benefits of the pesticide or food additive may not be considered. In addition, it set up a 'zero-cancer-risk' standard for food additives. If residues of carcinogenic pesticides are found to concentrate in processed foods, the US Environmental Protection Agency (EPA) cannot set a tolerance or maximum legal limit for that pesticide/food combination. Later, Congress added the same zero-cancer-risk clause for amendments governing new animal drugs and color additives (1960 Color Additives Amendment).

The birth of the Delaney Clause can be traced back to a 1950 resolution in the US House of Representatives that charged a House select subcommittee to investigate the use of chemicals in foods. Among the subcommittee's responsibilities was an examination of the 'nature, extent, and effect' of 'chemicals, compounds, and synthetics' on all facets of food production. The subcommittee was chaired by James J. Delaney, a New York Democrat.

While the Delaney Clause prevented the use of possibly dangerous chemicals such as diethyl-stilbestrol (DES), some prospectively useful substances were banned because improved analytical testing procedures were able to detect very small quantities of possible carcinogens. When the Delaney Clause was introduced, analytical testing procedures detected substances in concentrations of parts per million. It later became possible to detect substances in concentrations of one part per billion or trillion, making it far more probable that traces of a carcinogen be detected. Worsening this problem was the fact that tested substances are administered to animals at the maximum tolerated dose, far more than would be normally ingested. The Delaney Clause was criticized by many scientists who believed that its zero-tolerance standard was impossibly high.

The Delaney Clause was eventually replaced with a new law, the Food Quality Protection Act (FQPA) of 1996, which advanced a new standard of 'reasonable certainty of no harm'. Prior to the passage of the FQPA, the FDA had been employing Delaney in the case of food additives and animal drugs in a similar manner, that is, 'reasonable certainty of no harm'. FDA incorporated the idea of safety into its color additive regulations. Currently, under 21 CFR 70.3(i), a color additive is 'safe' if 'there is convincing evidence that establishes with reasonable certainty that no harm will result from the intended use of the color additive'. The EPA was following the zero-tolerance standard. The FQPA ended the application of the Delaney Clause to pesticide tolerance levels. FQPA would allow EPA to determine what level of risk will be adequate to protect the public health as long as the dietary risk posed to food consumers is negligible.

See also: Carcinogenesis; Food Quality Protection Act, US; Pesticides.

Further Reading

Merrill RA (1997) Food safety regulation: reforming the delaney clause. *Annual Review of Public Health* 18: 313–340.

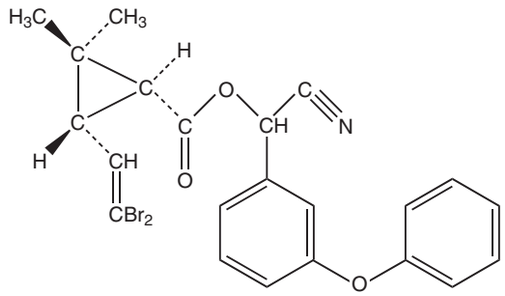
Relevant Websites

http://www.epa.gov – US Environmental Protection Agency (EPA). Environmental laws that establish EPA's authority.
http://vm.cfsan.fda.gov – US Food and Drug Administration (FDA). What are FDA requirements for Food Additives?

Deltamethrin

Paul R Harp

- Chemical Abstracts Service Registry Numbers: CAS 52918-63-5; CAS 62229-77-0; CAS 55700-96-4
- Synonyms: *S*-α-Cyano-3-phenoxybenzyl-(1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate; Butox; Decis; K-Othrine; Kordon; Sadethrin; AEF 032640; NRDC 161; OMS 1998; RU 22974; SHA 209400
- Chemical/Pharmaceutical/Other Class: Type II pyrethroid insecticide
- Chemical Structure:



Uses

Deltamethrin is a broad-spectrum insecticide used in a variety of agricultural applications. It is available as a wettable powder, granule, emulsifiable concentrate, concentrate for ULV application, and as a concentrate for thermal fogging.

Exposure Routes and Pathways

Exposure to deltamethrin has occurred through ingestion, inhalation, and dermal contact.

Toxicokinetics

Pyrethroids are poorly absorbed through the skin and are only moderately absorbed in the gastrointestinal tract. Metabolism of deltamethrin occurs rapidly through ester cleavage and hydroxylation. Deltamethrin is eliminated more slowly from adipose tissues than from other sites such as brain or blood. In one case of dermal exposure, absorption was estimated to be ~3%. Urinary excretion is the primary route of elimination.

Mechanism of Toxicity

Several mechanisms of action have been identified for the pyrethroids with the primary mechanism related to a selective high affinity for membrane sodium channels. Closing of the channel, which ends the action potential, is slowed resulting in a prolonged 'tail' current and repetitive firing of presynaptic and accompanying postsynaptic cells following a single action potential. High enough doses can cause complete depolarization and blockade of nerve conduction. Deltamethrin also inhibits Ca^{2+},Mg^{2+}-ATPase and type II pyrethroids such as deltamethrin have been shown to act on γ-aminobutyric acid-mediated chloride ionophores and voltage-sensitive calcium-independent chloride channels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Symptoms include hyperactivity, incoordination, choreoathetosis, and convulsions.

Human

Extensive dermal exposure causes temporary effects of paresthesia (stinging, burning, tingling) and numbness. Symptoms following ingestion include nausea, vomiting, tenesmus, diarrhea, unconsciousness, and death due to respiratory failure.

Chronic Toxicity (or Exposure)

Animal

Chronic effects following deltamethrin exposure have not been reported.

Human

Chronic effects following deltamethrin exposure have not been reported.

Clinical Management

Exposed skin should be washed promptly with soap and water. Dermal application of vitamin E oil preparations may be used for both prophylaxis and treatment of paresthesia. For contact with eyes, flush immediately and for an extended period with generous amounts of clean water or saline. Gastric lavage is indicated if patient has ingested a large amount of pyrethroids and can be treated soon after exposure.

For ingestion of smaller amounts or if treatment has been delayed, activated charcoal and catharsis are indicated. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenytoin or phenobarbital may be helpful for recurrent seizures. No specific antidotes for pyrethroid-induced neurotoxic effects have been approved for use in humans. Spontaneous recovery usually occurs with mild or moderate intoxication.

Environmental Fate

Deltamethrin degrades in soil within 1–2 weeks. Deltamethrin is rapidly adsorbed primarily by sediment, is rapidly taken up by plants, and evaporates from surface water. No detectable residues exist on plants after $\sim$10 days.

Ecotoxicology

Deltamethrin is toxic to bees. Fish and crustaceans are extremely sensitive to pyrethroid compounds in laboratory settings. However, various factors (e.g., sediment binding) may reduce pyrethroid toxicity to these nontarget organisms in a natural environment.

Exposure Standards and Guidelines

The acceptable daily intake for deltamethrin is $0.01\,\mathrm{mg\,kg^{-1}\,day^{-1}}$. The reference dose is $0.0001\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.

See also: Neurotoxicity; Pesticides; Pyrethrins/Pyrethroids.

Further Reading

Vijverberg HPM (2000) Pyrethroids. In: Spencer PS and Schaumburg HH (eds.) *Experimental and Clinical Neurotoxicology*, 2nd edn., pp. 1028–1044. New York: Oxford University Press.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Deodorants and Antiperspirants

Zhengwei Cai and Pertti J Hakkinen

The main function of sweating is to control body heat. Sweat glands are most numerous in the armpits, groin, and feet, and the numbers have a genetic basis varying from person to person. Sweat is not a significant route for eliminating toxins from the body. In addition to producing sweat, sweat glands have been implicated in being able to secrete sex pheromones and other substances. Some substances in sweat can react with the bacteria normally found in the armpit, and the reaction can produce an unpleasant odor; however, the genetic variation in the ability to smell various substances means that people may perceive the same body odor in different ways.

Reducing perspiration in the underarm does not affect the body's ability to regulate temperature, or the body's ability to excrete substances of any kind. There are many sweat ducts all over the body, and only a small percentage of them are in the underarms. Thus, reducing the flow of perspiration in the underarms does not affect the body's ability to regulate temperature since the sweat glands in other parts of the body can adequately control the overall body temperature.

There are basically two types of deodorants: simple deodorants and antiperspirants. Deodorants help control odor primarily by masking the odor caused by the bacteria interacting with perspiration and by reducing odor-causing bacteria. Deodorants have no effect on decreasing sweat. Antiperspirants help control wetness, and thereby odor, by slowing the flow of perspiration to the surface of the skin.

A simple deodorant consists of an antibacterial agent in a cream base. Antiperspirant ingredients ('aluminum salts') such as aluminum chlorohydrate, activated aluminum chlorohydrates, and aluminum–zirconium–glycine (AZG) complexes work by forming superficial plugs in the sweat ducts, reducing the flow of perspiration. Antiperspirants are available in four product types: cream, liquid, powder, or stick. They usually include aluminum salts, titanium dioxide, oxyquinoline sulfate, zirconium salt, alcohol, and antibacterial agents. Some liquid forms are propellant dispensed (aerosols). Waxes, soap, and humectants may be present in minor proportion in stick forms. Roll-on types may be added with

emulsifiers and thickeners. The amounts of ingredients present in these products are usually small, and unless a large quantity is ingested, no ill effect should ensue.

Deodorant efficacy is evaluated by sensory assessments performed by an expert panel. The sensory assessment by an 'expert panel' can be somewhat misleading since it can involve professional armpit sniffers, a profession classified by some websites as one of the 'jobs your mother may never have wanted you to have' and among the worst possible jobs! Various measurement methods are used to demonstrate the efficacy of antiperspirants, including a gravimetric method, water evaporation quantification, electrodermal measurements, staining procedures, dye injections, and cyanoacrylate skin surface strippings and casting replicas. Other useful methods include visualization of apocrine gland excretion, and the collection of sweat and volatile compounds. Microbiological assessments and chromatographic analysis are also performed.

In case of oral ingestion, the mouth can be rinsed out, and milk can be given for soothing and diluting effect. These products are nonirritating to most people, but sensitization may occur in some individuals. For these people, the preparation should be washed off thoroughly and a substitute brand may be chosen. Discontinuing use of deodorant may be necessary. Shaving the underarms can impact the outer layers of the skin that protect the body, causing susceptibility to cuts, rashes, and various forms of skin irritation. Because of this, some formulations now contain chemicals that help to renew and protect underarm skin. Aerosol products may cause eye irritation. The eyes should be washed carefully with lukewarm water for a few minutes, and soothing eye drops may be helpful.

The (US) Food and Drug Administration has issued a final rule in 2003, in the form of a final monograph establishing conditions under which over-the-counter antiperspirant drug products (including deodorants) are generally recognized as safe and effective. This rule has been in effect from December 2004.

Antiperspirants have been implicated in breast cancer and in the 'aluminum hypothesis' of Alzheimer's disease. However, various experts and health organizations, e.g., the American Cancer Society and (US) National Cancer Institute, have reported that there is no apparent link with breast cancer. Further, expert reviews have found no consistent relationship between Alzheimer's disease and exposures to aluminum-containing medications, antiperspirants, drinking water, or other materials or products.

A sudden change in sweat production can signal other problems ranging from damage to the autonomic nerves controlling the sweat pores, to various hormonal disorders and obesity. A high level of sweating is called hyperhidrosis, and botulism toxin injections have been used to control hyperhidrosis for several months at a time. Another approach used to control hyperhidrosis is endoscopic thoracic sympathectomy, which involves severing the nerves controlling the hyperactive sweat glands.

See also: Aluminum; Cosmetics and Personal Care Products.

Further Reading

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http://www.ctfa.org – US Cosmetic, Toiletry, and Fragrance Association (CTFA)

http://cis.nci.nih.gov – US National Cancer Institute, Cancer Facts on Antiperspirants/Deodorants and Breast Cancer.

http://hpd.nlm.nih.gov – US National Library of Medicine, "Household Products Database"; and "ToxTown." The Household Products Database links several thousand US consumer brands to health effects from Material Safety Data Sheets (MSDSs) provided by the manufacturers, and allows scientists and consumers to research products based on chemical ingredients.

http://www.heraproject.com – The Human and Environmental Risk Assessment (HERA) project.

Deoxyribonucleic Acid *See* Aneuploidy; Carcinogen–DNA Adduct Formation and DNA Repair; Chromosome Aberrations; DNA Phosphoramidites; Genetic Toxicology; Genomics, Toxicogenomics.

Dermal Toxicity Testing *See* Toxicity Testing, Dermal.

Desferrioxamine *See* Deferoxamine.

Desipramine *See* Tricyclic Antidepressants.

Detergent

Zhengwei Cai and Pertti J Hakkinen

Detergents are various surface-active agents (surfactants) particularly effective in dislodging foreign matter from soiled surfaces and retaining it in suspension. Soap, which is made from fats or fatty acids, is a detergent. However, in common usage the term 'detergent' applies to the synthetic nonsoap *substances*, not to soap, and also to *products* made from synthetic surfactants.

Surfactants and builders are the major components of cleaning products, with the builders serving to enhance or maintain the cleaning efficiency of the surfactants, primarily by reducing the water hardness. Other ingredients are added to formulations to provide functions such as increasing cleaning performance for specific soils/surfaces, ensuring product stability, and supplying a unique identity to a product. Examples include foam stabilizers, optical brighteners or whiteners, anti-redeposition agents, bleaching agents (chlorine-releasing agents) or bactericidal agents (mild concentrations of quaternary ammonium compounds), enzymes, fragrances, and dyes. Water is likely to be the major component of a liquid version of a detergent product.

Soaps and detergents are important for personal and public health. The (US) Soap and Detergent Association has noted that, through their ability to loosen and remove soil from a surface, soaps and detergents can (1) contribute to good personal hygiene, (2) reduce the presence of germs that cause infectious diseases, and (3) extend the useful life of clothes, tableware, linens, surfaces, and furnishings.

Soaps and detergents found in the home can be grouped into four general categories: personal cleansing, laundry, dishwashing, and household cleaning. The surfactants used in detergents have been developed to perform well under a variety of conditions, and are less sensitive than soap to the hardness minerals in water. Most surfactants will not form a film. Detergent surfactants were developed in response to a shortage of animal and vegetable fats and oils during World War I and World War II. In addition, a substance that was able to perform in hard water was desired to make cleaning more effective. Petroleum was used for the manufacture of these initial surfactants since it was widely available; however, detergent surfactants are now made from a variety of petrochemicals (derived from petroleum) and/or oleochemicals (derived from fats and oils).

Surfactants are usually classified by their ionic properties in water. Anionic surfactants are used in laundry and hand dishwashing detergents, household cleaners, and personal cleansing products. Linear alkylbenzene sulfonate, alcohol ethoxysulfates, alkyl sulfates, and soap are common anionic surfactants. Nonionic surfactants are low sudsing, and are typically used in laundry and automatic dishwasher detergents and rinse aids. The most widely used nonionic surfactants are alcohol ethoxylates. Cationic surfactants are used in fabric softeners and in fabric-softening laundry detergents. Other cationics are the disinfecting/sanitizing ingredients used in some household cleaners. Quaternary ammonium compounds are the major cationic surfactants. Amphoteric surfactants are used in personal cleansing and household cleaning products for their mildness, sudsing, and stability. Imidazolines and betaines are major amphoteric surfactants.

Even the different product types within a category of detergents are formulated in different product forms and with different ingredients selected to meet consumer desires for a selection of product types, to perform a broad cleaning function, and to deliver properties specific to that product. For example, laundry detergents and laundry aids are available as liquids, powders, gels, sticks, sprays, pumps, sheets and bars. They have been formulated to meet a variety of soil and stain removal, bleaching, fabric softening and conditioning, and disinfectant needs

under varying water, temperature, and use conditions. Further, the laundry detergents are either general purpose or light duty, with general-purpose detergents being suitable for all washable fabrics, and liquids working best on oily soils, and for pretreating soils and stains. Light-duty detergents are used for hand or machine-washing lightly soiled items and delicate fabrics.

Water alone will not remove oily, greasy soil on clothing since the oil and grease repel the water molecules; however, a surfactant's hydrphobic end is attracted to the oil and the hydrophilic end is attracted to the water molecules. These opposing forces loosen the soil and suspend it in the water. Warm or hot water helps dissolve grease and oil in soil. The agitation of the water and clothing in a washing machine, or rubbing clothing with the hands or an implement helps to pull the soil free from the clothing.

Exposure and risk assessors, and the developers of consumer products need to understand the reasonably foreseeable ways that consumers will use the products. Even a task as simple as dispensing a laundry detergent powder from its box into the washing machine could be done in several different ways by a consumer, resulting in different types and magnitudes of potential exposures. For example, the laundry powder could be poured from different heights above the washing machine, directly into the washing machine from the box, from the box into a measuring cup, etc. These and other possible differences in just how the product is dispensed could lead to meaningful differences in the types and magnitudes of inhalation and skin exposures to the powder.

Further, exposure and risk assessors, and the developers of consumer products need to understand the reasonably foreseeable ways that consumers will use a product in combination with other products. For example, laundry detergents are often used in combination with other products that might be useful for particular needs. For example, laundry aids contribute to the effectiveness of laundry detergents and provide special functions. Boosters enhance the soil and stain removal, brightening, buffering, and water softening performance of detergents, and are used in the washing machine in addition to the detergent. Enzyme presoaks are used for soaking items before washing to remove difficult stains and soils. Fabric softeners are added to the final rinse as a liquid, or to the clothes dryer on a nonwoven sheet. Pre-wash soil and stain removers are used to pretreat heavily soiled and stained garments. Starches, fabric finishes, and sizings are used in the final rinse or after drying. Water softeners are added to the wash or rinse to inactivate hard water minerals and increase cleaning power since detergents are more effective in soft water. Bleaches (chlorine and oxygen) are used to whiten and brighten fabrics and help remove stubborn stains, and liquid chlorine bleach (e.g., a sodium hypochlorite solution) can disinfect and deodorize fabrics.

In addition to laundry products, detergents are used in dishwashing products for hand and machine dishwashing. They are available as liquids, gels, powders, and solids. Further, many types of household cleaning products are available for consumers because no single cleaning product can provide optimum performance on all surfaces and soils. Thus, a broad range of products has been formulated to clean efficiently and easily, including liquids, gels, powders, solids, sheets and pads for use on painted, plastic, metal, porcelain, glass and other surfaces, and on washable floor coverings.

Human Safety

Human safety evaluations begin with the specific ingredients, and then move on to the whole product. The effects for all ingredients are considered as the product is formulated. Human safety-related data for a chemical used in a detergent or soap product (or in another type of consumer product), and for an entire formulation, can come from *in silico* data (from computer programs that estimate toxic properties based on data for similar chemicals, and/or from the physical chemical properties of the chemical of interest), *in vitro* data (from the results of 'alternatives to animal' tests, e.g., from cell cultures used to assess eye or skin irritation potential), animal (toxicological) studies (e.g., to assess eye or skin irritation potential), and human data (examples are discussed below).

The human data include premarketing (i.e., before a product has begun to be sold to consumers) clinical and 'controlled use' studies of the entire formulation. Further, the human data could include post-marketing (i.e., after a product has begun to be sold to consumers) studies conducted by physicians or dermatologists, and epidemiological studies developed by Poison Control Centers, companies, academia, etc.

Examples of human testing that may be very useful in the safety evaluation of detergents and other consumer products include human clinical studies, e.g., patch tests to confirm the absence of meaningful human skin irritation potential predicted from *in vitro* and any animal studies. Possible human studies also include 'controlled use' studies, e.g., from studies designed to assess the skin effects from wearing a type of fabric laundered with a new detergent formula. Further, examinations of the 'real-world' experiences consumers have had using a product are very helpful in confirming the absence of meaningful safety issues, or

could lead to changes in product composition, labeling, package design, etc., if the risk assessor judges that the data indicate a need to refine the product to lower risks. As noted above, these real-world data can come from human epidemiological studies and other studies developed by Poison Control Centers, companies, academia, and others to look at the health effects associated with the use of a consumer product under reasonably foreseeable conditions.

Even though manufacturers formulate and package their cleaning products to ensure that they are safe or have very low risk, human health effects can still result from normal uses and unintended exposures. To warn consumers about a specific hazard, household cleaning products carry cautionary labeling whenever necessary, e.g., CAUTION or WARNING or DANGER, along with first aid instructions. A laundry product label might look like:

Caution. Eye irritant. Harmful if swallowed. KEEP OUT OF REACH OF CHILDREN. If swallowed, give a glassful of water. Call a physician. In case of eye contact, flush with water.

The manufacturer's safety data and material safety data sheet (MSDS) supporting this labeling might indicate the following:

> Acute Health Effects:
>
> Inhalation: Transient irritation with prolonged exposure to concentrated material.
>
> Ingestion: May result in nausea, vomiting, and/or diarrhea.
>
> Eye Contact: May cause stinging, tearing, itching, swelling, and/or redness.
>
> Skin: Prolonged contact with concentrated material may be drying or transiently irritating to skin.

In addition, companies marketing detergents, soaps, and other products tend to work closely with poison control centers to assure that, should an accidental exposure occur, treatment information is available to health care providers and concerned parents.

While most laundry detergents are not strong enough to do significant harm, some laundry products, automatic dishwashing detergents, wall cleaners, drain or oven cleaners, disinfectants, and ammonia can cause extensive injury. In addition, extensive eye and skin exposure to some detergents may also cause toxic effects. Further, product interactions might occur. For example, the mixing of a toilet bowl cleaner or any acid with a chlorine-type bleach may produce chlorine gas, causing respiratory irritation with coughing, labored breathing, and inflammation of the eyes and mucous membranes, and the addition of ammonia to bleach produces a toxic gas, chloroamine. As the title of one risk communication book states, 'Read the label', especially if the product is a new one for a consumer.

Occupational Safety

Occupational allergy and occupational asthma were safety issues many years ago with the manufacture of detergents. However, comprehensive pre-clinical, clinical, and industrial hygiene programs have been developed to successfully control allergy and asthma to enzymes used in the detergent industry. The detergent industry has developed guidelines for the safety assessment of enzymes, control of exposure to enzymes, and medical surveillance of enzyme-exposed workers, and occupational allergy and asthma to enzymes in the detergent industry have become uncommon. The cases that have been documented in some manufacturing sites have had poor adherence to the guidelines. Those manufacturing sites that have adhered to the guidelines have had few cases of allergy and asthma to enzymes among exposed workers. Further, reviews of medical data from these sites have shown that workers who have developed IgE antibody to enzymes can continue to work with enzymes and remain symptom-free. The basic principles of these programs can be applied to other industries where occupational allergy and asthma to proteins are safety issues.

Environmental Safety

Most household cleaning products are formulated to be used with water and 'go down the drain' into wastewater treatment systems (municipal sewage treatment plants or septic tank systems). To assure that these types of products are safe for the environment, manufacturers evaluate the impacts of product ingredients in wastewater treatment systems, streams, rivers, lakes, and estuaries. Environmental risk assessment considers the exposure concentrations and effects of individual ingredients.

Two sets of information are used in these assessments. One set enables industry scientists to predict the concentration of the ingredient from all sources, including cleaning products, at various locations in the environment (the predicted exposure concentration). The other set is used to find the highest concentration of the ingredient at which no harm will occur to animals, plants or microorganisms living in the environment, i.e., the no-effect concentration. Comparing the predicted exposure concentration and the no-effect concentration enables scientists to determine whether the use of an ingredient is safe for the environment.

An example of an environment issue for detergents is the finding that high levels of phosphates in detergents discharged into water systems can lead to a build-up of nutrients that results in a large amount of algae and water plant growth (a complex process called eutrophication). Public, academic, and government concerns have led to adverse publicity, to legislation banning the use of phosphate detergents, and to the development of nonphosphate versions of products.

Environmental Quality

The (US) Soap and Detergent Association has noted that manufacturers of cleaning products have been leaders in reducing packaging waste and encouraging sound waste disposal practices. For example, "Advances in technology have resulted in products that are more concentrated, products that combine two functions in one, products with refill packages and packages that use recycled materials. Concentrated products need less energy to manufacture and transport, and require less packaging. Multifunctional products eliminate the need for separate packages. Refill packages allow consumers to reuse primary packages many times, decreasing the amount of packaging used and the volume of trash generated. Plastic and paperboard that would otherwise be thrown away become usable materials through recycling."

Finally, life cycle assessment (LCA) is being used to improve the environmental quality of detergents. LCA provides a 'cradle-to-grave' or 'cradle-to-cradle' evaluation of the environmental impacts of a product and its package, usually all the way from acquiring the raw materials, manufacture and distribution, and consumer usage and disposal. The use of LCA can help assess whether reducing an environmental impact in one area, e.g., manufacturing, moves the impact to disposal or another area. LCA also helps highlight where environmental improvement efforts should be focused.

See also: Alkalies; Consumer Products; Exposure Assessment; Life Cycle Assessment; Poisoning Emergencies in Humans; Surfactants, Anionic and Nonionic.

Further Reading

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Development Toxicity Testing *See* Toxicity Testing, Developmental.

Developmental Toxicology

Calvin C Willhite and Philip E Mirkes

The Problem of Birth Defects

Two-third of all infant deaths occur in the first 27 days of life. Congenital malformations and chromosome abnormalities account for 20% of all infant deaths, and infants born too small (<2.5 kg) or too soon (<37 weeks gestation) have much higher risk of death than those born at term. For the year 2002, the five leading causes of the 27 970 infant deaths in the United States were:

1. congenital malformations and chromosome abnormalities (5630 deaths);

2. premature birth and low birthweight (4686 deaths);
3. sudden infant death syndrome (2295 deaths);
4. maternal complications (1704 deaths); and
5. placental and membrane complications (1013 deaths).

In 2002, the three leading causes (congenital malformations/genetic defects, low birthweight, and sudden infant death syndrome) were responsible for 45% of all infant deaths. In 2001, United States infant morality was 6.8 per 1000 live births and this death rate increased to 7.0 per 1000 live births in 2002.

Non-Hispanic Black and American Indians experience the highest infant mortality rates. In 2002, infant death ranged from a low of 3.0 per 1000 live births to Chinese mothers to a high of 13.8 for Black mothers. For the years 1995–2002, infant mortality rates for Black mothers ranged from 13.3 to 14.6 per 1000 and those for American Indians ranged from 8.3 to 10.0 per 1000. In contrast, rates for Whites over those same years were 5.7–6.3 per 1000.

Infant mortality varies by location as well as race. Southern states have higher rates. Rates are lowest in Western and Northeastern states. Infant mortality is higher in Mississippi (10.5 per 1000 live births) than in Massachusetts (4.8 per 1000 live births). Between 2001 and 2002, the highest total rate was that in Washington, DC (11.4 per 1000 live births). Infants born to non-Hispanic Black mothers in Wisconsin experienced a rate of 17.9.

Other well-known factors also contribute to elevated risk of infant death. United States infant mortality rates increased significantly between 2001 and 2002 for teenage mothers (10.7–11.5). Infant mortality rates also increased for mothers who smoke tobacco (10.5–11.1). In 2002, infant morality was 68% higher for smoking mothers (11.1) than for mothers who did not smoke during their pregnancy (6.6).

Nearly 50% of the annual hospital charges ($29.3 billion) in the United States for delivery and neonatal care are associated with prematurity. Hospitalization costs for a normal delivery average $1300 where costs where for premature infants average $75 000. These initial charges do not include the public and private health care costs for the 25% of these infants who survive with blindness, cerebral palsy, and other chronic conditions.

The science of teratology (a word coined in 1832 by Geoffrey Saint-Hilares as literally 'the study of monsters') has a history predating that of medicine as we know it today. The contemporary definition of teratology is 'the science dealing with the causes, mechanisms, and manifestations of a structural or functional nature of abnormal prenatal development'. Teratology can be considered a subdivision of developmental biology. Developmental toxicology encompasses embryonic and fetal death, reduced fetal growth, and other manifestations of abnormal development brought on by exposure to xenobiotics (literally 'foreign chemicals'). Virtually all chemical compounds (including common sugars like glucose or normal amino acids like phenylalanine) can induce embryotoxicity and fetotoxicity if the dose given is sufficiently large and the time and duration of exposure in pregnancy is appropriate. Maternal disease like diabetes and phenylketonuria can predispose a patient to an abnormal pregnancy outcome. Nevertheless, a compound is not usually considered a teratogen (a chemical that causes birth defects) if the dose required causes maternal poisoning in animal studies. Human teratogens can be seen where there is frank intoxication; there is no better example of this than ethanol in alcoholic mothers and their offspring who exhibit features of the fetal alcohol syndrome (FAS). Thus begins the dilemma for the clinician, the teratologist, the government regulator, and the family of an affected child – teratogens may seem to be everywhere, but on close inspection they seem to be nowhere. The birth of a malformed child has always been a matter of intense concern and sorrow; the same question always follows: 'What caused it?' Ancient peoples formulated their own explanations on the cause(s) of these diseases and some remnants of their hypotheses are still with us today. As we will see in the following discussion, we have advanced from stoning or cremation of mothers of infants with birth defects. However, we have replaced the hypotheses of antiquity (termed superstition today) with more subtle (but in some instances equally preposterous) and damaging ideas in our search for responsible agents and parties.

It is often said that the cause of a particular birth defect is 'multifactorial', generally taken to mean that it is the interaction of one or more environmental agents (a drug, a hazardous waste site, or a drinking water contaminant) with the genetic makeup (genotype) of the mother and her embryo. This notion of multifactorial causation stems from the writings of the French surgeon Ambroise Pare (1510–90) in *Chyrurgery* (1579) recounting the influence of maternal impressions (see below), demonic intervention, and environmental or mechanical factors. Actually, until the thalidomide tragedy of the twentieth century, it was generally accepted that the embryo was well protected and that the placenta functioned as a 'barrier', which insulated the conceptus from noxious agents. Today, the multifactorial explanation is either applied correctly, when describing the interplay between a susceptible genotype (e.g., inborn errors of metabolism) and exposures of interest or out of

frustration on the part of teratologists, obstetricians, or pediatricians, who cannot otherwise account for the empirical observations at hand. This tendency taken together with the oft held public view that certain birth defects are inevitable, the result of random chance, or 'God's will', lead to dismay that these conditions can ever be 'cured' or prevented. In contrast, the frequency of these conditions and the natural desire to ascribe one or another particular factor or agent (e.g., a drug or a workplace chemical or practice) as the cause leads today to significant legal and financial consequences that, depending on the particular circumstance, may or may not be warranted. The science of teratology has at its core the emotional distress that accompanies the birth of a malformed child. This factor is neither lost in tort adjudication nor lost in the nation's abortion (right to life) debate. Professional scientific societies, such as the Teratology Society and the American College of Obstetricians and Gynecologists, are dedicated to the study and prevention of birth defects. Over the past 20 years, it has become clear that a great many of these common, costly, and deadly conditions can indeed be prevented. It is surprising that prevention can sometimes be accomplished by simple and inexpensive steps once the etiologic agent(s) has been identified.

Generally speaking, developmental toxicology focuses on abnormal morphogenesis induced by xenobiotics; however, a discussion of the extent or nature of birth defects must consider other possible causes – infectious microbes, abnormal chromosomes, radiation, hormones, maternal disease, and nutritional status. These factors must be added to the 'normal' or 'background' rate of embryonic demise known from the classic studies of Hertig AT and Rock J carried out during the middle of the last century in couples of proven fertility under optimal conditions for pregnancy. In these studies, 15% of the oocytes failed to fertilize, 15% of the fertilized oocytes started cleavage but failed to implant, and of the 70% that implanted 58% survived. Of those surviving, 16% were abnormal.

Embryos die for any number of reasons (e.g., degeneration of the corpus luteum or a defective trophoblast) and they are aborted spontaneously with the next menstrual period – usually without producing any of the maternal signs associated with pregnancy. Thus, by the end of the first expected menstrual period more than one-half of all human eggs exposed to sperm under the best of conditions die for one reason or another.

The biological context of the word 'development' covers the changes from conception through birth, neonatal life to adulthood, and to old age. The word is restricted here, however, to embryonic and fetal life ranging from subtle changes detectable only in studies of children or young laboratory animals to embryonic or fetal death. A brief discussion of functional delay or deficit (commonly referred to as behavioral teratology) is presented. It is these functional or behavioral deficits that represent insidious to overt manifestations of developmental toxicology.

To understand the causes and pathogenesis of congenital malformations, one must possess at least a working knowledge of embryology as can best be gained from completion of an undergraduate course in the subject or as is commonly taught in medical school anatomy. For purposes of the current discussion, a rudimentary understanding of biology and mammalian embryology is assumed.

Historical Lessons

While it may seem obvious, birth defects are not new. In the vast majority of cases, birth defects and their causes cannot be linked to modern consumer products, occupational exposures, therapeutic or recreational drugs, or environmental pollutants. Congenital defects are perhaps the greatest source to have influenced the myths of antiquity (second only to belief in divinity or the study of the heavens), fairy tales of Rumpelstiltskin and other dwarfs, elves and hunchbacks or otherwise twisted (arthrogryposis, torticollis, and scoliosis) trolls, or contemporary book and film scripts of the macabre.

Cyclops are first recorded as subterranean beings who serve Hephaestus (Sanskrit Yavishta and the Vedic god of fire), the Greek divine blacksmith. The sons of Uranus and Gaea (Arges, Steropes, and Brontes) are all cyclops. These cyclops forged the trident for Poseidon and the bronze helmet for Hades and were then killed in furious revenge by Apollo. The cyclops of Homer's *Odyssey* (Polyphemus, who Ulysses blinded with a sharpened, burning stake driven into his eye) inhabited the southwest coast of Sicily and lived in caves, killing and devouring any stranger who chanced upon them. According to Callimachus, the cyclops Brontes, Steropes, Acamas, and Pyracmon, who lived on Mount Etna (the active volcano near the Sicilian city of Taormina on the Ionian Sea), were

> *Enormous giants, big as mountains and their single eye, under a bushy eyebrow, glittered menacingly. Some made the vast bellows roar, others, raising one by one their heavy hammers struck great blows at the molten bronze and iron they drew from the furnace.*

One of the colonial American explanations regarding the etiology of cyclopia was hybridization

between species. The birth of a cyclopic infant or farm animal (whose mother lived near a person with features thought to resemble that of the malformed newborn) was suspect. In the *Records of the Colony and Plantation of New Haven* (1638–48), the story of one such unfortunate neighbor is recounted, Mr. George Spencer, who happened to live near a sow who gave birth to a cyclopic pig that had 'but one eye in the middle of the face'. The jury concluded that Mr. Spencer who 'had but one eye…the other hath (as it is called) a pearle in it' was guilty of bestiality. The 'pearle' apparently bore some superficial resemblance to the cyclopic pig's eye. The sow was 'slaine in his sight, being run through with a sworde' and poor Mr. Spencer was put to death for his crime on April 8, 1642.

Cyclopia is, of course, the most severe manifestation of holoprosencephaly – a condition in which the embryonic forebrain fails to separate into right and left hemispheres. The etiologic agent(s) of human cyclopia is not known. In ruminants (sheep, cattle, and goats), however, ingestion of the plant *Veratrum californicum* on even a single day (e.g., day 14 in ewes) reliably produces the condition. Subsequent investigations confirmed the presence of a teratogenic alkaloid, 11-deoxojervine, in the plant. That compound is termed most appropriately cyclopamine. Cyclopamine illustrates one of the many factors that must be taken into account for interspecies extrapolation of teratology data. Cyclopamine and its congeners cannot be held accountable for human cyclopia. Cyclopamine is not teratogenic in monogastric animals (e.g., rabbits) because it is degraded by stomach acid to an inactive compound (called veratramine). Nevertheless, when cyclopamine was fed to pregnant rabbits along with sufficient alkali so as to reduce stomach acid, cyclopamine was definitely teratogenic and, in fact, induced cyclopia.

Another example of historical explanations for birth defects is the theory of maternal impression. For better or worse, such theories find their way into laws, regulations, and even (in a modified fashion) into litigation. The eighteenth-century Philadelphia surgeon, Dr. John Morgan, described the birth of a 'piebald Negro girl with splotches all over her body' – a condition attributed to her mother's habit of evening star watching. Sirenomelia and ptergium colli were attributed to the mother seeing a snake or cobra during pregnancy and anencephalus to the mother looking at monkeys. Pregnant Spartan women were legally required to concentrate on statues and pictures of beautiful gods and warriors so as to ensure strong and healthy babies. One historical account involves an Italian nobleman whose wife happened to employ a black male servant. One day during her pregnancy, she attended a cultural exhibit at the local museum. After gazing at a portrait of a Moor, she subsequently gave birth some time later to a mulatto. The nobleman protested and the legislature responded, of course, by passing a law to the effect that pregnant women were to be banned from visiting art museums.

Although it may seem odd to consider it so, even structurally normal identical (monozygotic) twins can be considered a developmental aberration. Thirty percent of the 1.08–1.36% of the normal incidence of twin births in the United States are monozygotic. The factors responsible for splitting of the single fertilized egg (zygote) into two blastocysts (each with its own inner cell mass or embryo proper) are – like the vast majority of other developmental anomalies – unknown, but maternal genotype is a predominant contributor. The stage at which splitting occurs determines whether the embryos develop each with their own placenta and amniotic cavity, whether the embryos develop having a common placenta and separate amniotic cavities, or whether the embryos share a common placenta and a common amniotic cavity. Failure of complete separation of the inner cell mass results in any of a variety of conjoined (Siamese) twins (diploterata or 'double monster').

The term 'Siamese twins' was first coined by the nineteenth-century circus king, Barnum PT, in reference to Eng and Chang Bunker, who were joined at the sternoxiphoid. (After retiring from the circus, Eng and Chang farmed in North Carolina and married at age 44, fathered a total of 22 normal children by two sisters, and died of arteriosclerosis in 1874 at age 63 – within 2 h of one another.)

Conjoined twins can be joined at the chest (thoracopagus), lower spine (pygopagus), or skull (craniopagus), with the latter being variable in fusion at the dorsal (occipital craniopagus), parietal, or ventral (syncephalus frantalis) aspect. Symmetrical conjoined twins occur about once in every 50 000 births and craniopagus twins are born about once in 3 million births (one in every 58 cases of conjoined twins).

The most extreme occipital craniopagus (janiceps) is Janus, the Roman god of gates and doorways represented artistically with his double faces (*Janus bifrons*) each in opposite directions so as to observe the interior and exterior, the entrance and exit, of public buildings. Janus arose from the god Chaos when earth, air, fire, and water took form during the creation of the world. His two faces represent his confusion in his initial state; thus, not only was Janus the god of departure and return but also he was the god of daybreak and new beginnings. Janus was revered even more than Jupiter and was honored on the first

day of every month. The first month of the year (Januarius) still bears his name.

Dicephalic (double-headed) monsters populate not only 1950s matinee movies but also appear in sculpture, drawings, and carvings throughout history – Catal Huyuk (6500 BC) in southern Turkey and the South Pacific (dicephalus dibrachius), clay figurines in Mexico and South America (500 BC–800 AD), and figures on Babylonian clay tablets found near the Tigris (Cuneiform Texts from Babylonian Tablets, British Museum, London).

Neural Tube Defects

The belief that Satan, witches, sorcerers, and other diabolic and demonic forces were responsible for congenital malformations was prevalent during the fifteenth and sixteenth centuries, and this belief found its way to the new world. Not that these concepts were new – far from it. A mummified anencephalus – a condition considered the most severe malformation compatible with intrauterine life (**Figure 1**) – was discovered in 1825 at the Egyptian catacombs of the Hermopolis sarcophagus. This individual's area cerebrovasculosa (rudimentary brain) had been ceremonially removed through the nose, as was the custom. Based on the mummy's location and condition, and the inscription on the sarcophagus, the malformed individual was considered the product of fornication between the mother and an ape. This anencephalus was brought to the Berlin Museum by the King of Prussia and was unfortunately destroyed in the Second World War. By the 1600s, mothers of anencephalics were condemned in ways not dissimilar to the execution of witches in Salem, Massachusetts. One theory as to the origin of anencephalics was consanguinity with a troll – particularly dangerous being those who lived near roadways or under bridges – whose sons and daughters resembled their fathers.

Anencephalus is one of a constellation of malformations known collectively as neural tube defects (NTDs). Anencephalus is the end result of failure of neural fold elevation and fusion, a deficiency that can occur only in the most anterior region (**Figure 1**), along the entire axis (craniorachischisis totalis; **Figure 2**), or localized in areas along the spine (spina bifida; **Figure 3**). Spina bifida is a common

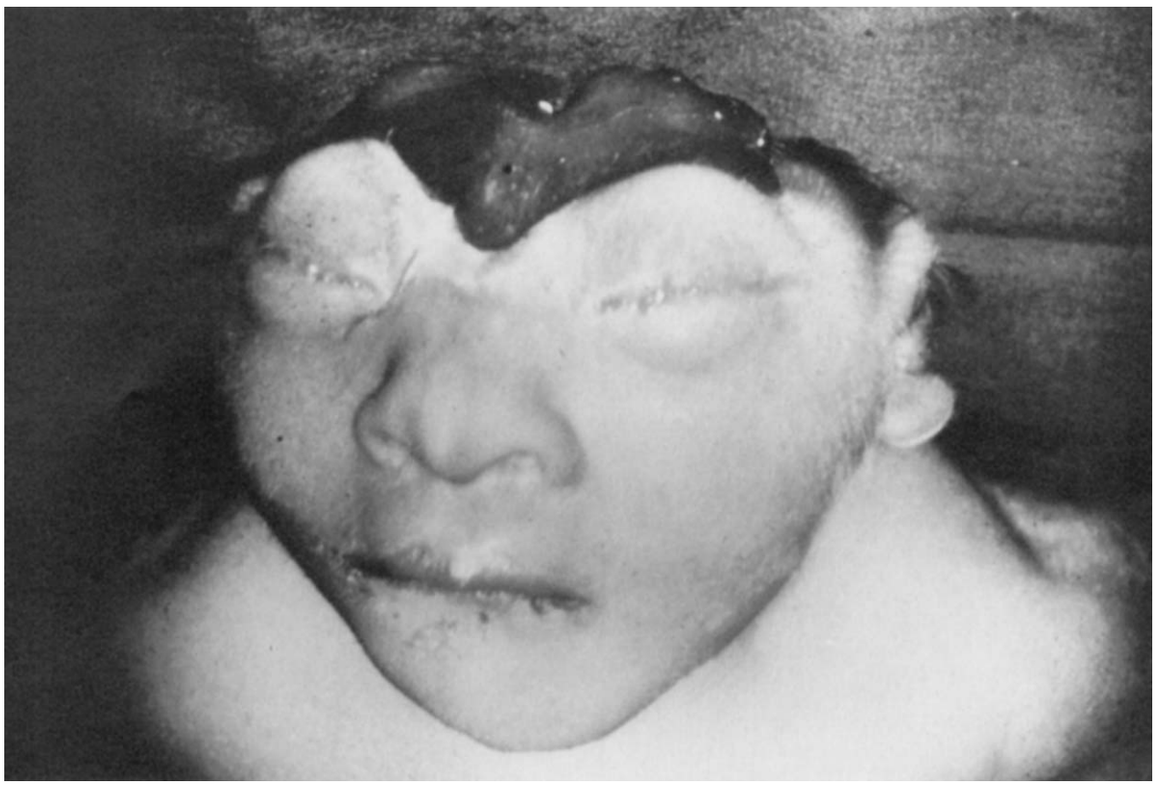


Figure 1 Anterior view of an anencephalic human fetus. Notice the low-set ears, elevated nose and maxilla, the short neck (due to anomalies of the cervical vertebrae), and the prominent, protruding rudimentary brain. (Reproduced from Marin-Padilla M (1991) Cephalic axial skeletal-neural dysraphic disorders: Embryology and pathology. *Canadian Journal of Neurological Sciences* 18: 153–169, with permission.)

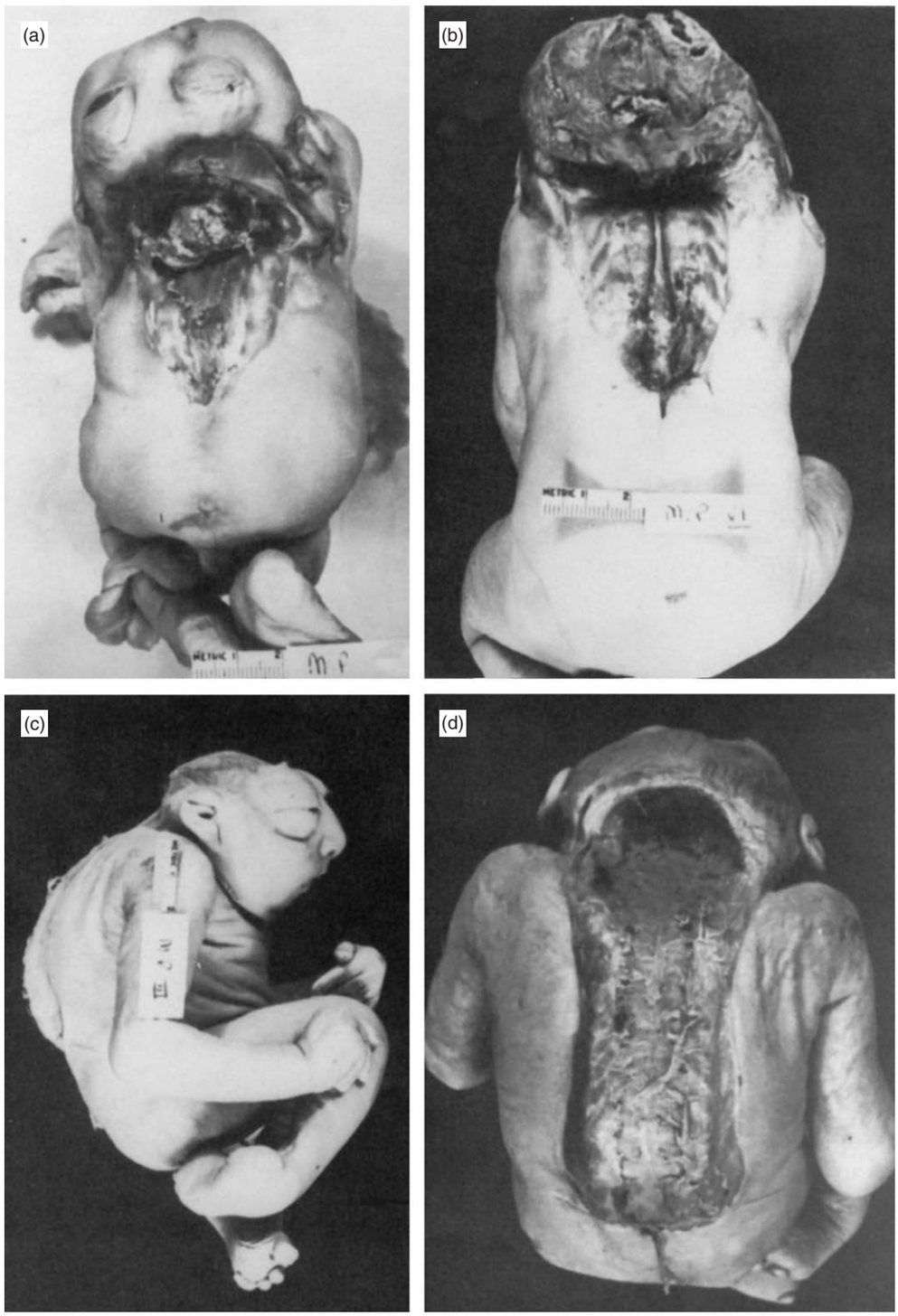


Figure 2 External appearance of various types of human craniorachischisis totalis (total myeloschisis) illustrating the severity of the dysraphic disorders. The first fetus (a) illustrates the severity of the lordosis and the shortness of the axial skeleton which can occur in these disorders. The exposed areas of the central nervous system are totally destroyed. In (b), note the exencephalic brain (termed area cerebrovasculosa). (c, d) Lateral and posterior view. Compare (c) with **Figure 1**. In (d), the destroyed areas of brain and spinal cord tissues have been removed to show the severity of the malformations of the vertebrae. (Reproduced from Marin-Padilla M (1978) Clinical and experimental rachischisis. In: *Congenital Malformations of the Spine and Spinal Cord. vol. 32. Handbook of Clinical Neurology*. Amsterdam: North-Holland, with permission from Elsevier.)

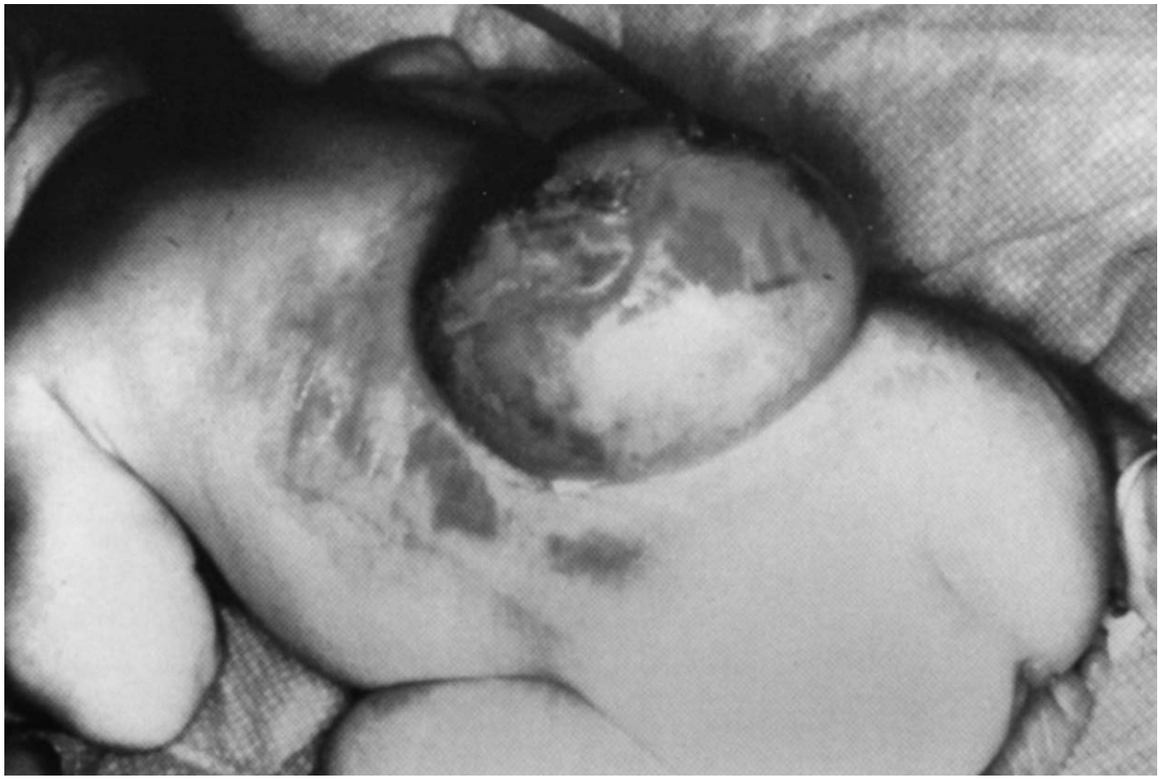


Figure 3 Newborn infant with spina bifida. Note the large meningocele on this child's back.

term used to describe a range of defects of the axial skeleton, involving the vertebrae and to various degrees the cord itself. If only the vertebrae show incomplete spinous process fusion and the subarachnoid space remains within normal limits, this is a subclinical condition known as occult spina bifida. If the vertebral arch is only rudimentary, the overlying tissues are weak, and cerebrospinal fluid pressure contributes to expansion of the subarachnoid space and the meninges herniate dorsally, this condition is diagnosed as spina bifida meningocele (cystica). If the vertebrae are so rudimentary that only the body of the bones is thickened, the spinal cord itself is displaced into the subarachnoid space (now a gross, protruding meningeal sac), the condition is classified as spina bifida with myelomeningocele (**Figure 3**). If there is complete failure of neural fold elevation in cranial, cervical, thoracic, and/or lumbar regions and the neuroectoderm is left exposed on its dorsal aspect, the spinal cord then develops with its ependymal layer in open contact with amniotic fluid and its lumen cannot be recognized. This latter condition (**Figure 4**) is termed spina bifida aperta (rachischisis or myeloschisis). Thus, spina bifida can range from a partial failure of neural tube closure, manifest as a benign subclinical condition of no practical consequence, to damage that is permanently disabling, affecting the patient's ability to walk and control normal bodily functions.

Just as partial failure of neural fold apposition and fusion can occur along the spine, it can also occur in the skull. Cephalic malformation can be complete in anencephalus (with little or no involvement of the lower spine) or incomplete as in encephalocele and Arnold–Chiari malformation (**Figure 5**). Encephalocele (**Figure 5**) is one such condition in which the brain extends through dura and membranous bone and comes into contact with the scalp.

From the early 1600s through the latter part of the nineteenth century, there was a great deal of interest on the part of physicians and surgeons in describing and classifying NTDs as well as in speculating on the cause(s) of NTDs and their pathogenesis. Dissections of fetuses with spina bifida and anencephaly were described in detail. These early studies provided a basis for understanding the morphogenesis of these malformations but gave little indication as to the actual etiologic agent(s) responsible. The 1801–96 manuscripts by the German authors Arnold J and Chiari H describe hindbrain anomalies in spina bifida and provide for four different categories of herniation of the cerebellum into the foramen magnum. In that case a cervical sac or encephalocele forms; in others there is a simple cerebellar hypoplasia

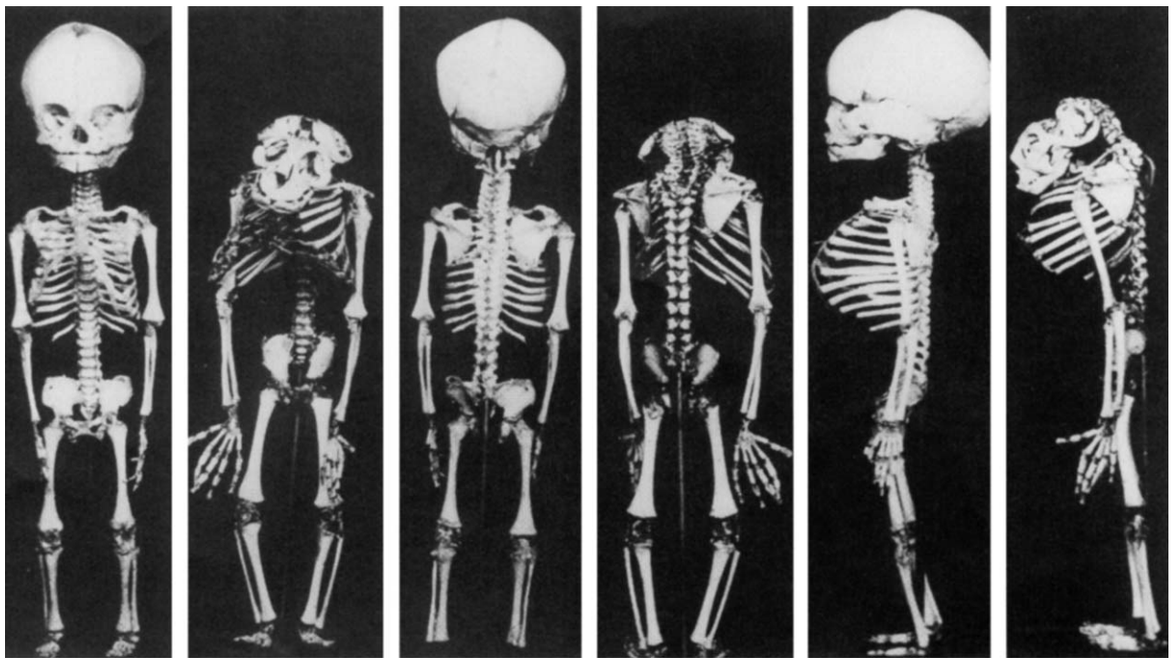


Figure 4 Posterior, anterior, and left lateral views of the entire skeletons of a 7 month premature infant and of a human anencephalic with cervical myeloschisis. Note the severity of the skeletal defects in the anencephalus, the anomalous facial bones, the short cervical column, and the absence of a proper skull. (Reproduced with permission from Marin-Padilla M (1991) Cephalic axial skeletal-neural dysraphic disorders: Embryology and pathology. *Canadian Journal of Neurological Sciences* 18: 153–169.)

(termed collectively Arnold–Chiari malformations). This condition often results in hydrocephalus (accumulation of cerebrospinal fluid followed by marked expansion and thinning of the skull with subsequent compression and atrophy of the brain). Blockage of the roof of the fourth ventricle and continued production of cerebrospinal fluid by the choroid plexus leads to increased intracranial pressure. As a result, the medulla oblongata is forced into the cervical canal, the herniated cerebellum is compressed, and interrupted cerebrospinal fluid flows into the subarachnoid space producing an extensive internal hydrocephalus (**Figure 5**). In those cases of Arnold–Chiari in which the lumen of the spinal cord is open at some point (e.g., myeloschisis), hydrocephalus does not occur since cerebrospinal fluid either accumulates at another point (**Figure 3**) or, in cases in which the cord is exposed on the surface of the skin, cerebrospinal fluid drains to amniotic fluid and relieves increased intracranial pressure. It is by this route that α-fetoprotein (a normal serum component synthesized in the embryonic yolk sac to 12 weeks and then by the fetal liver) escapes into the amniotic fluid. Radioimmunoassay of this protein is used in routine management of high-risk pregnancy where abnormally high concentrations are indicative of NTDs. Acute hydrocephalus is also a consequence of Dandy–Walker malformation, a condition characterized by defects of the ventricular system and stenosis (constriction) of the foramina of the fourth ventricle and it may present as a severe occipital encephalocele.

Anencephalus is a relatively common condition, affecting on average one in every 1000 births (or five or six embryos per 1000 pregnancies, given published studies of fetuses examined at 8 weeks gestation). Anencephalus occurs four times more often in males than in females and four times more often in Caucasians than in blacks. Even lower rates occur among North American Indians (0.5 per 1000), Japanese (0.4 per 1000), and Central and South Americans (0.1–0.3 per 1000). Spontaneous early pregnancy abortion of anencephalic embryos ranges from 54% (London) to 87% (Japan).

Although the term anencephalic suggests a lack of all but the bones of the face, in fact, all of the bones of the skull are present (**Figure 6**). The anomalies of the facial bones and those of the remainder of the skull are the consequence of early disruption of the notochord, the mesoderm, and the neuroepithelium. Early deficiency in neural tube closure exposes the developing brain (neuroepithelium) to mechanical abrasion from the fourth week of gestation until birth. The hindbrain (often enclosed and therefore protected by the rudimentary neurocranium) can remain intact – containing those structures responsible for control of respiration. Thus, the newborn

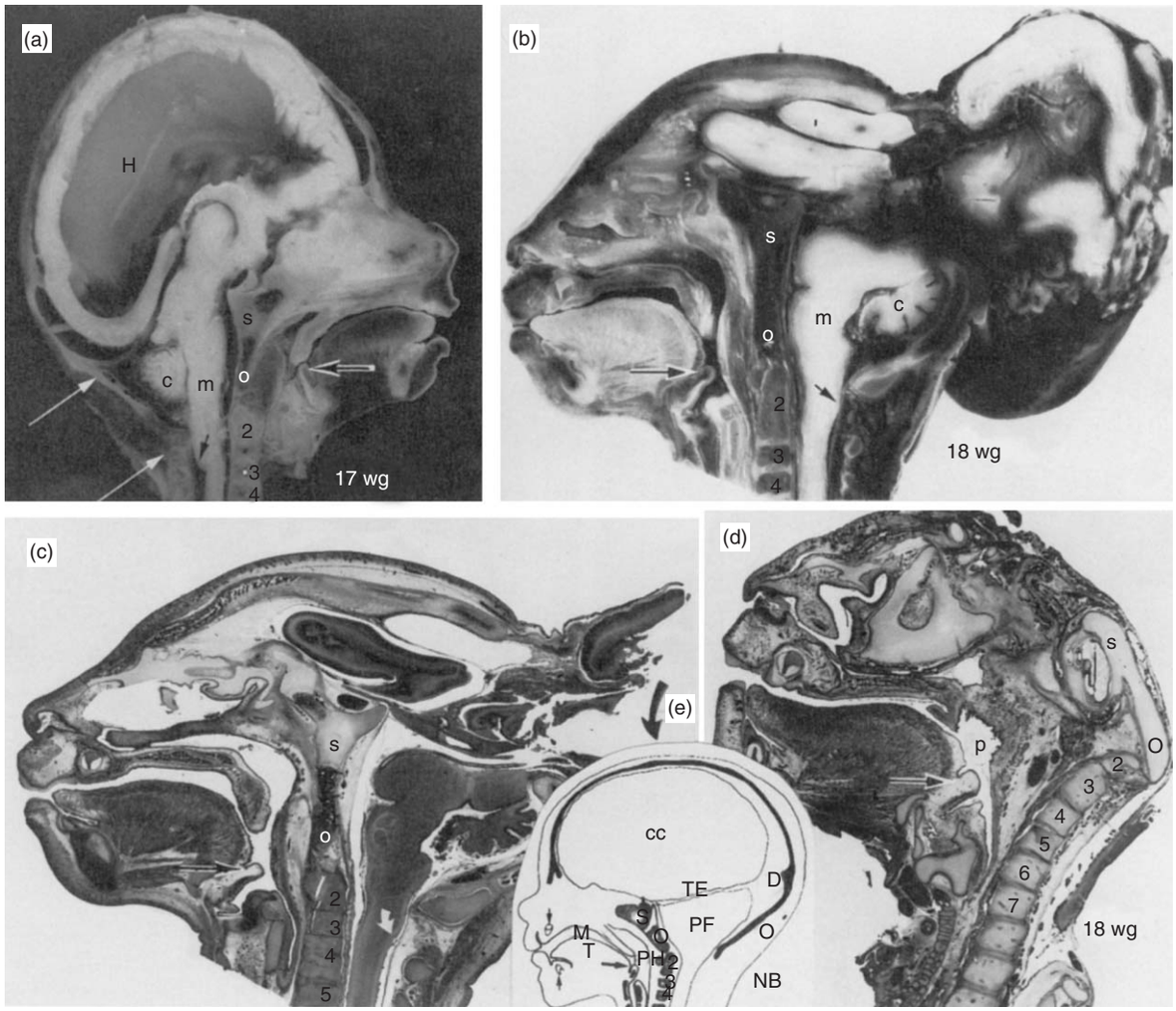


Figure 5 (a) Midsagittal section of the head of a premature infant with Arnold–Chiari malformation and secondary hydrocephalus. H, hydrocephalus; s, sphenoid; o, squama occipitalis; m, medulla; c, cerebellum; wg, weeks gestation. The cervical vertebrae are numbered. (b) Premature infant with partial failure of the anterior neural tube closure and a large occipital encephalocele. (c) Glass slide section of the infant shown in (b). (d) Premature infant with anencephalus, occipital schisis, and cervical rachischisis. The gross appearance of this infant was similar to that shown in **Figures 1** and **3**. (e) Arrow indicates schematic ink drawing of the midsagittal section of a normal newborn's head. For comparison to (a)–(d), locate the size and shape of the cerebral cavity (CC), the location of the tentorium (T), its angle in relation to that of the spine (D), the size and shape of the posterior fossa (PF), and the mouth (M), tongue (T), teeth (small arrows), and the nasal passage (P), pharyngeal (PH) and laryngeal (large arrow) cavities. NB, newborn. The black and white arrows in (a)–(d) point to the location of the epiglottis in relation to the base of the skull. The black arrows in (a) and (b) point to the bend in the medulla caused by the downward displacement of the subtentorial central nervous system. Note in (a)–(c) the short base of the skull, its angle to the spine, and the small posterior fossa. In (d), note the angle of the spine and relatively large facial skeleton compared to that shown in (e). (Reproduced from Marin-Padilla M (1991) Cephalic axial skeletal-neural dysraphic disorders: Embryology and pathology. *Canadian Journal of Neurological Sciences* 18: 153–169, with permission.)

anencephalic can survive hours to (at most) a few days in the absence of mechanical ventilation and aggressive life support. Anencephalic humans with larger skull bones (thus having a more representative brain) can survive for as long as a few weeks. The characteristic facial features of the anencephalus (low-set ears, protruding tongue, short maxilla, and elevated pointed nose; **Figure 1**) are direct consequences of the malformations of the bones of the base of the skull. The base of the skull (which in reality is composed of modified vertebrae) is small and, rather than forming a normal 90° angle with the spine, can be tipped to ~45°. The early collapse of the cephalic neural folds leads to gross malformation of the base of the skull. The normal sphenoid (with which all of these bones articulate directly or indirectly) resembles a bird in flight (**Figure 7a**), whereas the anencephalic sphenoid resembles a bat with folded

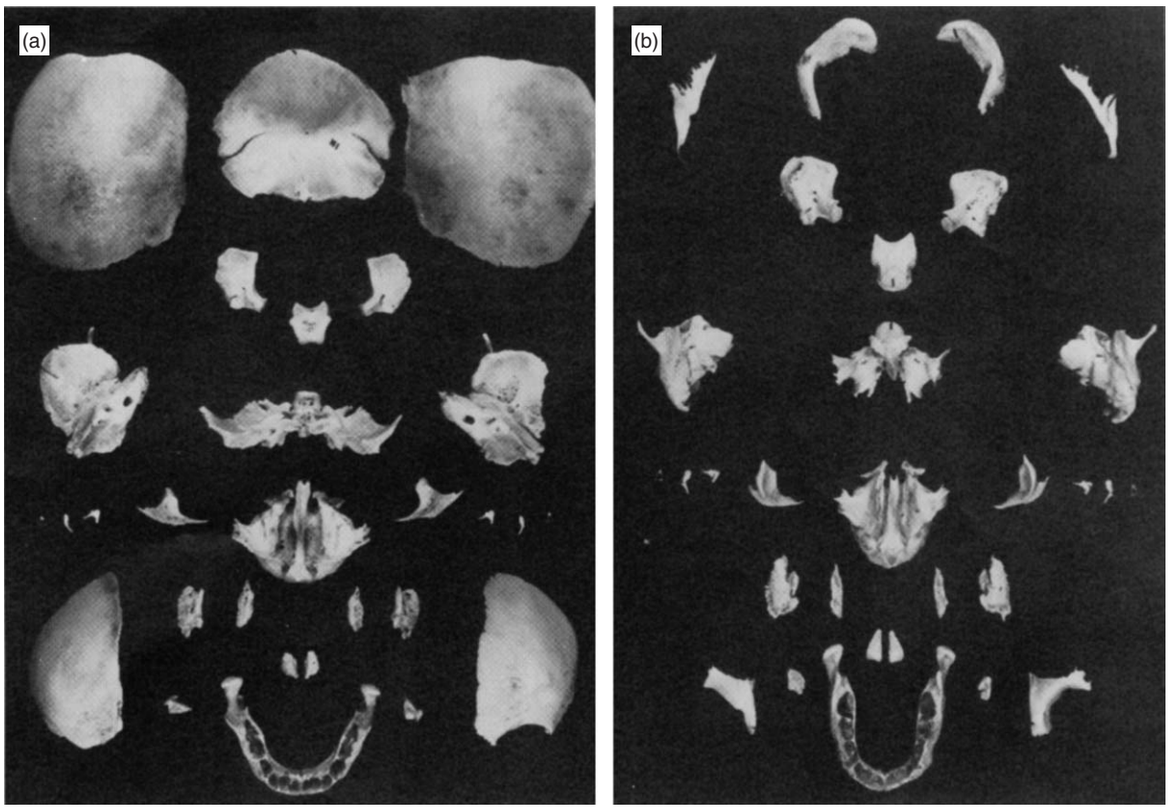


Figure 6 Disassembled skull of a (a) normal and (b) anencephalic infant. The following bones, starting from the upper corner, are the parietals and the squama of the occipital (which in the anencephalus are represented by two small fragments); the basilar portion of the occipital with its two lateral portions; the temporals (note the rudimentary squamas in the anencephalus); the sphenoid (located in the center of each figure); the ossicles, the zygomatics, and the maxilla with the vomer and palantines; the lateral masses of the ethmoid and the turbinate bones; and the frontals, the lacrimals, the mandible, and the two nasals. The rudimentary bones of the cranium and the normal (but narrowed) bones of the face are obvious. (Reproduced with permission from Marin-Padilla M, (1976) Morphogenesis of anencephaly and related malformations. In: *Current Topics in Pathology*, vol. 51, pp. 145–174. New York: Springer.)

wings (**Figure 7b–f**). Studies of disassembled normal and anencephalic skulls (**Figure 6**) show that it is the sphenoid malformations that precipitate the other gross malformations of the skull. The sphenoid is a bone that arises from mesodermal consolidation around the notochord prior to closure of the anterior neuropore. Since there is intimate communication between the embryonic brain (neuroectoderm), face (originating principally from neural crest), and the developing bones of the skull proper (neurocranium), teratogens that act on neuroectoderm, mesoderm, or both can initiate a cascade of abnormal events which ultimately give rise to anencephalus.

The geographic distribution of NTDs is most remarkable. The incidence among people living in Ireland is four times that of people living in the United States (including those of Irish descent living in New England). Such striking differences have precipitated speculations and generated hypotheses on the cause of NTDs. One such theory, advanced in the early 1970s, was that the high incidence of anencephalus and spina bifida in Ireland was caused by ingestion of potatoes containing unidentified teratogens, potatoes infected with fungi (blighted) after storage, or compounds produced by such potatoes in response to blight. Subsequent epidemiologic studies failed to support any of these theories; nonetheless, numerous studies have confirmed an excess number of NTD births in winter and a slow (but steady) decline in NTD births over the past five decades in the United States.

It is well known that the risk of NTDs is greatest in mothers 35 years or older (being particularly high in those who have borne several children) and that there is a marked increase in risk for mothers whose previous pregnancies ended in fetal or neonatal death (relative risk = 3.5–3.8). When one compares population prevalence of anencephalus and spina bifida (0.13–0.75% including New York, New England, British Columbia, Hungary, London, and South Wales), it is clear that recurrent risk is increased (from 0.1% in the general population to 1.8–7.1%)

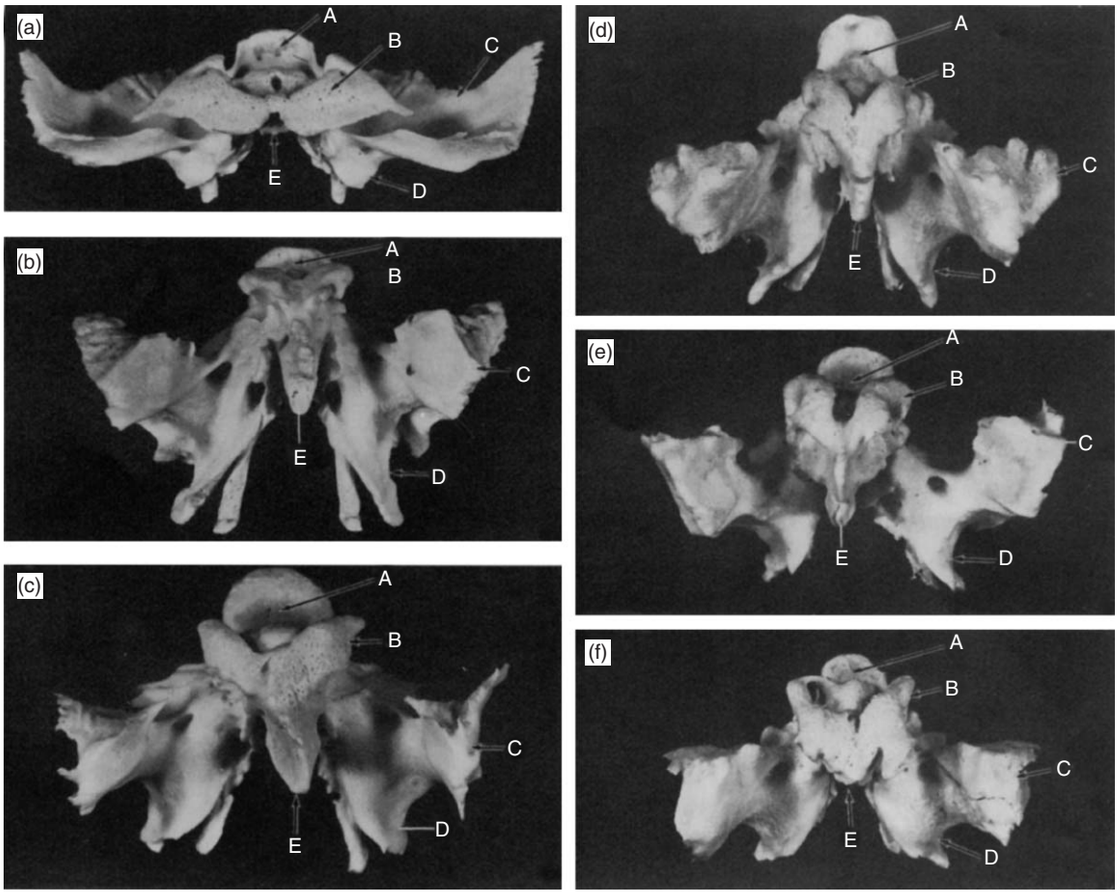


Figure 7 (a) Anterosuperior aspect of the sphenoid bone of a normal newborn infant as shown in **Figure 6** (a) (× 1.8). A Body of the sphenoid bone; B the lesser wings; C the greater wings; D the pterygoid process; E the rostrum. (b) Anterosuperior aspect of the sphenoid bone of a newborn infant with partial anterior cranioschisis (× 2). (c) Anterosuperior aspect of the sphenoid bone of a newborn premature infant with complete (simple) cranioschisis (**Figure 6**) (× 2.5). (d) Anterosuperior aspect of the sphenoid bone of a newborn premature infant with anencephalus and cervical spina bifida (× 2.5). (e) Anterosuperior aspect of the sphenoid bone of a newborn premature infant with complete open spina bifida and anencephalus. The gross appearance of this infant is shown in **Figure 3** c, d (× 3). (f) Anterosuperior aspect of the sphenoid bone of a newborn infant with complete open spina bifida, anencephalus, a diaphragmatic hernia, and a large omphalocele (× 3). (Reproduced from Marin-Padilla M (1965) Study of the sphenoid bone in human cranioschisis and craniorachischisis. *Virchows Archives in Pathological Anatomy* 339: 245–253, with permission.)

for siblings of fetuses with NTDs. In some families, as many as four anencephalics have been born to one mother. Although rare, anencephalics have been born to sisters who were daughters of women who had NTD pregnancies. Although the risk is greater among monozygotic compared to dizygotic twins, a simple genetic mechanism cannot be held responsible for NTDs. (The reader is reminded of those who advanced genetic causes for tuberculosis and Creutzfeldt–Jakob disease – a disease first found in the Fore cannibals of New Guinea.) More to the point, there is increased risk of NTDs in families with two or more affected pregnancies, and this increased risk for subsequent pregnancies is consistent "with a causal role of an environmental agent to which certain families are more exposed than others" (Yen S and Macmahon B as quoted in Elwood and Elwood, 1980). Indeed, one clue to the etiology of NTDs came from the observations of increased incidence in urban compared to rural areas – an observation consistent with a lifestyle or dietary hypothesis.

In 1992, the results of a landmark study by Czeizel A and Dudas I were published in the *New England Journal of Medicine*. Using a carefully controlled double-blind protocol (considered the 'gold standard' by which clinical trials of new pharmaceuticals are routinely conducted), these investigators found that 0.8 mg day^{-1} of folic acid (a water-soluble B vitamin) in a multivitamin preparation prevented spina bifida and anencephalus in the Hungarian women in their study. A subsequent study by Werler M and associates of Boston University (published in

1993) confirmed that folic acid supplementation of the diet could have prevented a large proportion of spina bifida and anencephalus that occurred in the United States from 1988 through 1991. These results also confirmed those published by the United Kingdom Medical Research Council, which had conducted a randomized controlled clinical trial among women who had previously experienced an NTD pregnancy in 1991. The Hungarian trial was so successful that ethical considerations dictated its prompt discontinuation and those women who had been assigned to the placebo group were given the vitamin. These studies were extensions of previous trials on multivitamin supplements conducted in Europe and the United States in the mid-1960s and 1970s. Although it had been known since the 1950s that two cancer chemotherapeutic drugs known to induce folate deficiency (methotrexate and aminopterin) also induced terata in animals and in humans, interpretation of those data was confounded by the fact that folate antagonists have a number of pharmacologic actions, NTDs are not produced uniformly after exposure to either of these drugs, and NTDs are not the only malformations produced. In previous prospective studies of serum and erythrocyte folate, two important epidemiologic and clinical observa tions emerged as indicators of maternal folate status: (1) Folate deficiency can be documented in 66% of mothers of NTD pregnancies and (2) low folate mirrors the woman's socioeconomic status. When matched with mothers of normal children for age, parity, time of conception, and pregnancy, 69% of mothers with NTD pregnancies were folate deficient compared with 17% of the referent controls.

In 1992 and 1993, Australian, Scottish, and Welsh departments of health and social services recommended widespread folic acid supplementation of breakfast cereals and breads. In order to avoid possible excess folate in one's diet, these groups recommended that some unfortified breads and breakfast cereals continue to be available. These groups recommended that women with spina bifida, or those who had a previous child with an NTD, consume 5 mg of folate each day if pregnancy was possible and that supplementation continue through the 12th week of gestation. It was recommended that all other women consume 0.4 mg daily, increase their consumption of folate-rich foods (fruit and vegetables), and avoid overcooking these foods. The United States Public Health Service recommended that all women capable of becoming pregnant should consume 0.4 mg of folic acid per day for the purpose of reducing their risk of having a pregnancy affected by spina bifida or other NTDs. Nevertheless, the debate on folate supplementation of women's diets continues. Money is not an issue (400 μg of folate is currently sold at 4/100ths of a penny and food fortification for 50 servings at 200 μg per serving amounts to one US cent). Rather, the benefits of folate supplementation are weighed against the possibility of masking vitamin B_{12} deficiency. This debate takes place in light of the following: only 30% of low-income women consume the US recommended daily amount of folate and fewer than 10% of women of childbearing age consume 400 μg of folate per day. Only half of the women with incomes 130% of the poverty line or less ate one serving of any vegetable over any 4 days; 18% did not eat any vegetables. With regard to fruit, fully one-third did not consume any fruit or juice and only 5% of White women 19–29 years of age and 4% of Black women of that age ate two or more fruits or three or more vegetables each day. To compound the problem, cigarette smoking reduces blood folate levels. Cigarette smoking is increasingly popular among young women and is found more often among lower socioeconomic groups. Thus, numerous social considerations confront the practical implementation of the prevention of NTDs by folate. It is not known how folate prevents NTDs or what precise role folate plays in early embryonic neural fold elevation and fusion.

Although we are far from understanding why folic acid plays such a significant role in preventing NTDs, some clues are available. To understand the discussion that follows and why clues are so hard to come by, it is necessary to appreciate the complex pathways involving folate metabolism (see **Figure 8**). For example, ~150 genes and their respective proteins are involved in folic acid metabolism and transport. Because gene mutations are known to result in alterations in proteins that are related to various diseases, scientists have focused on key genes involved in folic acid metabolism and transport in an effort to determine whether specific mutations (termed single nucleotide polymorphisms) in 'folic acid genes' are causally related to NTDs. The most extensively studied 'folic acid genes' include folate receptor alpha (FRα), reduced folate carrier (RFC), 5,10-methylene-tetrahydrofolate reductase (MTHFR), methonine synthase (MTR), methionine synthase reductase (MTRR), methylenetetrahydrofolate dehydrogenase (MTHFD), and serine hydroxymethyltransferase (SHMT).

Folates are transported from the extracellular milieu to the inside of cells by either receptor-mediated (FRα) or carrier-mediated uptake (RFC). Because folates are essential for proper cellular function and necessary for neural tube closure, it is easy to envision how mutations in these receptor genes could lead to reduced levels of intracellular folates and thereby NTDs. Thus, several groups have searched for

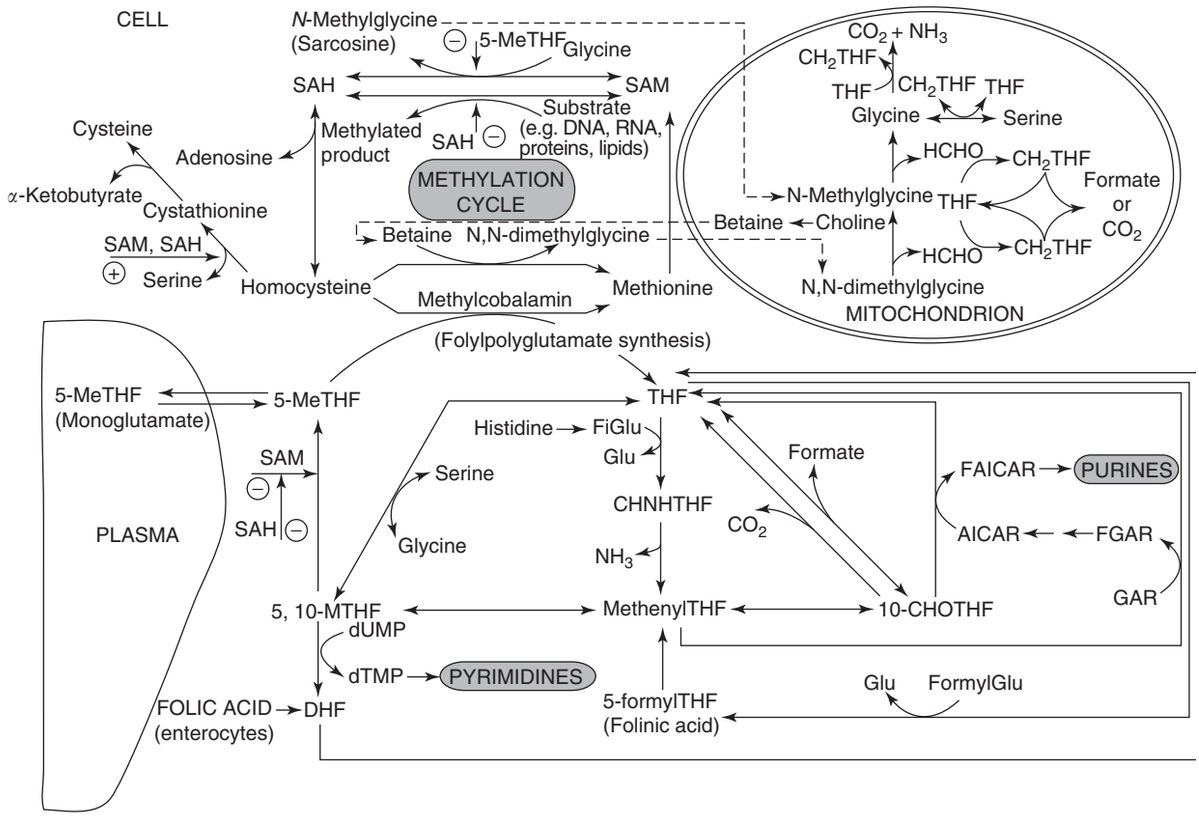


Figure 8 Extended folate metabolism, including compartmentation. *MTHFR*, methylenetetrahydrofolate reductase; *SHMT*, serine hydroxymethyltransferase; *BHMT*, betaine homocysteine methyltransferase, *MAT*, methionine adenosyltransferase; *SAH-hydrolase, S*-adenosylhomocysteine hydrolase; *MT*, methyltransferase; *CBS*, cystathionine β-synthase; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; THF, tetrahydrofolate; and 5-MeTHF, 5-methyltetrahydrofolate. (Reproduced from Van der Put *et al.* (2001) Folate, homocysteine and neural tube defects: An overview. *Experimental Biology and Medicine* 226: 243–270.)

nucleotide polymorphisms in FRα and RFC to determine whether specific mutations are associated with an increased risk for NTDs. Several polymorphisms were found in FRα (631T > C, 610A > G, and 762G > A); however, none of these alterations was associated with increased NTD risk. In addition, other studies failed to find any polymorphisms in RFC. Although studies completed to date have not identified any polymorphisms in FRα or RFC associated with NTD risk, it is important to remember that complete loss of the folate binding protein 1 gene (the mouse homolog of human FRα) resulted in mice with NTDs. This leaves open the possibility that specific mutations in FRα and/or RFC may play a role in the genesis of NTDs.

Once inside the cell, folates participate in a number of interconnected metabolic pathways involving (1) thymidine and purine biosynthesis necessary for DNA synthesis, (2) methionine synthesis via homocysteine remethylation, (3) methylation reactions involving *S*-adenosylmethionine (AdoMet), (4) serine and glycine interconversion, and (5) metabolism of histidine and formate (see **Figure 8**). Via these pathways, folates play an indispensable role in such critical processes as DNA synthesis and gene expression, to name a few. Because DNA synthesis and gene expression are critical processes during embryogenesis, one can see how alterations in these processes due to limited folates could negatively affect important developmental events like neural tube closure. Because methionine is such a critical amino acid, researchers have focused considerable effort on characterizing nucleotide polymorphisms in MTHFR, the rate-limiting enzyme in the synthesis of methionine from homocysteine. Mutations in MTHFR can result in elevated levels of homocysteine, which in turn have been associated with an increased risk for NTDs. The first and most well-studied MTHFR polymorphism is the 677C > T, with TT homozygotes exhibiting reduced MTHFR activity and increased homocysteine. Although several research groups have reported a three- to sevenfold increased risk for NTDs associated with the 677C > T mutation, other studies have reported either a smaller or no associated risk for NTDs. More research is required to determine the

linkage between this mutation and increased risk for NTDs; however, currently available information confirms that this mutation is associated with an increased risk. In addition to this mutation, complete sequencing of the MTHFR coding sequence identified a second common polymorphism, 1298A > C. The homozygous CC allele is, like the TT allele, associated with decreased MTHFR activity, but this CC allele it is not associated with increased homocysteine or an increased NTD risk. Polymorphisms in two other genes, MTR and MTTR, have also been studied. A polymorphism in the MTR gene 2756A > G was found; however, this polymorphism is not associated with increased homocysteine levels or increased risk for NTDs. In contrast, a common polymorphism in MTTR, 66A > G, is associated with increased risk for NTDs when the cobalamin (B_{12}) levels are low or the MTHFR 677 TT allele is present in the infant. Finally, SHMT catalyzes the reaction of serine and tetrahydrofolate to form glycine and 5,10-methylene-tetrahydrofolate and is, therefore, a major entry point for one-carbon units from serine into folate-dependent metabolism. Because of this key role in folate metabolism, SHMT could be a candidate gene involved in NTDs. Two polymorphisms have been identified in the cytosolic form of SHMT, 1181G > A and 1420C > T and two in the mitochondrial form of SHMT, 850C > T and a 4 base pair deletion (delTCTT); however, none of these alterations were associated with an increased risk for NTDs.

While there is little doubt that genetic factors are involved in the etiology of NTDs, proving a causal link between a specific gene (mutation) and an increased NTD risk remains a challenge. Nonetheless, some putative genetic risk factors have been identified. Hopefully, future studies in humans taking advantage of information gained from animal studies will identify not only genetic risk factors but also environmental factors that together culminate in an increased risk for NTDs. Identification of genetic and/or environmental factors contributing to an increased risk for NTDs is a critical step in reducing the numbers of babies born with NTDs.

The Cause of Birth Defects

In the vast majority of cases, the cause of a birth defect is unknown (**Figure 9**).

Maternal infections account for no more than 3% or 4% of the total load of congenital malformations. The most well known of these infections is rubella (German measles). Infection during various stages of gestation corresponds to the particular malformations produced. Malformation of the eye, including cataract and microphthalmia (literally small to nearly absent eye), can be induced by infection during the sixth week. Congenital deafness is induced by infection during the ninth week. Malformations of the heart (patent ductus arteriosus and ventricular septal defect), teeth, and brain (resulting in profound mental retardation) follow infection during weeks 5–10 and weeks 13–28, respectively. As with all known teratogens, not all children exposed to rubella *in utero* develop congenital defects, but the risk of malformation is greatest (to at least 47%) when rubella exposure occurs shortly after implantation.

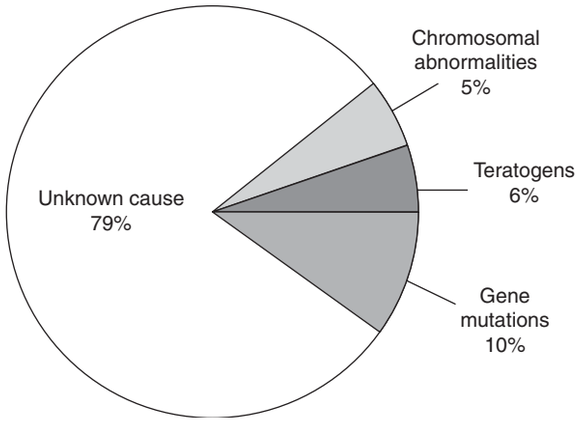


Figure 9 Pie chart of the relative percentages of the causes of human birth defects. It has been estimated by various authorities that cytogenetics contributes to no more than 5% of all malformed live births, Mendelian inheritance to no more than 15–20%, maternal infections 3% or 4%, maternal disease 3% or 4%, problems of constraint *in utero* (amniotic bands) 2%, and all drugs, chemicals, and radiation no more than 1% of the total load of structural birth defects in human beings.

Other infections clearly associated with increased risk of terata include toxoplasmosis, cytomegalovirus, and herpes simplex. Microphthalma, blindness, hydrocephalus, and cerebral calcification can occur after infection with these organisms.

The contribution of genetic disorders is estimated to account for no more than ~15% of the total load of congenital malformations (**Figure 9**). Inherited conditions account for (at most) 20% of the total, and abnormal cytogenetics accounts for no more than 5% of the total. An entire branch of the science of genetics is concerned with abnormally high or low numbers of chromosomes. Variable degrees of mental retardation, frank structural malformation, and sterility are common consequences of abnormal numbers of somatic chromosomes (autosomes) or of sex chromosomes. Two well-known syndromes arising from an abnormally high number of sex chromosomes are Klinefelter's (male only) and Triple X (female only), which originate as failures in normal chromosome number reduction (meiosis) during in spermatogenesis and oogenesis. A syndrome resulting

from too few sex chromosomes is Turner's syndrome, a condition characterized phenotypically by a webbed neck and congenital absence of the ovaries.

An excess number of autosomes or the absence of one or more autosomes can either be lethal to the embryo or result in well-known conditions. For example, the risk of Down's syndrome (trisomy 21 or mongolism) increases with maternal age – being one in 2000 for mothers aged 40 or more years. Extra chromosomes 17 and 18 result in micro- or anophthalmia (congenital absence of the eyeball), mental retardation, cleft lip, cleft palate, and deafness; this condition occurs on average once in every 5000 births.

Phenylketonuria is a genetic disease that results in abnormally high concentrations of the amino acid phenylalanine in the blood. Children of mothers with high plasma phenylalanine ($>3\,\mathrm{mg\,dl^{-1}}$) are at increased risk for microcephaly, mental retardation, heart malformations, esophageal atresia, tracheoesophageal fistula, and low birth weight. There is a clear concentration–response relationship between plasma phenylalanine and abnormal pregnancy outcome; head circumference and low birth weight are inversely (and linearly) related to maternal blood phenylalanine concentrations. Offspring of mothers with plasma phenylalanine $>20\,\mathrm{mg\,dl^{-1}}$ experience a 92% incidence of congenital heart disease; those exposed *in utero* to maternal plasma phenylalanine levels higher than 3 but lower than $11\,\mathrm{mg\,dl^{-1}}$ experience a 21% incidence of congenital heart malformation. These defects can be prevented with adherence to a strict diet to control phenylalanine intake, total energy, protein, and weight gain during pregnancy; however, normal pregnancy outcome can occur only when dietary control occurs at or before conception. Women who consume a 'relaxed diet' experience a 0.6% malformation rate in their children (compared to 0.0% for those on a strict diet). Women placed on a phenylalanine-restricted diet after conception (but before the second trimester) typically experience pregnancies with malformation rates on the order of 19–20%.

Maternal disease – not necessarily of either microbial or genetic origin – can cause or contribute to adverse pregnancy outcome. One common example is diabetes mellitus. If uncontrolled, diabetes mellitus can result in mental retardation, congenital malformation, and embryonic death. Glucose appears to be the teratogenic agent (perhaps potentiated by acetone and β-hydroxybutrate ketone bodies) in uncontrolled diabetes. The extent of hyperglycemia is related directly to the risk of holoprosencephaly (also usually accompanied by microcephaly, cleft lip and palate, mental retardation, and epileptiform seizures), situs inversus (complete transposition of the viscera), and ureteral duplex (complete or partial double ureter). These risks are 400, 84, and 23 times that in uncomplicated pregnancy, respectively. Sacral, vertebral, and pelvic malformation are also associated with elevated maternal glucose levels. To illustrate that stage of development determines susceptibility to teratogenic insult, women who develop diabetes relatively late in gestation (second or third trimester) do not have an increased risk of adverse pregnancy outcome. Only those women with uncontrolled diabetes prior to conception and through the first 8 weeks of pregnancy are at risk. Insulin control of maternal diabetes produces marked reduction in neonatal mortality (from 33% in the decade 1920–30 to 6.5% from 1975 to 1979). By careful management of these mothers, the number of diabetes-induced late fetal deaths (stillbirths) and depressed or abnormally high birth weights (macrosomia) have been reduced to the point that birth defects are now the leading cause of death among offspring of diabetic mothers.

Another example of a maternal condition that contributes to birth defects is low circulating iodine. Cretinism is one of the most profound, but completely preventable, syndromes of malformation known. Characteristic consequences of prenatal iodine deficiency include pervasive mental and physical retardation, deaf-mutism (due to primary malformation of the inner ear), lack of muscle tone with a spastic or rigid walk, and failure to attain a height at maturity of more than 1 m. Today, this condition (known as endemic cretinism) is most prevalent in impoverished areas of African and East Asian countries. Prior to implementation of a national program of iodized salt in the early part of the twentieth century, endemic cretinism was commonplace in Switzerland. After institution of iodized salt, deaf-mutism declined 50% within 8 years and no cretins have been born in that country since 1930.

Maternal disease in addiction contributes to infant morbidity and mortality. Two of the most common agents, ethanol and tobacco smoke, are illustrative. A host of clinical and epidemiologic studies have confirmed a distinctive pattern of congenital malformations in babies born to alcoholic mothers. These malformations include microcephaly, short palpebral fissures, epicanthal folds, maxillary hypoplasia, cleft palate, micrognathia, joint disease, cardiac anomalies, capillary hemangiomata, anomalous genitalia, and retarded fetal and neonatal growth and development (a pattern referred to as fatal alcohol syndrome (FAS)). FAS children have abnormal motor and psychological development and abnormal dermatoglyphic characters. Miscellaneous terata also found among these children are arthrogryposis, limb

reduction defects, and gastroschisis. With ethanol, there is a definite dose–response relationship: a 9% malformation rate in light drinkers, a 14% rate for moderate drinkers, and a 32% rate for heavy drinkers. In addition, as with almost all teratogens, the severity of the malformations is greatest in those infants born to mothers consuming the highest dose. Some authors have argued that consumption of one type of alcoholic beverage (e.g., wine, beer, and schnapps) was more or less dangerous than another; however, the relationship holds true for all drinking. Pregnancy outcome is directly related to the quantity of absolute ethanol consumed per day. Ethanol doses consumed by these mothers range from 'social' (1 oz of absolute ethanol day^{-1} or $350\,mg\,kg^{-1}\,day^{-1}$) to 'heavy' (2.3–15 oz of absolute ethanol day^{-1} or $800–5600\,mg\,kg^{-1}\,day^{-1}$). Statistically significant reductions in birth weight (91 g for ethanol exposure before pregnancy and 160 g for exposure in late pregnancy), decreased infant length, increased stillbirth and second-trimester spontaneous abortion (0.5–1 oz of absolute ethanol day^{-1}), and increased risk of early spontaneous abortion (1 oz of absolute ethanol, twice per week) are well documented. Notwithstanding these data, results from the National Institute of Child Health and Human Development (NICHHD) study of drinking habits and pregnancy outcome in 32 870 women who had two drinks or less each day showed that those women had the same overall risk of birth defects as pregnant women who did not drink ethanol at all. NICHHD defined moderate drinking for purposes of the study on a daily basis (e.g., wine with a meal) but excluded binge drinking (foregoing drinking during the week but consuming several drinks on the weekend).

The most common addiction during pregnancy is tobacco smoking; 30.9% of all US women smoke before pregnancy and 25.5% continue to smoke during pregnancy. This practice continues despite the fact that the first reports of adverse effects on the human fetus were published more than 80 years ago and despite the legally required warnings on tobacco advertisements and product packaging. At least 19 major epidemiologic studies of more than 300 000 pregnancies have been published. The results of those studies lead to the following dose–response conclusions. Smoking 10–20 cigarettes per day throughout pregnancy increases the risk of early spontaneous abortion and reduces birth weight by as much as 92 to 316 g. Women who cease smoking during the early part of their pregnancy deliver babies with birth weights near those of babies born to mothers who have never smoked. The frequency of spontaneous abortion is directly related to the number of cigarettes smoked; the frequency for pack-a-day mothers being double that of nonsmoking mothers. The data hold true after correcting for maternal age, race, height, weight gain during pregnancy, socioeconomic status, gestational age, and parity. It appears that it is the nicotine and carboxyhemoglobin content of maternal blood that is responsible for decreased placental blood flow and anoxia leading to or contributing to the low birth weight. Perinatal mortality increases exponentially with decreasing birth weight; perinatal mortality is increased 20% in offspring of mothers who smoke less than one pack per day, and it is increased 35% in mothers who smoke more than one pack a day. Tobacco use is the single most important preventable determinant of low birth weight and its associated perinatal mortality in the United States. Of the 39 000 excess low-birth-weight babies in the United States, 5900 could be prevented each year by smoking cessation. The hospitalization and medical cost on a national basis for these babies is enormous – so much so in fact that for every $1 spent on smoking cessation during prenatal care, at least $6 in hospitalization and related medical care costs can be saved. This one intervention alone could double the cost savings gained by prenatal care.

Fear of Birth Defects

Two of the most questionable practices by those who are not familiar with the principles of teratology are (1) making lists of chemicals or other agents known or suspected to be teratogenic or otherwise toxic to the embryo and fetus and (2) assuming that the results of animal studies mimic those in human beings.

Perhaps the most obvious example of the first practice is California's Proposition 65, a set of laws enacted by public vote in 1986 (known as the Safe Drinking Water and Toxic Enforcement Act). Proposition 65's list, like other similar lists, includes chemical compounds ranging from known human teratogens to compounds having adverse effects demonstrated to occur only in laboratory animals. This list reinforces the notion that compounds are either 'positive' (teratogenic) or 'negative' (nonteratogenic) and that providing printed or other warnings will prevent birth defects. This is simply not the case. All compounds can cause the various manifestations of developmental toxicity provided the exposure (dose) is large enough, the route of exposure is appropriate, and the timing of exposure occurs during a susceptible period of development.

One clear example of a known teratogen for which warning can be effective is ethanol, but warnings alone are not the whole story. Alcohol consumption

during pregnancy is a definite problem that has increased over time (**Figure 10**). Studies of children in the general population indicate that from 0.1% to 0.2% show signs of FAS, but this figure does not represent the true measure of this problem. Among Native American tribes of the Great Plains or in northern British Columbia, the Yukon and the Southwest, studies have found 5% of the children affected. On the Pine Ridge reservation in South Dakota, more than 25% of the children show signs of FAS. Many of these mothers are so disabled by their disease that they are unable to care for their children. These children are difficult to place in foster homes because of their mental deficiency and inability to develop appropriate emotional and logical responses to everyday situations.

In the case of compounds that are merely suspected teratogens, however, there can be dark consequences. For example, in August 1973, the United States Consumer Product Safety Commission (CPSC) banned the sale of spray adhesives and published national warnings that these products caused birth defects and chromosome abnormalities. The CPSC warned all pregnant women who may have had contact with these sprays to see their physician and inquire about the chromosomes of their fetus. The minimum consequences of this regulatory action were 1273 working days logged by 130 US diagnostic and genetic counseling centers on spray adhesives, at least 380 chromosome studies, 11 amniocenteses, and at least nine elective abortions out of concern for exposure to spray adhesive. Eight of these abortions were performed without first performing diagnostic amniocentesis, and one was performed in a woman who had chromosome breaks in her amniotic fluid. The genetic counselor in the latter case had informed the mother that he was unable to determine the health of her fetus with the information he had on hand; she elected abortion because of fear of possible birth defects and without telling the counselor of her decision. The aborted fetus was fixed in formalin and a detailed autopsy was performed: not only was there no evidence for any congenital abnormality, but the chromosome change first observed was also found to be due to viral contamination of her amniotic fluid sample. In those areas of the country where local newspapers had given the CPSC warnings the greatest publicity, the greatest numbers of inquiries to local genetic counseling services were made. Six months later, the CPSC withdrew the ban on these sprays because no toxicity of the substances in the spray could be demonstrated and the original observations on chromosome damage could not be confirmed.

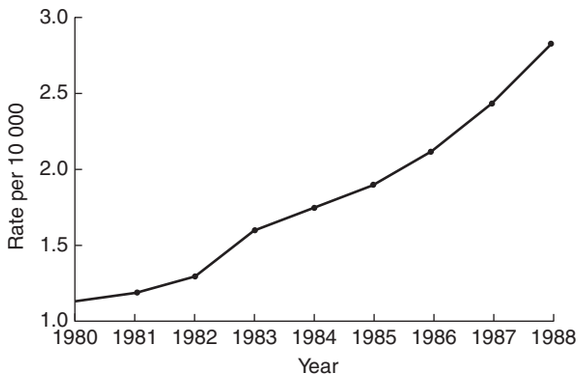


Figure 10 Fetal alcohol syndrome rates in the United States, 1980–88. These rates have shown a steady increase due to increased recognition and reporting of this condition by physicians and not necessarily due to increased alcoholism among women of childbearing age. (Reproduced from *Birth Defects and Infant Mortality*, Infant Mortality Report Series, vol. 1, no. 2, March of Dimes Birth Defects Foundation, with permission from March of Dimes.)

The 1973 US CPSC action and its consequence is not an isolated or rare example. Pregnant women are often very worried about birth defects. Women not exposed to any teratogenic agent appearing on any lists believe they have a one in four chance of having a child with major structural malformations – a risk equivalent to that after prenatal thalidomide exposure. Single women have a significantly higher (probability of less than 0.05) tendency to terminate their pregnancy than do married women; published studies demonstrate a greater willingness on the part of single mothers to abort their fetus when exposed to nonteratogens compared to married women in similar circumstances. The data show that the economic and social factors cited by single mothers in decisions to continue their pregnancy are compounded by distorted perceptions of teratogenic risk.

That the young, the minority, and the educationally and economically disadvantaged are placed in a particularly vulnerable position with respect to this misinformation is highlighted by the following example. United States hospitalization and census data show that 61% of all pregnancies end in live birth, 26% in induced abortion, and 13% in early embryonic and late fetal death. Definite ethnic- and age-related differences underlie these overall rates. United States pregnancy rates for nonwhite women (80% of whom are Black) average 68% higher than for White women. Although current census data show reduced numbers of births to teenage mothers (a 10% decline in the number of teenage pregnancies since the 1970s), those figures mirror the decline in the total number of teenagers (9%) over the same period. Pregnancy rates among teenagers as a group increased because of the decline in use of oral

contraceptives and increased sexual activity. For US teenagers 15 years of age, more than 50% of all pregnancies terminate in elective abortion; for all US teenage pregnancies, 40% end in abortion and 10% in fetal loss. For women aged 15–19 years, there has been a 24% increase in elective abortion since 1976. Rates of induced abortion for US nonwhite women are significantly greater than those for White women with the differential increasing to age 34 (after which this differential declines). These patterns underlie the fact that, in North America and Europe, there is one induced abortion for every live birth.

Among the most problematic issues raising the specter of death and disability is radiation. After the Hiroshima and Nagasaki atomic bombs, spontaneous abortion in survivors who were pregnant increased to the point that one-third of the embryos died and of those that lived, at least 25% were afflicted with a structural malformation of one type or another (microcephaly, spina bifida, ocular defects, or oral cleft). There is no question that exposure to radiation from atomic bombs, or from X-rays or other medical procedures, has been responsible for instances of human congenital malformation. Other experience illustrates an ironic association between radiation and abortion. Following the Chernobyl meltdown and disaster in the former Soviet Union, fear and rumor were responsible for the abortion of at least 2500 otherwise wanted pregnancies in Greece. This occurred despite the fact that the radiation drifted north, to Scandinavia, and that effective exposure in Greece was 100 mrem – much less than the amount that could cause terata. In all of Western Europe, the total number of panic-induced abortions resulting from that episode has been estimated from hospital records at 100 000–200 000.

The second questionable practice by those unfamiliar with the principles of teratology concerns an overemphasis of animal data. Several thousand compounds have been identified as developmentally toxic in animal bioassays, but only relatively few are known human teratogens. There is a tendency among those who have not actually conducted laboratory studies, those who substitute a strength-of-evidence approach for the weight-of-evidence approach, or those who are otherwise unfamiliar with the principles of teratology to assume that the effects seen in animal (including bird) studies do or could occur in people. This is evident in: (1) epidemiology studies in which investigators focus on a specific malformation in human populations after those defects have been observed in animal studies and (2) laboratory studies in which investigators have attempted to confirm or reproduce the human syndrome in animals. Concordance between animal and human data is the exception rather than the rule. Five teratogens are offered here to illustrate this point: acetazolamide, aspirin, caffeine, lead, and trypan blue. Finally, the retinoids (a large and diverse number of compounds of which vitamin A is a member) are presented to demonstrate the fact that although species concordance is relatively rare, it does occur.

Acetazolamide (Diamox or Hydrazol), a prescription diuretic, is a classic example of a teratogen that produces malformations in a highly species-dependent fashion. Administration of acetazolamide to pregnant mice, hamsters, or rats causes right forelimb postaxial ectrodactyly (absent digit) and only on very rare occasions is any other malformation induced. Acetazolamide is not teratogenic in monkeys and, despite its widespread use in the 1950s in early and late human pregnancy, there has been no evidence that acetazolamide caused developmental toxicity in humans.

Acetazolamide exerts its pharmacologic action through inhibition of the enzyme carbonic anhydrase. It is believed that inhibition of this enzyme in rodent placenta results in disruption of the normal potassium ion balance, producing the malformation of the right forepaw. Replacement of the potassium lost to acetazolamide inhibition of carbonic anhydrase prevents the terata that would otherwise be induced by acetazolamide. Because the rodent fetus remains in constant orientation *in utero* with its right side against the placenta, it is thought that local disruption of potassium in that area dictates the constant malformation of only the right digit. In those rodent strains having genetic situs inversus, only the corresponding left digit is affected. In primate embryos, there is no detectable carbonic anhydrase at the stage of development where acetazolamide would be expected to induce these malformations.

Aspirin is the most widely used of any medication in the United States. Aspirin is a reliable, reproducible teratogen in rodents, cats, dogs, ferrets, and monkeys when 50–500 mg kg^{-1} oral doses are administered on a susceptible day of gestation. Malformations of all major organ systems, growth retardation, embryonic/fetal death, and behavioral deficits in the survivors are all consequences of prenatal aspirin exposure in common laboratory animals. Epidemiologic studies have failed to demonstrate any syndrome of terata; increased embryonic, fetal, or neonatal mortality; or reduced birth weight that could be attributed to *in utero* aspirin exposure. Some epidemiologic data indicate that mothers of children with congenital malformations actually consumed less aspirin during the first trimester than did mothers of normal children. To be sure, there are case reports of limb reduction defects, cardiac malformations, and even

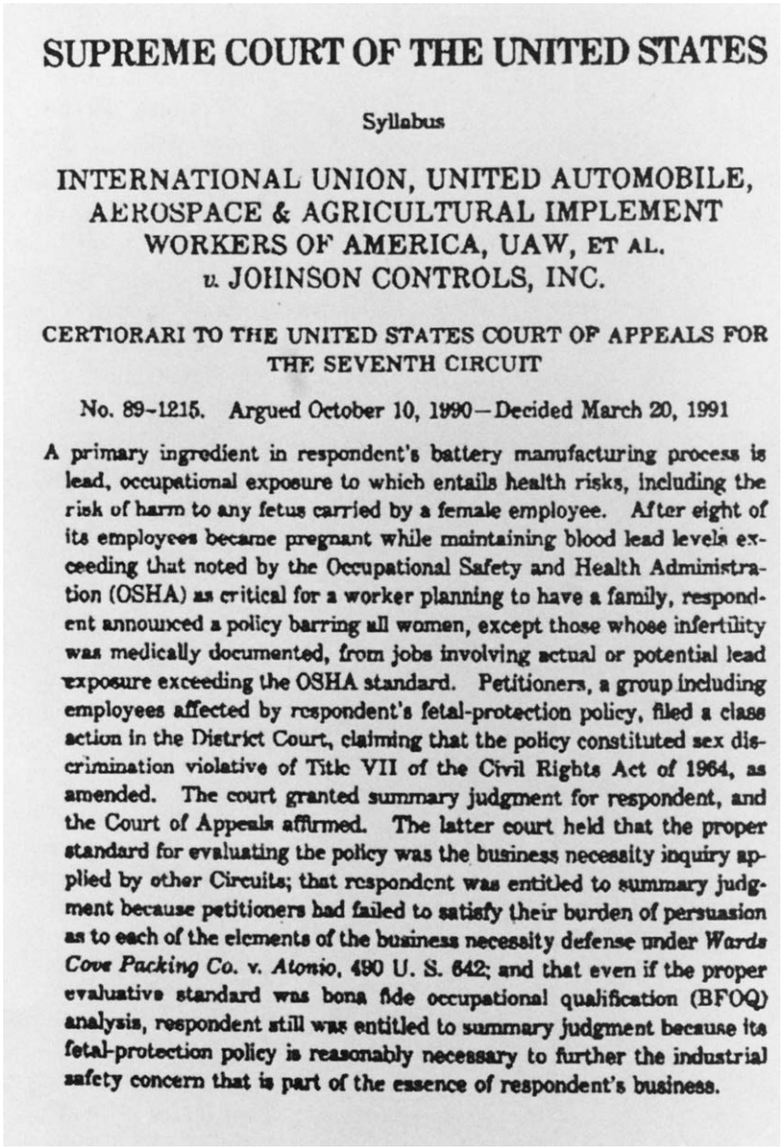
cyclopia associated with prenatal aspirin exposure. When circulating aspirin concentrations in the blood of pregnant rodents or monkeys given teratogenic doses of aspirin are compared with concentrations found in human blood, the values in the animals are of the same order of magnitude as (and in some instances even less than) those found in human blood. Aspirin can be present in human umbilical cord blood and in normal newborn blood at concentrations of up to 10 times those associated with embryotoxicity in animals.

It is acknowledged that, given aspirin's widespread, unrestricted use among the 6 million pregnant women per year in the United States and given the 7.5% total malformation and minimum 13% fetal loss (spontaneous abortion and stillbirth) rates, a great many of these adverse pregnancy outcomes will have experienced aspirin exposures. It is also acknowledged that, if aspirin were a newly developed drug submitted today for regulatory evaluation, it is highly unlikely that it would be approved for marketing. The data published to date demonstrate that, at the aspirin doses usually consumed and at doses less than those causing overt intoxication (salicylism), the risk of adverse pregnancy outcome is no greater than the norm. Like acetazolamide, it appears that there is a physiologic insensitivity on the part of the human embryo compared to other species (which explains the differential teratogenic potency).

Caffeine is, without question, teratogenic in common laboratory animals. It is in the methylxanthine of chocolate and cocoa (2–20 mg/5 oz serving), coffee (74 mg/8 oz serving), soft drinks (30–58 mg/12 oz serving), prescription drugs (32–100 mg), and over-the-counter drugs (30–200 mg). In rodents and rabbits, oral doses of 80–150 mg kg^{-1} day^{-1} induce a consistent pattern of limb reduction defects and delayed development (usually measured as reduced skeletal ossification). A single, large oral bolus is more effective in producing limb malformations than is the same total dose given over several days. More than a dozen published epidemiologic and clinical studies on thousands of pregnant women and their caffeine consumption have failed to confirm any relationship between caffeine and human congenital malformation. Some studies have shown a possibility of increased risk (1.7 times normal) of early spontaneous abortion in selected subgroups consuming total doses in excess of 3 mg kg^{-1}; however, these studies failed to take into account covariates like ethanol and cigarette smoke or measured caffeine consumption before (but not during) pregnancy. A study of offspring of 1529 women who consumed three, four, or six cups of coffee per day (corresponding to 222, 296, and 444 mg caffeine per day, respectively) during early and mid-gestation at birth through age 7 and adjusted for use of ethanol, tobacco, aspirin, acetaminophen, prescription drugs, and also dietary composition found no evidence of functional deficit. There is no question that caffeine crosses the rodent and human placenta and that caffeine has been detected in human umbilical cord and newborn blood. Here dose is the parameter that underlies the differential response in humans and laboratory animals; it is theoretically possible that daily ingestion of caffeine at concentrations equivalent to that of 75 cups of coffee, 125 cups of tea, or 200 cans of a caffeine-containing soft drink could induce terata in humans. Exposures to high doses such as these occurred in the early 1980s from ingestion of mail-order caffeine pills (each pill containing $\sim$500 mg caffeine). These doses produced stroke, convulsions, tachycardia, coma, and at least 12 deaths in acute cardiopulmonary arrest. None of the individuals involved appear to have been pregnant at the time.

Lead is another example of an agent that induces terata in laboratory animals that have no direct concordance in human beings. When pregnant hamsters are given a sufficiently large dose of a water-soluble lead salt early in gestation, the otherwise normal young are born without tails. When first reported, some advocated the position that since humans do not normally grow tails, the observations in hamsters were only a laboratory curiosity. Today, however, the effects of lead on the developing human are well documented. There are at least six major prospective epidemiologic studies on prenatal lead exposure and postnatal cognitive development. Deficits in Bayley Mental Development Index scores and problems with language skills in young children are linked with maternal or umbilical cord lead concentrations in the 20–30 $\mu g\,dl^{-1}$ range. In some children, slowed intellectual development has been noted after exposure to umbilical cord lead concentrations of as little as 10 $\mu g\,dl^{-1}$ during gestation. (By comparison, background blood lead in healthy US adults without occupational lead exposure ranges from 2 to 6 $\mu g\,dl^{-1}$.) It is important to note here that these studies also show that the early postnatal delay in intelligence is not permanent and generally cannot be detected at more than 5 years of age. These data have had a profound influence on social policy worker protection and reproductive health in the United States (**Figure 11**).

Trypan blue, a teratogenic azo dye, is yet another example of a compound which produces malformations in a highly species-specific fashion. Exposure of pregnant rodents to trypan blue during early gestation produces a uniform lumbosacral spina bifida in their offspring (**Figures 12–14**). These malformations are anatomically indistinguishable from those in



SUPREME COURT OF THE UNITED STATES

Syllabus

INTERNATIONAL UNION, UNITED AUTOMOBILE, AEROSPACE & AGRICULTURAL IMPLEMENT WORKERS OF AMERICA, UAW, ET AL. v. JOHNSON CONTROLS, INC.

CERTIORARI TO THE UNITED STATES COURT OF APPEALS FOR THE SEVENTH CIRCUIT

No. 89-1215. Argued October 10, 1990—Decided March 20, 1991

A primary ingredient in respondent's battery manufacturing process is lead, occupational exposure to which entails health risks, including the risk of harm to any fetus carried by a female employee. After eight of its employees became pregnant while maintaining blood lead levels exceeding that noted by the Occupational Safety and Health Administration (OSHA) as critical for a worker planning to have a family, respondent announced a policy barring all women, except those whose infertility was medically documented, from jobs involving actual or potential lead exposure exceeding the OSHA standard. Petitioners, a group including employees affected by respondent's fetal-protection policy, filed a class action in the District Court, claiming that the policy constituted sex discrimination violative of Title VII of the Civil Rights Act of 1964, as amended. The court granted summary judgment for respondent, and the Court of Appeals affirmed. The latter court held that the proper standard for evaluating the policy was the business necessity inquiry applied by other Circuits; that respondent was entitled to summary judgment because petitioners had failed to satisfy their burden of persuasion as to each of the elements of the business necessity defense under *Wards Cove Packing Co.* v. *Atonio*, 490 U. S. 642; and that even if the proper evaluative standard was bona fide occupational qualification (BFOQ) analysis, respondent still was entitled to summary judgment because its fetal-protection policy is reasonably necessary to further the industrial safety concern that is part of the essence of respondent's business.

Figure 11 Summary statement of the famous Johnson Controls decision by the United States Supreme Court in the matter of lead and developmental toxicity.

humans with spina bifida (**Figure 2**d). After trypan blue treatment, the dye distributes to all maternal organs except the brain because it is unable to cross the blood–brain barrier. The embryo does not contain any of the dye, but the cells of the trophoblast, the parietal and the visceral yolk sac, and derivatives of the gut show visible accumulation of the compound. The site of action of trypan blue is the visceral yolk sac and the associated endoderm where it inhibits pinocytosis, causes increased mineral ion absorption, disrupts local osmotic balance, uncouples oxidative phosphorylation, and ultimately interferes with histiotrophic nutrition. Trypan blue does not reach or act on the embryo itself and there are no cellular or subcellular pathologies that can be attributed to the temporal presence of the dye. The interference with embryonic nutrition results in accumulation of fluid (intercellular edema) in the paraxial mesenchyme that is located above the affected endoderm. This is followed by extravasation from the blood islands of the yolk sac placenta into these expanded intercellular spaces, giving rise to failure of neural fold apposition and fusion in that area leading to myeloschisis (**Figure 11**). The cell cycle in the neuroepithelium of the affected area maintains normal generation times and the neuroepithelium continues to grow, only in a mechanically distorted fashion.

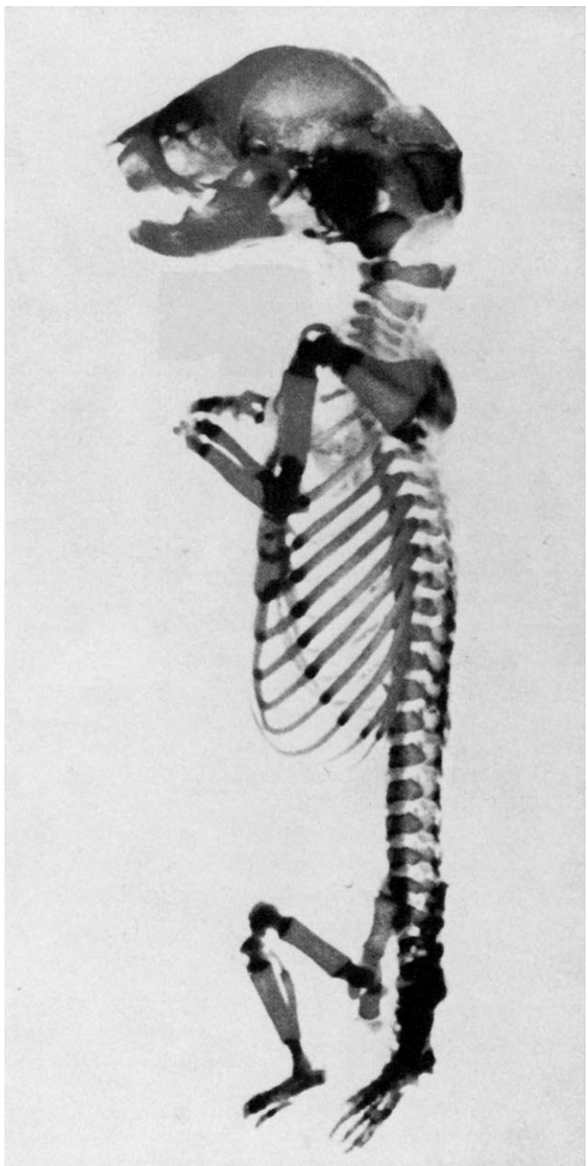


Figure 12 Skeleton of a day 20 control rat fetus. The ossification in the skull, vertebrae, ribs, and sternebrae is nearly complete. Endochondral ossification of the pectoral and pelvic girdle and limbs is progressing ($\times$ 3.2).

In rodents and rabbits, two placentas function during organogenesis: the yolk sac (choriovitelline) and the chorioallantoic placenta. In humans, the chorioallantoic placenta is the organ in which exchange between maternal and embryonic blood takes place. The choriovitelline placenta of rodents and lagomorphs is a selective adaptation in those species which have a very high reproductive potential of short gestation (hamster = 17 days) and large (six to 17 littermates) litters. Trypan blue does not induce its teratogenic response in those species that do not depend on the (comparatively primitive) yolk sac placenta.

Retinoids (vitamin A and its congeners), on the other hand, are agents capable of inducing terata across diverse species. Excess retinoic acid (RA) in human, hamster, and macaque embryos induces a nearly identical syndrome of craniofacial, cardiac, and central nervous system (CNS) terata. The craniofacial defects are due to disruption of the neural crest. The gestational stage during which embryos must be exposed to elicit craniofacial terata corresponds to a time when neural crest cells are still associated with the neuroepithelium. Retinoid teratogenesis displays a classic U-shaped dose–response relationship and has a definite structure–activity relationship. Experimental (proprietary) retinoids have been synthesized which are among the most powerful teratogens known, some being as potent as the most active dioxin. Retinoid deficiency, however, is also teratogenic. Retinoids related to RA (including the 13-*cis* isomer and the oxidized and glucuronide metabolites) are normal constituents of human and animal tissues; thus, one can debate what constitutes a 'safe' dose.

The effects of retinoids on embryos occur through changes in gene expression. Two families of nuclear receptors, termed retinoic acid receptor (RAR) and the related RXRs, have two endogenous retinoids, RA and its 9-*cis* isomer, as normal ligands. These receptors mediate retinoid changes in gene expression. When these receptors bind their particular ligand, they induce transcription by complex with the promoter region of the target genes (termed response element or RARE). The nucleic acid sequence GGTCA is present in at least two parts of the response element and this DNA sequence is the minimum (termed half-site) for binding of one receptor molecule. Subdivisions (RAR-α, RAR-β, and RAR-γ) of each family are recognized, each with a number of isoforms. Retinoid nuclear receptors are subsets of the steroid/thyroid hormone superfamily of nuclear receptors, all of which recognize an AGGTCA sequence, and it is the spacing of this sequence which determines response element specificity for the cognate receptor. The RXR receptor recognizes the AGGTCA half-site separated by one base pair and the RXRs form heterodimers with those nuclear receptors like RARs that bind to direct repeats of the half-site. RARs require heterodimerization with RXRs for efficient DNA binding; after RXR–RAR interaction, binding to the specific DNA sequence is stabilized. The RAR–RXR heterodimers are gene activators in the presence of ligand and are repressors in the absence of ligand.

The expression of the retinoid nuclear receptors and related proteins for cytosol retinoid transport,

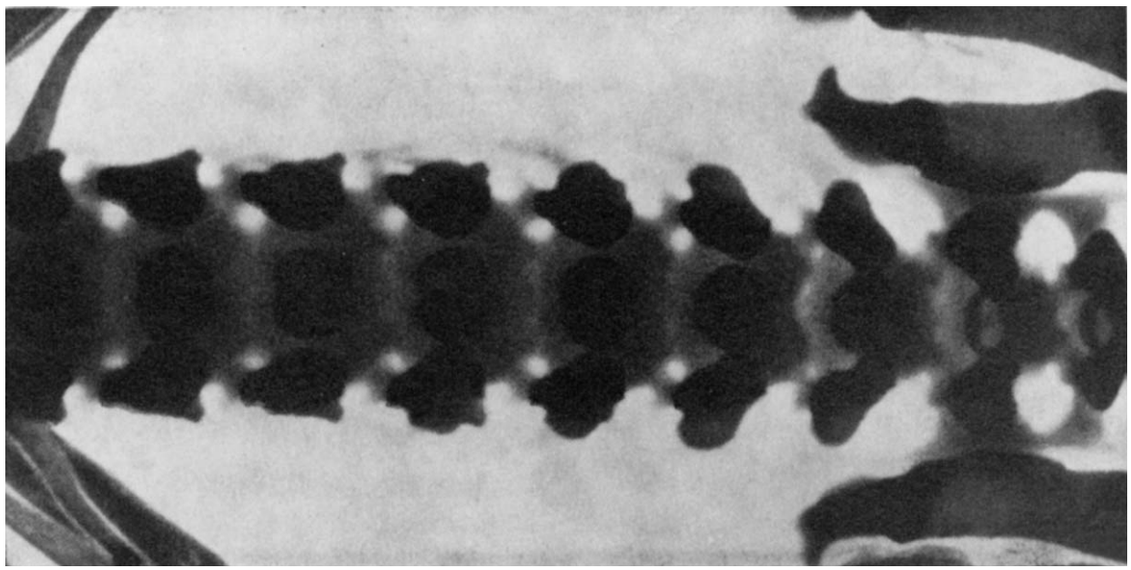


Figure 13 Higher magnification of the vertebrae, ribs, pelvic girdle, and limbs of the fetus shown in **Figure 12**. Note how the ossification centers of the bodies and arches of the vertebrae are regularly oriented ($\times$ 9).

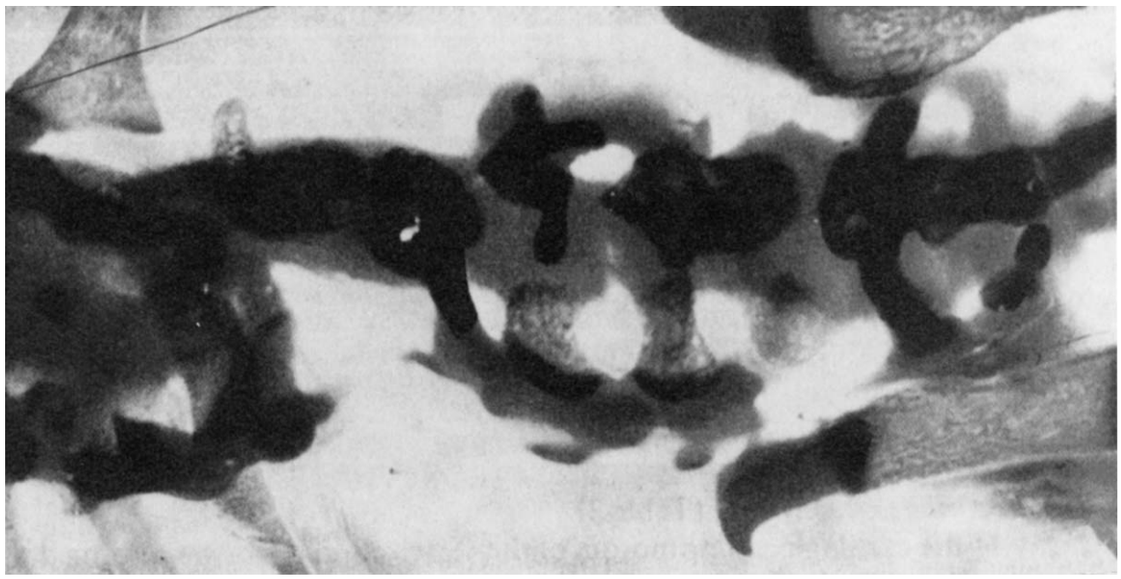


Figure 14 Magnification of the thoracic, lumber, and sacral regions of the spine of a rat fetus with trypan blue-induced spina bifida aperta on day 21 of gestation. Note the severe malformations of the vertebral column when compared to the control (**Figure 13**). The vertebral arches and bodies are so malformed that it is difficult to identify those structures. (Reproduced from Peters PWJ, Verhoef A, De Liefde A, and Berkvens JM (1981) Development of the skeleton in normal rats and in rats with trypan blue-induced spina bifida. *Acta Morphological Neerlando-Scandinavica* 19: 21–34, with permission.)

metabolism, and control of local concentrations of free ligand (termed the CRABPs and CRBPs) in developing embryos are carefully controlled in space and time. Embryonic CRABP is differentially expressed in those structures sensitive to retinoid teratogenesis (e.g., branchial arch mesenchyme and portions of the embryonic CNS), but this cellular binding protein is not directly involved in retinoid mechanism of action. The RAR-α serves a common 'housekeeping' function, and it is present in nearly all embryonic tissues. RAR-β is abundant in the lateral nasal processes and hindbrain neuroectoderm. RAR-γ predominates in somitic mesoderm, frontonasal and precartilaginous mesenchyme, and skin. RAR-β

does not colocalize in embryos with RAR-γ and neither does it occur in the limb, except in the interdigital mesenchyme (which is doomed to die in the formation of normal digits). RAR-β is expressed in inner ear mesenchyme, which gives rise to structures damaged by retinoids and producing deafness in those children who survive (**Figure 15**).

It is the binding and transactivation by the retinoid nuclear receptors on target genes in embryos which is held responsible for retinoid teratogenicity. It is the disruption of normal embryonic segmentation and alterations of normal gene expression by exogenous retinoid which leads to the defects (**Figure 15**). Homeobox (Hox) gene expression is one of the targets. The gene *Hox 2.9* (found normally in rhombomere 4 during stages sensitive to RA insult) is inappropriately expressed in rostral areas, and *Krox-20* (a gene found normally in rhombomere 3) is suppressed after exposure to RA. *Hox 2.9* expression is also disrupted in neural crest and mesoderm – tissues that are known targets in retinoid teratogenesis. Normal segmented patterns of temporal gene expression are either disrupted or lost altogether in RA-treated embryos and it is these genes which have roles in normal cardiac and craniofacial and CNS morphogenesis.

The paradigm emerging is that of a cascade (a series of biochemical steps using secondary, tertiary, or more messenger molecules, each successive step amplifying those of the preceding step). After retinoid absorption from the gut, biotransformation in the liver, transport through the blood and across the

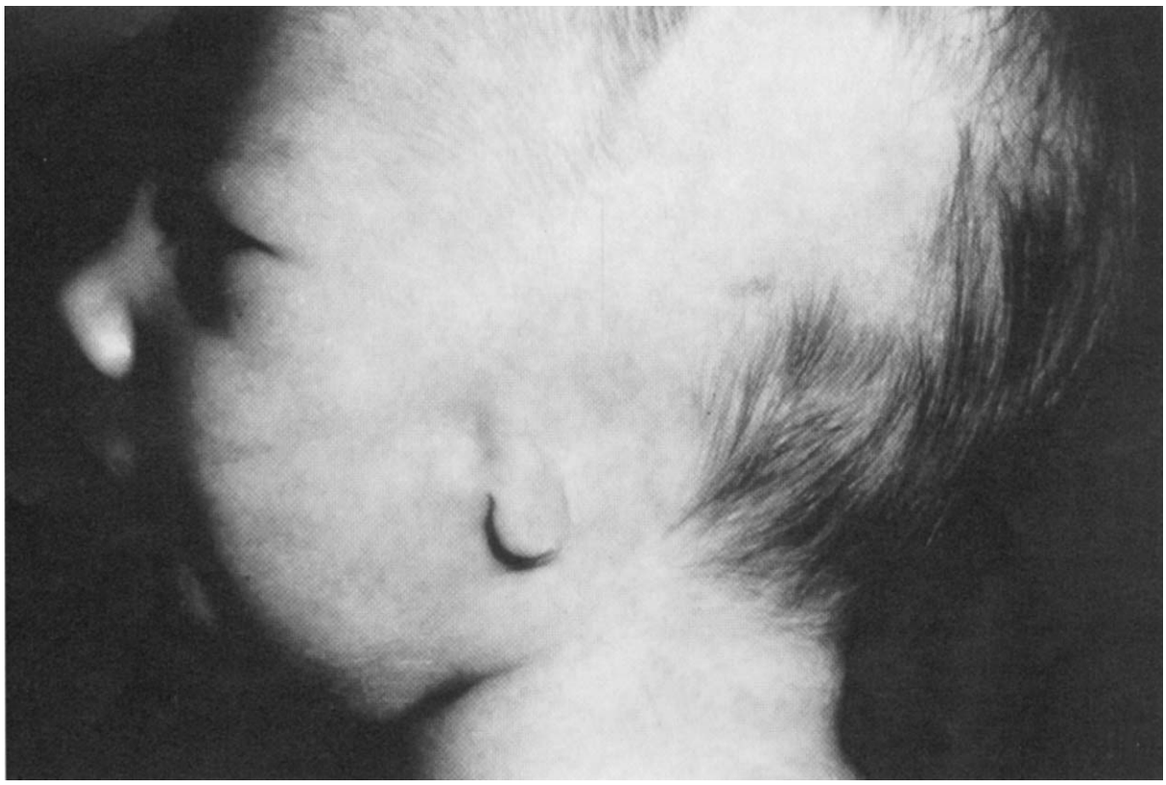


Figure 15 Lateral view of a 2.8 kg newborn at 38 weeks gestation exposed to 80 mg day^{-1} isotretinoin (13-*cis*-retinoic acid) from days 0 through 42 of pregnancy. Note the rudimentary pinna, imperforate external auditory canal, micrognathia, depressed nasal bridge, prominent occiput, and narrow sloping forehead. This child is also afflicted with paralysis of the left side of the face, abnormal visual-evoked potential, hyperterlorism, latent auditory brain stem response, reduced muscle mass and tone in his legs, and eyes that cannot follow. This child also has a cleft palate, ventricular septal defect, aortic stenosis, pulmonary stenosis, dysplastic pulmonary aortic arch, and at 2 months of age he required a tracheostomy and gastrostomy. This collection of malformations is known as retinoid embryopathy. The syndrome is common in infants born to mothers ingesting from 20 to 50 mg day^{-1} isotretinoin in treatment of acne from conception (day 0) or shortly thereafter (days 14–16) through the first trimester (days 35–84). Identical malformations can be induced in fetal hamsters after isotretinoin exposure during equivalent stages of embryogenesis (early primitive streak stage in hamster occurs on day 8); in humans, retinoid embryopathy occurs after exposure during implantation (day 7), the primitive streak stage (day 14), closure of the anterior neuropore (at 10 somites or about day 25), and through appearance of limb buds (days 27 and 28). Thus, the most sensitive time for exposure occurs before the mother knows she is pregnant (i.e., from the first missed menstrual period). (Reproduced from Willhite CC, Hill RM, and Irving DW (1986) Isotretinoin-induced craniofacial malformations in humans and hamsters. *Journal of Craniofacial Genetics and Developmental Biology* 2(Suppl.):193–209.)

placenta, and sequestration in target cells in the embryo the first biochemical actions are triggered by retinoid binding to their cognate nuclear receptor(s). The retinoid–receptor complex binds as a heterodimer to specific upstream regions of the clusters of Hox genes. Hox genes contain the information for patterning of the vertebrae, brain, and branchial arches (Hox codes). The proteins produced by transcription of Hox genes are themselves transcription factors (called homeoproteins). The homeoproteins have DNA-binding domains that are remarkably consistent from amphibians to birds, rodents, and primates – just as are the highly conserved regions of the retinoid nuclear receptors. (It is the highly conserved nature of the morphogenetic mechanism of action of this cascade which forms the basis for the similar teratogenic profiles of retinoids across diverse species – a reflection of the most ancient of vertebrate pattern formation maintained through prehistoric evolution.) Abnormal expression of Hox codes in target cells precipitates magnified local disruption of the ordinary careful control and coordination in vertebrate body pattern formation. Once the small window in space and time has closed and the affected progenitor cells have not arrived at their appointed station in sufficient numbers, all is lost. There is a cascade of abnormal events at the next higher level of organization – when the neural crest cells remain clustered together just under the neuroepithelium instead of migrating down to the branchial arches, insufficient numbers of these cells are available for proliferation and differentiation into bone, muscle, cartilage, thymic, and cardiac tissue and the syndrome known as retinoid embryopathy ensues.

For decades, it has been known that high levels of vitamin A during pregnancy (on the order of 40 000–250 000 international units (IU); 10 000 IU = 3.3 mg retinol) cause structural and behavioral disorders (termed functional deficits or behavioral teratology) in rodents. Prenatal hypervitaminosis A delays and disrupts motor coordination, activity, and learning-related development. In fact, vitamin A has been used for years as a reference teratogen or 'positive control' in the study of potential behavioral teratology of drugs, workplace chemicals, or other agents. After vitamin A doses that do not cause overt structural malformation, neonatal survival and weight may not be affected, but performance of the offspring on a variety of tests designed to assess mental and physical ability (e.g., rotorod balance, righting reflex and other reflex development, running wheel, water T maze, active avoidance, open field, and shock avoidance) is reduced. Thus, postnatal growth and development can be affected by exposures (dose) to retinoids less than those causing obvious structural terata. Behavioral terata induced by retinoids in rodents mirror the human experience. The risk for major craniofacial, cardiac, thymic, and CNS malformations after prenatal isotretinoin (13-*cis*-retinoic acid) exposure (**Figure 15**) is at least 25%, a value excluding those spontaneously or otherwise aborted embryos and fetuses exposed to the drug. Longitudinal follow-up of children who were exposed to isotretinoin *in utero* and survived to 5 years of age show that at least 20% are mentally retarded and that more than 50% are of substandard intelligence (**Figure 16**). The retinoids are an example of the principle that postnatal growth and development can be the most sensitive endpoint in teratogenesis. Retinoids are perhaps the best example of species concordance, dose–response in behavioral disorders at lower exposures, structural terata and growth retardation at somewhat increased exposures, and increased embryonic/fetal death at higher exposures. The abnormal transactivation of the retinoid nuclear receptors and subsequent molecular events precipitate abnormal events at the level of the cell, leading to abnormal anatomic or mechanical disorganization in tissues (e.g., the cranial neural crest) precipitating abnormal morphogenesis of whole organs and systems relating to or surrounding those abnormal organ structures (e.g., malformation of the inner and external ear, the jaw, and CNS). As in retinoid-induced or other CNS malformation (**Figures 4–7**), malformation of the skeleton cannot be separated in the final analysis from malformation of the brain.

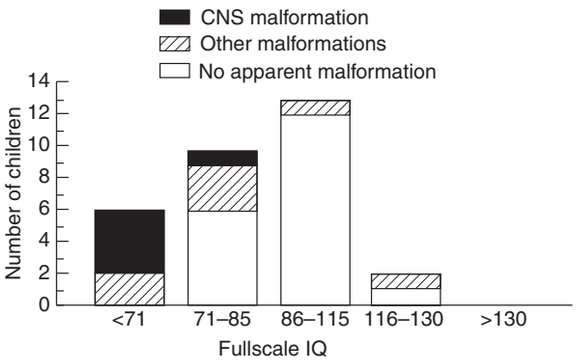


Figure 16 Relationship between congenital malformation and functional deficit in isotretinoin-exposed children at 5 years of age. All of the children classified as mentally retarded (IQ of less than 71) have major malformations. Of those with marginal intellectual ability (full-scale IQ of 71–85), 40% have major structural malformations. Among all children with significant intellectual deficits, 38% have no major malformations. (Reproduced from Adams J and Lammer EJ (1991) Relationship between dysmorphology and neuropsychological function in children exposed to isotretinoin *in utero*. In: *Functional Neuroteratology of Short-Term Exposure to Drugs*, pp. 159–170. Tokyo: Teikyo University Press.)

Principles of Teratology

Evaluation of new pharmaceuticals, pesticides, and the like for developmental toxicity is required by law. Such testing is actually a special type of toxicity testing and the rules for these studies are based on a few generally accepted principles and a number of assumptions. The principles are listed in this section and the assumptions implicit in these studies are discussed. The overall predictive ability of the animal studies to give reliable indication of potential adverse effects in humans is then presented.

There are four general principles of developmental toxicity testing:

1. Developmental stage determines susceptibility to insult.
2. There is a dose–response continuum. The magnitude and duration of the exposure determines the response, which can range from subtle change to frank malformation to death.
3. Genotype influences response.
4. Maternal systems may or may not be influenced by exposure to the insult. Although the ultimate target in the conceptus, the primary site of toxic action may be elsewhere.

Developmental toxicity testing is carried out using animal bioassays. Guidelines published by domestic and international regulatory agencies specify whole animal, mammalian systems. A number of *in vitro* screening methods using invertebrate, amphibian, chick, and cultured normal or neoplastic mammalian cells have been developed, but none of these are currently accepted by regulatory agencies as adequate measures of potential developmental toxicity. These policies stem from experience; as one moves away from whole animal mammalian systems, prediction of teratogenic potential in humans becomes tenuous. *In vitro* and other systems (nematodes (*Caenorhabditis elegans*), fruitfly (*Drosophila*), amphibians (*Xenopus*), and Hydra) are valuable in studies of teratogenic mechanisms of action. Nearly all the basic work in understanding homeotic gene complexes, their arrangements in tandem clusters, their segmented expression in anteroposterior domains which determine position, and the basic tenant that transcription of these genes in space and time is related to their order on the chromosome comes from *Drosophila*. Using homeobox and zinc finger probes developed in *Drosophila*, research is underway to characterize homologous and related genes in mammals, including those homeotic gene complexes which control anteroposterior orientation and organization of the neural tube. The examples discussed in the previous section (aspirin, acetazolamide, caffeine, lead, trypan blue, and retinoids) illustrate that as knowledge of a mechanism is increased, the accuracy of interspecies extrapolation of laboratory data to human beings is increased and one can extend with confidence those conclusions to related compounds or exposure situations. Since the genes involved in embryogenesis have been conserved during evolution, alteration in homeobox sequence and expression as measured in nematode worms, sea urchins, mollusks, annelid worms, fish, chickens, and mice can have similar consequences in human embryos.

There is no perfect animal model for accurate prediction of human teratogenic sensitivity – not all primates respond to all known or suspected human teratogens. Monkeys yield the most predictive dose–response in no more than half of all cases. New methods offer much promise in improving this situation. Using homologous recombination to 'knock out' particular genes in embryos can yield chimeric offspring that provide excellent models of phocomelia, osteogenesis imperfecta, and congenital degeneration of Purkinje cells; inappropriate gene expression is most often lethal but, in some cases, the offspring are phenotypically normal. After insertion of bacterial genes like that for β-galactosidase (lacZ) under control of a weak constitutive promoter, those genes which fall near a strong promoter region are transcribed and the mRNA is translated into a functional enzyme detectable by histochemical methods – producing an insoluble, blue salt in those groups of cells (like neurons) expressing those DNA sequences that can be isolated. Injecting lacZ constructs into fertilized eggs and screening the transgenic embryos yields models like 'Blue mice', giving straightforward indications of disrupted genes. The tools of molecular biology and the application of rigorous interspecies dose scaling based on the principles of physiologically based pharmacokinetics (discussed in a following section) are keys to accurate identification of those agents and levels of exposure with which the public needs to be concerned. The high degree of uncertainty currently associated with interpretation of developmental toxicity data leads to marked discord between the regulated community and those agencies and groups charged with protecting the public health.

Recent developments in biomedicine promise to reduce this uncertainty. One of these new developments involves the completed human genome sequencing project. As a result of this project, we now know, or will shortly know, all of the genes that combine to orchestrate human development. This knowledge, combined with similar information from a variety of animals, has led to the development of a new field

called genomics, literally the study of the genome. Because of recent developments, researchers have tools, for example, DNA microarrays (so-called DNA chips), to study the thousands of genes that must be turned on and off according to a precise development schedule to ensure normal development. Currently, these DNA chips, actually microscope slides, are engineered to contain sequences representing 20 000 or more genes. In fact, it will not be long before all the genes for humans and a variety of animal species will be contained on chips smaller than the size of a microscope slide.

Why is this such a significant development? For an answer, we must go back to the central dogma of biology, that is, DNA makes RNA makes protein makes phenotype. During development from the fertilized egg to the newborn, the genes in the human genome are temporally and spatially read out and translated into their respective proteins. Subsequently, a complex interaction of proteins culminates, in ways only dimly understood, in the phenotype. Prior to the advent of genomics, investigators typically studied genes one at a time, attempting to relate the expression of a particular gene and its encoded protein with a particular developmental phenotype, for example, the eye. Scientists used techniques such as Northern blot analysis, *in situ* hybridization, and immunohistochemistry to study one particular gene or at most a small group of genes. This approach was taken despite the knowledge that literally thousands of genes and proteins were operative at any particular stage of development. With the advent of DNA microarray technology, scientists can now study all genes in the genome simultaneously. How is this capability of monitoring gene expression on a global scale going to reduce the uncertainty in extrapolating developmental toxicity data from animals to humans? One possibility is to use microarrays to identify patterns of gene expression that are associated with congenital defects in animal models (e.g., mouse, rat, rabbit) induced by different individual or classes of developmental toxicants. Such patterns of gene expression could then be used to design appropriate experiments in nonhuman primates to determine whether specific genes or groups of genes are related to abnormal phenotypes. In the end, the hope is that gene expression profiling using DNA microarrays will identify biomarkers of exposure/effect/susceptibility so that data from animal studies can be used to more accurately predict whether a particular developmental toxicant poses a risk of causing birth defects in humans. Gene expression profiling using DNA microarrays has enormous potential; however, much research needs to be completed before we know whether this hope is realized.

Although genomics in general and gene expression profiling in particular offer great promise, scientists are already looking beyond genes and their messenger RNAs to the cellular molecules that actually produce the phenotype, that is, proteins. Although it is currently estimated that there are $\sim$30–40 000 genes in the human genome, it is also estimated that there may be an order of magnitude more proteins because of alternative splicing of RNA and various post-translational modifications of proteins, for example, phosphorylation. Thus, the task of monitoring changes in all cellular proteins, termed the proteome, is daunting. Nonetheless, new developments in protein analysis, particularly developments in mass spectrometry (MS), have spawned a new field of research called proteomics. The goal of proteomics is to develop high-throughput techniques for the simultaneous analysis of all proteins (and their modifications) in the proteome. While currently far from this goal, exciting new developments are coming online frequently. One example is ICAT analysis. ICAT stands for isotope-coded affinity tag. ICATs are small molecules that consist of three elements: (1) an affinity tag (biotin), which is used to isolate ICAT-labeled proteins (peptides), (2) a linker region that can incorporate stable isotopes (e.g., light = hydrogen or heavy = deuterium), and (3) a reactive group that can form a covalent linkage with cysteine residues in proteins. ICATs exist in two forms, heavy (containing eight deuteriums) and light (containing no deuteriums). Using these ICATs, two protein mixtures representing two different states (e.g., embryos exposed to a developmental toxicant compared to unexposed embryos or exposed embryos of a sensitive strain of mice compared to exposed embryos of a resistant strain) are treated with isotopically light and heavy ICAT reagents (e.g., lysates from treated embryos labeled with light ICAT compared to lysates from untreated embryos labeled with heavy ICAT). In each lysate, any one protein is covalently tagged at cysteine residues with either a heavy or light ICAT. The two 'labeled' protein mixtures are then combined and proteolyzed to peptides. ICAT-labeled peptides are isolated utilizing the biotin tag and then separated by microcapillary high-performance liquid chromatography (LC). Each pair of ICAT-labeled peptides is chemically identical, but can be distinguished by LC–MS on the basis of the 8 Da mass difference between the heavy and light ICATs. Because the ratios of the original amounts of proteins from the two lysates are strictly maintained in the peptide fragments, the relative levels of any protein in the two lysates can be determined from the ratio of the peptide pairs. Every other scan is devoted to recording sequence information about an eluting

peptide (tandem mass spectrum), which is subsequently used to identify the parent protein by computer searching the recorded sequence information against large protein databases. Although ICAT analysis has not yet been used to identify specific proteins that play important roles in developmental toxicity, this approach has tremendous potential. Moreover, the combination of gene expression profiling using DNA microarrays and protein expression profiling using ICAT analysis offers the real possibility of identifying genes and their respective proteins that orchestrate the developmental processes that culminate in structural and/or functional birth defects. It is the hope that such information will lead to new strategies for preventing birth defects.

Developmental toxicity testing in laboratory animals is based on the assumption that those species are, in one way or another, similar to humans in their reaction to the compound or agent of concern. The apparent concordance between human and animal data is quite poor – even when normal maternal–placenta–embryo relationships remain intact. In some cases, rodents are more sensitive to the particular chemical insult than rabbits, monkeys, or humans; in other cases, the converse is true. Drug registration, pesticide registration, and other regulatory testing protocols make use of either articulated or implicit assumptions. Two of the assumptions made in the absence of rigorous data are that the metabolic fate and pharmacokinetic parameters of the chemical in animals are similar to those in humans, and that the embryonic and fetal structural and metabolic development in animals is similar to humans. Since no one species is always suitable, regulatory agencies require testing in more than one species in the hope that at least one species will show some relevant adverse effect at a particular dose on which a regulatory decision can be based. In practice, this is usually the no-observed-adverse-effect level (NOAEL), the lowest-observed-adverse-effect level (LOAEL), or a benchmark dose (BMD) in the most sensitive species tested.

Two practical compromises occurred at the outset of these testing protocols. First, the laboratory species used most commonly (rodents and rabbits) are relatively inexpensive, can be bred year-round, and are small, which allows for efficient husbandry, dosing, and ease of handling and permits use of the rather large numbers needed for statistical evaluation. Second, dosing is carried out for a prolonged period of gestation (usually days 6–15 of pregnancy in rats and 6–18 days in rabbits) to cover organogenesis and early fetal life, even though it is recognized that a single exposure during a critical period can be sufficient to induce terata. The latter practice stems from the fact that, at the outset, the investigator cannot be sure which particular gestational stage will be most sensitive; however, this approach is compromised by the possibility that some compounds (like retinoic acid and cadmium) induce or otherwise alter their own metabolism after repeated exposures so that teratogenic potency can be altered.

Methods

Of the 50 or so mixtures, compounds, or agents known to elicit human developmental toxicity under a particular regimen or exposure, the majority were first identified by astute physicians. Some human teratogens have been identified by epidemiologic studies and were only later confirmed in animals. Only a few were identified first in animal studies. Nonetheless, for every known human teratogen, there is at least one animal model. The only way the predictive ability of animal studies can be evaluated is by epidemiologic studies of sufficient statistical power. Some believe that the results of animal developmental toxicity studies have little value in predicting human response; others maintain the opposite point of view. This divergence is due to the physiologic and metabolic differences between humans and common laboratory animals and the differences in how the animal studies have been designed. For example, scientists have generally been unable to reproduce folic acid deficiency-induced NTD in wild-type rodents, probably not because these animals are insensitive to the reproductive consequences of inadequate dietary folate, but because rodents practice coprophagy (feeding on dung) from which they obtain microflora-produced nutrients. Recent studies with 'knockout' (genetically modified) mice lacking normal folate binding and transport capabilities found craniofacial defects in these animals.

The general application of epidemiologic methods to developmental toxicity is described below and followed by a discussion of laboratory studies in rodents and rabbits. The bulk of data available in developmental toxicology are based on these protocols. Since the difference in human and animal response appears to rest in large part on differences in behavior, physiologic parameters, and xenobiotic absorption, distribution, metabolic fate, and elimination, a brief description of transplacental pharmacokinetics is also provided.

Human Studies

The major advantage of studies of humans is that we have the data in the right species. The major

disadvantage is that usually we have no reliable quantification of exposure (dose). It is, therefore, difficult if not impossible to construct a dose–response relationship.

Clinical Data

Malformations like cyclopia occur on average 60 times more often in early, aborted embryos than in infants at term. Cleft lip and palate occur 10 times more often in early abortions than at term. Study of early human embryos could be a very useful tool in identification of developmental toxins, but tabulation of anomalies in aborted embryos and fetuses is not routine. Since the total malformation rate in these spontaneous abortions ranges from 3% to 5%, it is difficult to sort out cause and effect for the comparatively small contribution of exposure to one compound or situation of relatively low teratogenic potential. It is, by comparison, relatively straightforward to identify potent, unique, or unusual teratogens in contrast to a general or common effect (e.g., ventricular septal defect). In some instances, the more rare the malformation (e.g., cyclopia), the more difficult it is to correlate etiology because of the very small number of affected infants at term; conversely, a cluster of very unique findings (e.g., thalidomide-induced phocomelia) has been the best clue historically to identify human teratogens.

Epidemiology

Birth certificates are notoriously poor sources of information because of unintentional and intentional deletion of important data. Three descriptive approaches are useful for generating hypotheses: case reports, correlation studies, and birth defect registries. Four methods are useful in testing these hypotheses: cohort studies, case–control studies, cross-sectional studies, and intervention studies. The greatest limitation to all these approaches is quantifying exposure and correcting for potentially confounding factors (e.g., inherited disorders, maternal parity, disease, age, prepregnancy weight, weight gain during pregnancy, socioeconomic status, ethnicity, and diet).

Case reports can be very useful and have been the mainstay in identification of human teratogens. Usually there is no information on confounding variables, and the actual cause and effect relationship cannot be established by the case report alone. Many known human teratogens were established by a consensus of case reports, later to be confirmed by epidemiologic evaluation. Correlation studies seek relationships between geographic location, time of exposure, personal characteristics, and pregnancy outcome, but it is extremely difficult to correct for those correlations that have no actual influence on the malformation of interest. Birth defect monitoring programs (surveillance registers) can be conducted on an area-wide basis (e.g., California Birth Defects Monitoring Program) or on an industry-wide basis (e.g., Finnish Occupational Registry); however, dose estimation and comparison to control populations are difficult. These programs can provide promising leads only through laborious, expensive follow-up of the mothers with efforts to correct for recall bias. All these methods rely on identification of subgroups placed at increased risk because of their lifestyle, habits, occupations, or medical needs.

Cross-sectional studies can identify prevalence rates based on the distribution of a particular syndrome or malformation, but the study design makes it difficult to identify cause and effect relationships. Case–control protocols match the affected pregnancy to an unaffected pregnancy but, again, it is difficult to account for maternal recall bias and perhaps equally difficult to control for bias (even unconscious) on the part of the investigator. Cohort studies, while suffering from problems of dose determination, are usually prospective, the largest, the most expensive, the slowest, and usually the most statistically powerful to detect reliable associations. Double-blind intervention studies, like those conducted with folic acid and prevention of NTD, usually yield the most conclusive data.

All these epidemiologic approaches are judged by the following criteria:

1. consistency with other epidemiologic findings;
2. specificity of risk with those having highest exposure;
3. strength of statistical association;
4. dose–response relationship;
5. biological plausibility;
6. temporal relationship between exposure and outcome; and
7. statistical significance.

Animal Bioassays

The first step in the laboratory study of developmental toxicity is determination of the substance to be tested. This might be straightforward in drug or pesticide registration studies because only a single pure compound is of concern. In the case of complex mixtures, however, selection of the test substance(s) for the particular condition(s) and route(s) of exposure can be very difficult. For example, gasoline, diesel fuel, or aviation fuel each contain more than 250 diverse hydrocarbons, which change with source of the crude oil, the products are formulated differently

by different refineries and the composition can change with the season of the year. While bioassays can be carried out with complex mixtures or technical-grade materials, interpretation or extension of those data to related or slightly altered materials can be problematic.

The second step in the laboratory study of developmental toxicity is selection of the test species and strain. International (and even state) regulatory requirements differ; most look favorably on data from outbred rats and rabbits from laboratories with cumulative and comprehensive histories of malformation incidence in untreated or control animals. All regulatory agencies recommend selection of a test species that absorbs, distributes, and metabolizes the compound(s) of concern in a manner similar to that of humans; however, human pharmacokinetic data are rare and even those parameters in animals may be lacking. The animals should be housed and treated in accordance with the *Guide for Care and Use of Laboratory Animals* (US National Institutes of Health (NIH) Publication No. 85-23; DHEW Publication No. 74-23). The only difference between the treated and control groups should be exposure to the agent of concern; both groups should be provided with controlled humidity, temperature, light cycle, and the same basic, nutritionally adequate diet.

The third step in the laboratory study of developmental toxicity is the determination of dose, exposure route, and number of animals to be studied. Most regulatory agencies require at least three dose levels in addition to a sham or vehicle control group. The highest dose is expected to produce toxicity in either the offspring or the dams; a reduction in maternal body weight gain or increased mortality (usually not more than 10% maternal death) are accepted as the highest required dose. Some agencies permit substitution of a 'limit test'; that is, if a dose of 1000 mg kg^{-1} fails to induce embryotoxicity or teratogenicity, then a full developmental toxicity bioassay may not be necessary. The lowest dose should elicit no adverse effect. Initial dose selection can be based on the anticipated therapeutic dose (or multiple thereof), on results of a range-finding toxicity study, or on an LD_{50} value in the same or a closely related species. The route of administration should be the same as the route by which humans are exposed. If the compound is to be given orally, it is preferable that intubation be used since incorporation into the feed can yield palatability problems and it provides only an approximation of dose. Regulatory agencies stipulate that 20 litters for rodents and eight to 12 litters for nonrodents per dose group are necessary. Taking note that rodents can have as many as 15–18 offspring per litter, large numbers of animals at each dose can be required for statistical evaluation. It is the litter – not the individual implantation site – that is considered the experimental unit. However, when one encounters a potent teratogen, as few as five litters per dose group may be sufficient to provide meaningful results.

The fourth step in the laboratory study of developmental toxicity is selection of duration of exposure. The reader should be aware that different laboratories count the days of gestation differently; some count the day after breeding as day 0 and others count it as day 1. Regulatory agencies stipulate that treatment begin on day 6 (first day counted as day 0) and continue each day through day 15 in rodents and from days 6 through 18 in rabbits to cover organogenesis and early fetal life. The animals should be weighed prior to dosing, regularly during treatment, and at term. Careful daily inspection for clinical signs of intoxication is needed. Just before term (e.g., day 18 in mice and day 15 in hamsters), the fetuses are collected by cesarean section. Conventional teratology study in rodents requires that the dams and their fetuses be killed just prior to term since malformed or otherwise defective offspring are preferentially cannibalized by the mother. Regulatory requirements for sacrifice of the dam date from 1970 US Food and Drug Administration (FDA) safety evaluation guidelines. Cannibalism of malformed offspring occurs during the immediate postpartum period perhaps precipitated by the pup's inability to move about and/or nurse normally. If the dams are disturbed during this period, this will also precipitate cannibalism.

In contrast to primates, which abort dead embryos, dead rodent embryos are resorbed and the implantation site is recorded as a resorption site. The numbers of living and dead fetuses, and the number of resorption sites, are counted, and fetal weight, sex, and external malformations are recorded. Fetuses can either be inspected fresh in evaluation of internal soft tissues (known as the Staples technique) or can be placed in a fixative (usually Bouin's solution) and sectioned at a later time (Wilson technique). Other fetuses are fixed in ethanol, cleared in potassium hydroxide, and the cartilage and bone are stained with Alcian Blue and Alizarin Red, respectively. The US FDA recommends that one-third of the rodent fetuses be subjected to visceral examination and that two-thirds be studied for abnormalities of cartilage and bone. The US Environmental Protection Agency (EPA) recommends that one-third to one-half of each litter be examined for skeletal anomalies. For rabbits, all fetuses are to be examined for both visceral and skeletal malformations.

There is no rigid, universal definition of what constitutes a malformation, a deviation, a retardation,

an aberration, or a functional disorder. Only death is unequivocal. All species show anatomic variations, particularly in patterns of skeletal ossification. In some cases, delays in ossification can be a reflection of retarded growth, often found in concert with reduced fetal body weight; however, in other cases these changes may be a reflection of terata found after exposures to higher doses.

Just as with an LD_{50} in adult animals where, by definition, one-half of the animals die and one-half live after acute chemical insult, and the reason(s) why one particular animal that is apparently identical to the other members of the group lives and another dies is not known. Some fetuses in a litter can be grossly malformed and the neighboring fetus can be normal. The 'litter effect' is a term applied to the finding that, at some dose, different females treated in the same way vary in the degree and even type of response.

Mention of *in vitro* methods is appropriate here. Study of teratogens with chick embryos can be extremely valuable, as illustrated by the elegant mechanistic studies of retinoids in cultured wing bud; however, the chick embryo suffers from ill-defined metabolic capabilities and the numbers of false positives due to the placement of the test substance directly on the embryo and toxic surface actions are high. Two of the more useful *in vitro* methods are cultured whole rat or mouse embryos and cultured rat and mouse limb buds. For whole embryo culture, the embryos can develop normally during 48 h of head-fold stage and precise control of exposure and experimental conditions can be achieved. The technique is inexpensive and it has been said that, with a little practice, one skilled person can explant and culture 50 embyros in 1 day. The embryos are placed in bottles containing culture medium and the bottles are attached to a rotating drum; during incubation, oxygen and 5% CO_2 are bubbled continuously into the bottle to maintain adequate oxygen and a steady pH. The embryos are very sensitive to changes in temperature and artifacts can be induced. Although novel approaches, like including human serum from patients being treated with various drugs can help in relating the rodent culture results for human health risk assessment, the patterns of malformation induced in cultured rodent embryos can be quite different from those seen in embryos exposed to the same compound *in vivo*. A great many *in vitro* methods have been developed: cultured ova, cultured embryonic pancreas, palate, teeth, lens, kidneys, gonads, bone, thyroid, fish, sea urchin, dissociated adult invertebrate cells, micromass (dispersed embryonic limb and lung), and whole invertebrate or amphibian tail regeneration. None of these, however, have been accepted by regulatory agencies in safety assessment. *In vitro* studies of developmental toxicants are generally regarded as methods for elucidation of mechanism of action and those data can be used to explain and support conclusions reached from conventional protocols.

It has become increasingly important to collect pharmacokinetic and transplacental transfer data in studies of developmental toxicants. These approaches require that quantification of parent compound and metabolites be carried out in a rigorous manner; measurements of total radioactivity from labeled test compounds in blood and tissues are of little utility here. The exception is use of autoradiography of early embryos, where one can obtain an approximation of compound localization in tissues of developing embryos that cannot be otherwise achieved. It may be very important to determine species-specific plasma or erythrocyte binding of the active compound since small differences here can make for large differences in the concentration of free drug and can explain what otherwise looks like a major difference in species sensitivity to the teratogen.

Classical methods for analysis of pharmacokinetic data ('box models') fit sums of exponential functions for two or three mathematical compartments, and these methods usually have no identifiable compartment other than that from which the data arise (e.g., plasma). These methods are useful first steps, yielding concentration:time curves with rates of uptake, the maximum concentration, the rates of elimination, and total dose (measured as the area under the concentration:time curve, AUC). Box models are not particularly useful in scaling dose between species as there are at least four different kinds of dose: administered dose, absorbed dose, metabolized dose, and delivered (or target tissue) dose. Physiologically based pharmacokinetic (PBPK) models have been developed to overcome these limitations (**Figure 17**). These methods require anatomical information (organ-specific blood flow and volume) and physiologic information (rate of metabolism), whereas traditional compartment models assume all input to a central compartment (blood) and free distribution into other compartments. The results from PBPK analyses show the influence of dose-dependent changes in drug behavior and it may be that one species appears most sensitive when compared using an administered dose basis ($mg\,kg^{-1}$ body weight), but these species may be of equivalent sensitivity when one compares the two on a target tissue (delivered) dose basis (e.g., embryonic AUC). Advantages of PBPK analyses include considerations of blood flow and metabolic limitations, anatomic volumes, and other characteristics (such as plasma

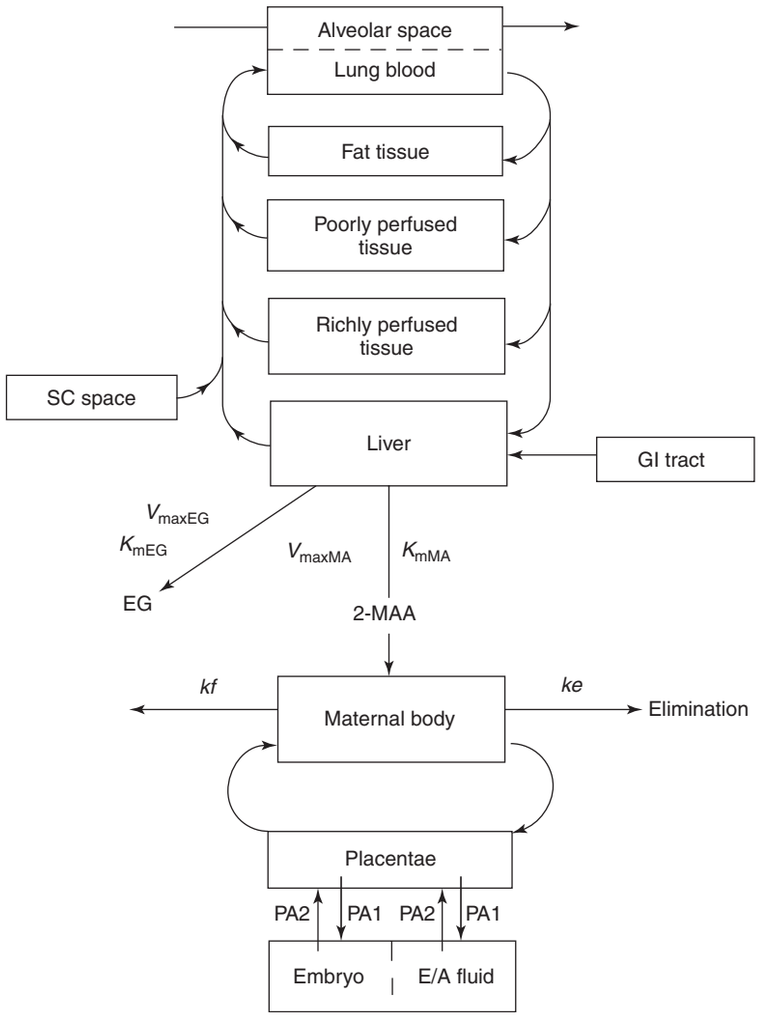


Figure 17 Schematic diagram of a physiologically based pharmacokinetic model used to describe the uptake, distribution, transplacental transfer, and elimination of a 2-methoxyethanol (2-ME) and its teratogenic metabolite, 2-methoxyacetic acid (2-MAA) in the pregnant mouse. Extraembryonic (E/A) fluid is the combined execoelomic and amniotic fluids that surround the embryo. The results of pharmacokinetic studies show that it is the total exposure (concentration $\times$ the exposure time) of the embryo to 2-MAA rather than the peak concentration of this toxic metabolite which is most closely related to teratogenic outcome. (Reproduced from Clarke DO, Elswick BA, Welsch F, and Conolly RB (1993) Pharmacokinetics of 2-methoxyethanol and 2-methoxyacetic acid in the pregnant mouse: a physiologically based pharmacokinetic model. *Toxicology and Applied Pharmacology* 121: 239–252, with permission from Elsevier.)

protein binding). These methods provide increased accuracy for interspecies extrapolation of dose.

Lessons

Three examples of mistakes known to every teratologist and the major lessons learned from each are described below. These lessons provide a warning and a caution to the reader. These lessons demonstrate how data can be misinterpreted or missed altogether and illustrate the responsibilities borne by those who make mistakes in developmental toxicology.

Diethylstilbestrol

For a great many years, the only thought given to transplacental carcinogenesis was the teratoma. Teratomas are – in a word – bizarre. Ordinarily, the primitive streak mesenchyme degenerates at its caudal terminus, but in rare cases (and mostly in female embryos and under unknown circumstances) some of these cells can persist and can become malignant either *in utero* or in infancy. In other cases, the primordial germ cells (which migrate by ameboid movements from the yolk sac along the dorsal mesentery to the gonadal ridge at week 3 and normally differentiate into male and female germ cells) remain

and give rise to teratomas in or on testes or ovaries. These uncommon tumors can arise from all three germ layers, giving rise to a disorganized mass or ball of pancreas mixed with teeth or an eyeball, cartilage, and neural tube. Teratomas can also present as an independent growth, as a partial 'parasitic twin' or 'fetus in fetu'.

Diethylstilbestrol (DES) changed forever the view of transplacental carcinogenesis. DES is a drug that was prescribed to 1 or 2 million pregnant women at doses ranging from 1.5 to 150 mg day^{-1} from 1945 to 1970 to reduce the risk of spontaneous abortion and premature delivery. Female offspring of these women have risk for vaginal and cervical clear-cell adenocarcinoma of 1.4 per 1000. Latency is between 7 and 30 years and peak age of diagnosis is 19 years. These women may also be afflicted with structural terata of the uterus, fallopian tubes, cervix, and vagina, and may present with adenosis. Male offspring experience an increased incidence of urogenital, hypotropic, and capsular induration to testicular tissue and have associated terata of the reproductive system. The total DES dose in mothers of women with vaginal adenocarcinoma ranges from 135 to 18 200 mg.

Conventional developmental toxicity protocols can be conducted as independent studies or as part of a multigeneration or continuous breeding reproduction toxicity test. The animal bioassay for developmental toxicity has traditionally focused on gross congenital malformations, usually induced by exposure during early embryogenesis. By contrast, DES exposure late in gestation by oral dosing or subcutaneous injection in pregnant mice and rats (days 17–21), hamsters (days 14 or 15), and rabbits (days 12–14) with 0.1–40 mg kg^{-1} can increase embryonic mortality, induce cryptorchidism, and cause feminization of male genitalia. Histologic examinations of female and male mice or hamsters born to DES-treated dams reveal cystic metaplasia and neoplasia in segments of the reproductive tract. In animals raised to maturity, reduced fertility, ovarian tumors, endometrial hyperplasia, and uterine adenocarcinoma develop. In female rhesus monkeys born to mothers given oral DES at 1 mg day^{-1} from day 130 to term, vaginal ridging, cervical hooding, and vaginal adenosis develop.

Three lessons are demonstrated by the DES experience:

1. Transplacental carcinogenesis can and does occur in humans and animals.
2. To focus attention on gross terata is to miss important manifestations of developmental toxicity.
3. Exposure during late gestation can have at least as great a consequence as exposure during early embryogenesis.

As a result of lessons learned from DES, an *in utero* exposure phase was added to the carcinogenicity testing requirements for food additives, drugs, and pesticides. The results of multigeneration reproduction studies must be considered together with those from carcinogenicity bioassays and conventional developmental toxicity studies in hazard evaluation.

Thalidomide

No discussion of developmental toxicity can be complete without mention of the thalidomide epidemic of the 1950s and early 1960s, which affected 10 000 children. Thalidomide ranks with methylmercury as the most infamous of all teratogens. Thalidomide was prescribed in Western Europe and Australia as a sedative antiemetic in early pregnancy; it is still used today under careful supervision in the treatment of Hansen's disease (leprosy) and other special situations. Thalidomide phocomelia and associated limb reduction defects (oligodactyly and bone fusions) usually of the radius, humerus, and ulna occurred in at least 96% of those embryos exposed. These defects were usually bilateral and symmetrical and occurred after consumption of 0.5–1.0 mg kg^{-1} day^{-1} (corresponding to maternal circulating concentrations of 1 μg ml^{-1}) from days 34 to 50 of pregnancy. Of the children whose mothers received thalidomide only after day 50, 103 of 104 were normal. Of equal (but perhaps less dramatic) importance were the drug-induced malformations of the ear (anotia and microtia), the congenital deafness, epilepsy, anophthalmia, and the eye muscle and facial paralysis. Nearly one-half of these infants died in their first year, due principally to patent ductus, aortic and ventricular septal defects, and other cardiac malformations that occurred in at least 30% of these infants.

Common laboratory rodents are refractory to thalidomide embryopathy; doses up to 4 g kg^{-1} increased the numbers of abnormal rat and mouse fetuses in only a few of the more than 60 studies. Rabbits do show limb reduction defects (classified as terminal incomplete longitudinal paraxial radial hemimelia) after thalidomide administration, and it is this observation that forms the basis for inclusion of rabbits in current animal testing requirements. Many species have been studied, including the armadillo, baboon (*Papio cynocephalus*), bonnet monkey (*Macaca radiata*), bushbaby, cat, cynomolgus monkey (*Macaca fascicularis*), dog, ferret, green monkey (*Cercopithecus aethiops*), guinea pig, hamster, Japanese monkey (*Macaca fuscata*), marmoset (*Callithrix jacchus*), mouse, pig, rabbit, rat, rhesus monkey (*Macaca mulatta*), and stump-tailed monkey (*Macaca arctoides*). However, only certain strains of rabbit

(given 150 mg kg^{-1} day^{-1}) and nonhuman primates (given 4–45 mg kg^{-1} day^{-1}) respond in a manner similar to that of humans. Not all primates respond to thalidomide (e.g., *Galago crassicaudatus*).

The mechanism of the species-specific action of thalidomide is not known. The compound crosses the placenta and has similar pharmacokinetic parameters in susceptible species (rabbit) and resistant species (rodent). The molecular structure of thalidomide and requirements for malformation are very specific. Thalidomide decomposes rapidly in water to at least 12 different products – all of which are inactive as teratogens and all of which undergo biotransformation *in vivo* to other products. These data prompted the suggestion that it is the parent thalidomide molecule that is the ultimate teratogen. Studies with supidimide analogs and other members of the phthalimide family (**Figure 18**), including side chain or phthalimide ring-modified analogs, show precise structural requirements. Of the numerous phthalimides with their *in vivo* metabolites and related compounds examined, only thalidomide and its EM12 congener are teratogenic. Using topological parameters, geometric parameters, electronic parameters, and physiochemical parameters, several groups have attempted to derive generalized predictors for structure–activity relationships of teratogens; the structure–activity relationships with phthalimides

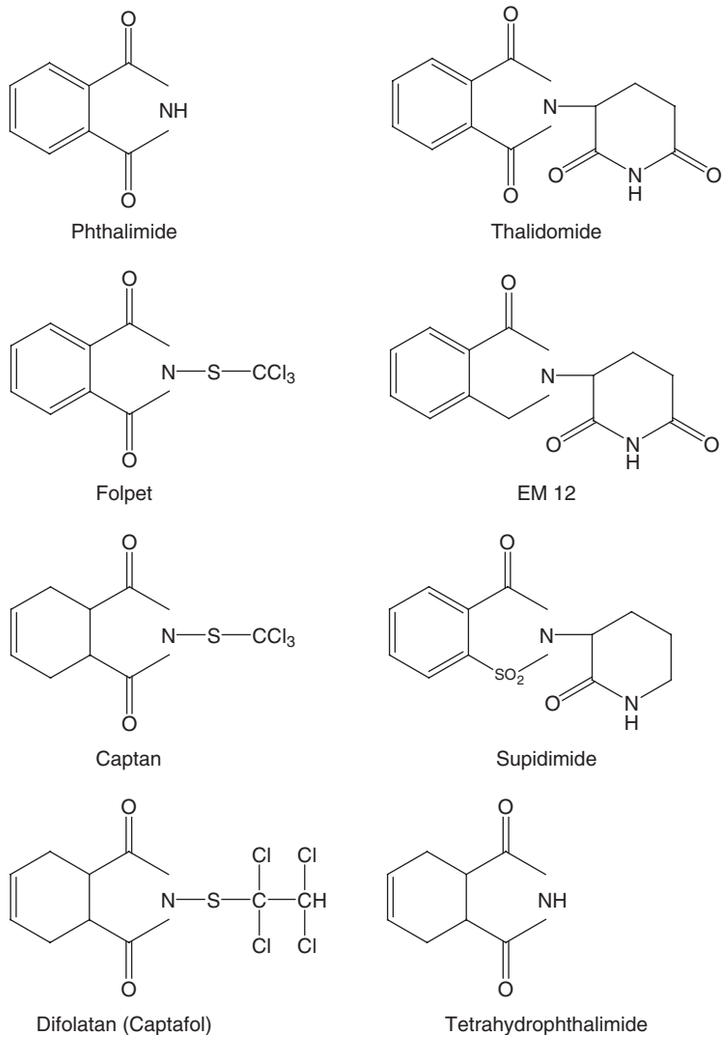


Figure 18 The phthalimide family. In the presence of heat and ammonia, cyclic anhydrides lose a molecule of water and the two acyl groups (R–C = O) become attached in a ring to the ammonia nitrogen, forming an imide. In this way, phthalimide is produced from phthalic anhydride. Phthalimide is acidic and when heating with potassium hydroxide in alcohol along with an alkyl halide, the corresponding *N*-substituted imide is produced. Neither phthalimide nor tetrahydrophthalimide are teratogenic; no derivative of tetrahydrophthalimide (captan and difolatan) is teratogenic. The *N*-substituted imides, folpet and supidimide, are not teratogenic. Of the phthalimides, only thalidomide and its close relative *N*-(2′,6′-dioxopiperiden-3′-yl)-phthalimidine (EM 12) are teratogenic.

point out the severe limitations of this approach. Regulatory agencies use structure–activity relationships to guide recommendations for collection of certain kinds of toxicity data; structure–activity relationships (even for compounds like retinoids in which these are well described) are no substitute for actual developmental toxicity data.

Four lessons are demonstrated by the thalidomide experience:

1. Terata can occur in the offspring of apparently healthy mothers.
2. Human teratogens may have little or no such activity in common laboratory animals.
3. Human terata can occur after exposures less than those used in animal studies.
4. Just because a compound has a chemical structure that appears closely related to an established teratogen does not imply that the congener is teratogenic.

Bendectin

Bendectin (Debendox) was the name given to the 1:1:1 mixture of dicyclomine hydrochloride, doxylamine succinate, and pyridoxine hydrochloride, a prescription drug first marketed in 1956 used to control nausea and vomiting in pregnancy. In 1976, dicyclomine hydrochloride was dropped from the formulation after large clinical trials showed that it did not contribute to the efficacy of the drug. Bendectin use was very common; from 20% to 25% of all expectant mothers used the drug. Approximately 30 million pregnancies were exposed over the 27 years that the drug was available. The customary daily dose was one to four tablets day^{-1}, each containing 20 mg of active ingredient (1 or 2 $mg\,kg^{-1}\,day^{-1}$).

Bendectin is not teratogenic in laboratory animals (including nonhuman primates), but developmental toxicity (reduced fetal body weight and delayed skeletal ossification) can be induced in animals after exposures (500–800 $mg\,kg^{-1}\,day^{-1}$) that also cause frank maternal intoxication and/or increased maternal death. There are six published *in vitro* studies of Bendectin using cultured rodent embryos or embryonic cells; of those, only one, of mesenchymal cells, showed any indication of toxicity and that occurred after exposures to concentrations far greater than can be achieved in humans after ingestion of therapeutic doses (25 mg or more). There are at least 14 cohort and 18 case–control epidemiologic investigations on Bendectin and pregnancy outcome in addition to one, by the NIH, in which the occurrence of congenital malformations was prospectively studied in 31 564 newborns. The results of the NIH study, like those of others, found that the odds ratio for any of 58 major categories of malformation and Bendectin exposure was 1.0 – exactly that which is expected by chance alone. Of those categories with trends or suggestive positive associations, the magnitude of those associations was as great as that from vomiting during pregnancy without Bendectin use as with Bendectin use. There was no increase in malformation rate after exposure to Bendectin *in utero* than would otherwise be expected by chance; there are no objective data to conclude that exposure to Bendectin in animals or humans has any adverse effects on embryonic or fetal development at the doses used by these 30 million women.

As of 1987, at least 300 lawsuits had been filed alleging that Bendectin caused congenital malformations. The drug was dropped from commerce not because of lack of efficacy, or because it caused toxicity or because there was no market for the drug, but because of the excessive cost incurred by the manufacturer in defending the drug in litigation. The major focus here was the allegation that prenatal Bendectin exposure caused phocomelia and assorted limb reduction defects. Given the 'background' or 'spontaneous' rate of major congenital malformation in the United States (3%), it would be expected that 900 000 malformed children would be born to those 30 million mothers even in the absence of any drug use. Given the US background rate for limb reduction defects (one per 3000 births), 10 000 such defects would be expected to occur even in the absence of any drug use.

How then can it be that a compound which has no detectable teratogenic activity in either animal or human studies be held responsible for human congenital malformations? The history of Bendectin can be traced to ignorance of the principles of teratology, compounded by precedential case law following the first erroneous decision and to two articles appearing in the popular press. In the September 1979 issue of the *National Enquirer*, the following was published:

> *Experts Reveal…Common Drug Causing Deformed Babies. In a monstrous scandal that could be far larger than the thalidomide horror, untold thousands of babies are being born with hideous defects after their mothers took an anti-nausea drug (Bendectin) during early pregnancy.*

At least seven women are known to have elected abortions as a consequence. In the magazine, *Mother Jones* ('The Bendectin Coverup', November, 1980), the authors counseled women to use – instead of Bendectin – 'natural alternatives' including 100 mg of pyridoxine (a dose 10 times that of the same compound in the Bendectin formulation).

The Bendectin lawsuits stem from the age-old question that always follows the birth of a malformed child, 'What caused it?', together with today's substantial monetary rewards that can befall successful litigation or out-of-court settlement, particularly with a large, impersonal, and wealthy corporation. Compounded by parental shock, denial, anger, and sadness, the potential astronomical medical costs and social costs for the child, the financial rewards offered by expert witness testimony, the inadequate research and reporting necessary for sensationalization in the popular press, and successful settlement or judgment in preceding cases all combine to perpetuate the myth. Juries – being people – are by nature very sympathetic to the plight of the child and his or her family who are through no fault of their own in need of financial and other assistance to address their situation. The desire to help can be overwhelming. The original Bendectin lawsuits arose from failure to understand proper interpretation of the animal data, the desire to identify a responsible agent, and from substituting a strength-of-evidence approach for the weight-of-evidence evaluation of the data. Fundamental here was the failure to apply properly the concept of dose response. Only after the accumulated weight of many consistent epidemiologic findings of sufficient statistical power, accounting for confounding factors and where exposures are of sufficient magnitude and during appropriate periods in gestation, can conclusions on excess risk be drawn. In the strictest sense then, the best the science of teratology can offer on the concept of safety is the statement that the exposure of concern represents no measurable excess risk.

Three lessons demonstrated by the Bendectin experience are the following:

1. Human teratogens can be confirmed only by consistent findings in epidemiologic studies, recognizing that at the 95% confidence limit, the results in one of 20 studies of equal statistical power can differ by chance alone.
2. Maternal intoxication in animal studies in and of itself can contribute to abnormalities in the offspring.
3. Overall weight of evidence should be used in identification of compounds, agents, and exposures of concern.

Data Interpretation and Regulatory Policy

Some have criticized those groups charged with protection of the public health for failure to designate a code or notation which can be assigned to a chemical showing it to be a reproductive or developmental toxicant (see Further Reading, US EPA, 1991). Developmental toxicity is usually not the only kind of toxicity associated with exposures of concern. A 1985 US EPA reevaluation of 18 pesticides (avermectin, cacodylic acid, captafol, captan, cyanazine, dinocap, EDBC, endrin, fenarimol, folpet, fusilad, nitrofen, pentachlorophenol, 2,4,5-T, silvex, TPTH, triadimefon, and warfarin) and six industrial chemicals (arsenic, two glycol ethers, lead, chloromethane, and mercury) found that in no case was embryonic or fetal toxicity the sole documented effect. Teratogenic activity almost always occurs in tandem with other adverse effects on health, including mutagenicity, male or female reproductive toxicity or carcinogenicity. Gender-specific requirements like the protective work clothing promulgated for women in the case of endrin and silvex cannot be justified, not only on the basis of US Supreme Court decisions (**Figure 11**) or because gender-specific regulations concerning 'women of child-bearing age' restrict the activities of those women who are not fertile but also because such regulations carry with them high social costs. Regulations concerning only pregnant women would most likely fail to protect the embryo since it is exposures which occur before the patient recognizes that she is pregnant which are most likely to damage her embryo. Correct determination of safe levels of exposure rests with identification of the end point of toxicity associated with the LOAEL, regardless of whether it is developmental toxicity or another expression of toxicity.

The conventional method for interspecies extrapolation of developmental toxicity data involves empiric determination of an NOAEL in the test species followed by application of an uncertainty (or 'safety') factor to calculate the safe dose for the species or individual of interest. These calculations are often based on the administered dose and are usually expressed on a body weight basis. The default safety factor used can range from 100 to 1000 or more. Dose scaling on a body weight or surface area basis is fraught with problems. For example, scaling of a child's sulfonamide dose to the adult by body weight, surface area, age, or caloric expenditure (as a surrogate for metabolic rate) yields an adult daily and grossly excessive dose of 7 g. In the case of teratogens, the problem is even more acute. The absorbed dose can be so small (e.g., cyclopamine) or the site of drug action so restricted (e.g., trypan blue) that the quantitative differences between the species are so great that the result is judged an inherent qualitative difference. In the case of vitamin A and its metabolites, the conventional safety factor approach results in calculation of vitamin A doses so small that the risk of vitamin A deficiency-induced terata is increased.

Thirty years ago, Karnofsky D listed the two basic tenets used today in interpretation of animal developmental toxicity data. All compounds can produce embryotoxicity if applied in sufficient dosage at an appropriate stage of development, and the purpose of evaluating chemicals for teratogenic potential is not to eliminate from use, but rather to estimate the hazard its use presents to the human embryo. Teratologists believe that developmental toxicity can be viewed most accurately from the threshold concept; that is, there exists an exposure below which no adverse effect will occur. This belief arises from experience with human teratogens, from pharmacokinetic considerations, and because embryos – to a point – have the remarkable ability to compensate for lost or damaged cells. For instance, early rodent and rabbit embryos can develop quite normally after surgical obliteration of at least 50% of the entire inner cell mass. Implicit here is that one must distinguish between theoretical risk and practical risk. Using statistics, arbitrary safety factors, or subjective means, theoretical risk can always be calculated and upper-bound confidence levels assigned. Multiple and consistent epidemiologic studies of high quality showing no association between developmental morbidity or mortality and the exposure of concern can never prove that no hazard exists; these data can be used only to demonstrate that the risk, if any, is so small that for all practical purposes it can be disregarded.

The weight-of-evidence evaluation takes into account the human experience and the animal data. Interpretation of human data depends on the design of the study, definition of the cohort, quantification of exposure, validity of ascertainment, control for confounding factors, size of the study population, and appropriate statistical methods. In practice, identification of human teratogens has relied on answers to two questions:

1. Is there a distinctive pattern of malformation which can be associated with the exposure of concern?
2. Have there been sufficient numbers of cases to substantiate the conclusion?

The principal disadvantage to using human data in this way is, of course, that recognition occurs after the fact. Interpretation of the animal data is carried out with the following in mind. Without exception – even with very odd, rare, or unique malformation – there is no case in which the defect has not occurred sporadically; there are no known examples of agents which cause malformation that cannot also be caused by some other agent; some species are prone to particular types of congenital malformation (e.g., cleft palate in mice); and chemicals that are not teratogenic in animals (e.g., coumarin anticoagulants) can be teratogenic in humans. Animal data can only be used to provide an approximation of risk in humans. Interpretation of the animal data rests on answers to these seven questions:

1. Were the studies carried out in a species that handles the compound in a manner similar to that in humans?
2. Were the studies carried out using a route of administration applicable to anticipated human exposure?
3. Does the effect occur in more than one species?
4. Does the effect occur after doses less than those which elicit other types of toxicity?
5. Was a significant increase in the numbers of litters containing abnormal outcome observed?
6. Was a dose–response relationship identified?
7. What populations are at risk of exposure and what is the magnitude of their exposure?

Of great concern are those teratogens, like thalidomide, which are able to induce terata in the absence of any apparent disturbance in maternal well-being. A large difference in the dose required to cause embryotoxicity and a much higher dose required to cause maternal toxicity can be key in identification of those agents. Where maternal toxicity occurs after exposures equivalent to those causing increased embryonic morbidity and mortality, then estimates of safe exposure can be derived using safety factors applied to the NOAEL for adults. Even studies with low statistical power can contribute to the total weight of evidence; a strength-of-evidence (picking and choosing data to support one or another conclusion) approach cannot be justified.

In summarizing the results of a risk assessment, it is important that the reader look for the following three key points: a discussion of the quality of the studies supporting the concern for risk of developmental toxicity, the confidence that one can place in the NOAEL derived, and the list of uncertainties in the assessment. Animal studies (like those for aspirin and caffeine) usually employ doses much greater than those to which humans are or could be exposed. When extremely high doses are used, drug actions in the mother can elicit systemic poisoning which by itself may or may not be responsible for the effects seen. Even very reliable animal teratogens may have no counterpart in humans since the human exposures are relatively small.

Conclusions

What is the solution to the problem of birth defects? If not lists of developmental toxins, printed and electronic warnings, or new laws or judicial decree, then what? Although answers to the question can be simple, true solutions to the problem are complex. Only by reductions in the numbers of infants with birth defects can significant inroads to reducing infant mortality be made. The solutions are complex and expensive, but not as expensive as the cumulative charges for labor, postpartum care, surgery, hospitalization, prostheses, lost wages, and the extra social and educational services required by these patients. For every $1 spent on research into the cause and prevention of birth defects, published data show that $11 can be saved in public expenditure, insurance claims, and legal and medical payments made by individuals to address or attempt to rectify the problem.

Only by knowing the cause of a problem can an intelligent solution be devised. Basic research into the 80% of all birth defects that are of unknown cause (**Figure 9**) sponsored by groups like the March of Dimes, the Deutsche Forschungsgemeinschaft, and the US Public Health Service is key. This effort has two fronts: (1) the systematic collection and evaluation of human data and (2) basic laboratory investigation. These efforts include central birth defect registries in Australia, California, Canada, Finland, Japan (Ishikawa, Kanagawa, Osaka, Tottori, and the National Association for Maternal Welfare), New York, Sweden, the Commission on Professional and Hospital Activities (Michigan), the US Centers for Disease Control (Georgia), and the March of Dimes Nationwide Information Center (Massachusetts). For example, the first indication of valproic acid teratogenicity was detected by the Rhone-Alpes birth defect registry.

Understanding how embryos grow is fundamental to understanding and preventing birth defects. How does the embryo know its back from front, top from bottom? How does it control shape change from an initial ball of rapidly dividing cells to form the segmented cylinder of the torso? It is remarkable that the molecular biology of *Drosophila* has a direct bearing on understanding human embryonic development. Of equal import is understanding what the animal data are trying to tell us; as has been said by toxicologists on more than one occasion, 'The animals never lie' – it is only our flimsy interpretation of the results of the animal studies upon which doubt can be cast. To make use of those data and to develop credible public health standards based on those data, fundamental knowledge of control mechanisms in normal embryonic development and rigorous methods for interspecies extrapolation of animal and *in vitro* data are urgently needed. It makes little sense to generate animal developmental toxicity data if in the end we do not know how, if at all, those data relate to human beings. Fortunately, progress in two areas of research provide optimism that we may, in the not too distant future, gain the information needed to make accurate predictions of human risk. One advancement comes from developmental biology, a field of study that focuses on how the fertilized egg develops into an adult capable of producing gametes of the next generation. Over the past 20 years, research using roundworms (*C. elegans*), fruitflies (*Drosophila*), fish (zebrafish), frogs (Xenopus), birds (chicken), and mice has revealed several revolutionary findings. First, research has shown that development in these widely divergent organisms proceeds using similar mechanisms of communication both within and between cells of the embryo. Second, communication within and between cells in embryos from the roundworm to the mouse (and undoubtedly the human as well) involves a relatively small number (estimated to be 17) of signal transduction pathways. These pathways are used at different times and in different contexts in different organisms; however, in all animals these pathways orchestrate the complex processes that facilitate development from the fertilized egg to the newborn. Third, these signaling pathways are highly conserved from roundworms to chordates. What is different between roundworms and chordates is the way in which these pathways are used. Different organisms use different combinations of pathways at different times and in different places in the embryo to achieve species-specific gene expression patterns and thereby normal development. Nonetheless, the study of how specific signaling pathways are regulated during normal and abnormal fruitfly development, for example, has direct relevance to the study of human development and how developmental toxicants cause birth defects. Thus, the addition of new information from developmental biology continues to generate new insights relevant to understanding human development and preventing birth defects. Another avenue of great promise is the development of accurate PBPK models (**Figure 17**) scaling dose from rodent to rabbit to nonhuman primate to human. Only on very rare occasions is there an opportunity to measure the compound(s) of concern in human tissue after quantifiable maternal exposure, but it has been done and every effort must be made to use the results to confirm and validate the confidence that can be placed in the PBPK results. While PBPK interspecies scaling of dose can be handled, these methods cannot scale response.

Once one knows the problem and has devised a solution, then the real job begins. National Center for Health Statistics data show a decline in total US infant mortality from 1982 to 1992, but marked geographic and racial differences remain. The 1992 overall US rate of infant death was 8.5 per 1000 live births (California, 6.9; Texas, 7.7; New York, 8.5; New Jersey, 8.5; Pennsylvania, 8.6; Ohio, 8.7; Florida, 9.1; Illinois, 10.0; Georgia, 10.4; Michigan, 10.5) – a decline attributed not to reductions in the numbers of birth defects or premature births but to improved neonatal intensive care units and the introduction of synthetic pulmonary surfactants and consequent reductions in death from acute neonatal respiratory distress syndrome. Still, the years of potential life lost due to birth defects ranks fifth, just behind that of homicide and suicide (1, unintentional injury; 2, cancer; 3, cardiovascular disease); prematurity/low birth weight ranks sixth and sudden infant death syndrome seventh. Ethnic discrepancy remains pronounced; rates of White (5.8 per 1000 live births) and Cuban Hispanic (3.7 per 1000 live births) infant death are similar, but the 2002 rate for Blacks (13.9 per 1000 live births) increased compared to the previous year.

Aggressive public health efforts to combat cigarette smoking during pregnancy can pay for themselves at least six times over. Targeting low-income and other women to increase their consumption of fruit and vegetables and to improve their folate status can prevent at least 50% to perhaps 80% of all cases of anencephalus and spina bifida. Ethanol has without doubt been responsible for congenital mental retardation for thousands of years; compassionate and performance-driven intervention is critical for Indian reservations where whole towns and villages are affected. Not that these measures are popular or easy; they are not attractive high technology and they suffer from the fact that one cannot identify a villain or other responsible party upon which to place blame, extract compensation, and with whom one can take free journalistic license. That these steps are not easy can best be illustrated by iodine. Today's residents of wealthy industrialized countries give not a second thought to iodized salt; supplementary dietary iodine in prevention of goiter and cretinism is so common that these problems have disappeared. Why, then, does this completely preventable problem persist in the Third World? In impoverished countries, it is routine that a salt merchant equipped only with sack, shovel, and camel or mule should go to the salt mine and return to the market to sell his wares. The consumer, whose total annual income may be only a few hundred or less US dollars and who is likely illiterate, faced with the choice between the more expensive iodized variety (which looks, tastes, and for all intents and purposes works just as well as the 'natural product') and the less expensive native rock salt, will select the less expensive item. The cultural resistance and practical problems faced by the World Health Organization and United Nations in widespread dissemination of iodine or vitamin A supplements are legend, but their successful introductions can make and have made tremendous improvements in the quality of life and have reduced the incidence of birth defects.

While it remains important to identify anthropogenic and iatrogenic contributions to the etiology of birth defects, it must be recognized that despite all the resources devoted to laboratory testing, regulation, banning, restricting or listing of drugs, pesticides, industrial chemicals, and hazardous waste, birth defects occurred long before these chemical products and their wastes were introduced to our environment. Despite our best efforts, only modest progress has been made in preventing congenital malformations. Embryogenesis is exceedingly complex – after one observes the heart form from cardiac jelly (literally by growing back on itself and tying itself into a knot), the partitioning of aortic sac and conotruncus, migration of neural crest to aorticopulmonary septum, and development of associated circulation, it is a wonder things go as well as they do as often as they do. In the final analysis, it is becoming apparent that factors like inadequate or improper nutrition, ethanol, and other lifestyle factors contribute far more to the load of human congenital malformations and other manifestations of reproductive failure than has been appreciated to date.

See also: Carcinogenesis; Chromosome Aberrations; Dominant Lethal Tests; Dose–Response Relationship; Environmental Hormone Disruptors; Epidemiology; Levels of Effect in Toxicological Assessment; Molecular Toxicology–Recombinant DNA Technology; Reproductive System, Female; Reproductive System, Male; Risk Assessment, Human Health; Toxicity Testing, Developmental; Toxicity Testing, Reproductive; Toxicology, History of.

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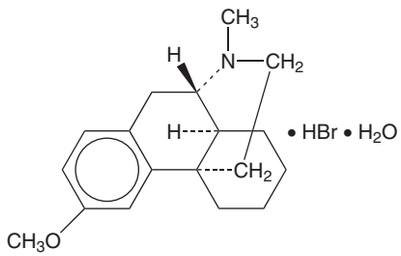
Dextromethorphan

Michael Wahl

This article is a revision of the previous print edition article by Carole Wezorek, volume 1, pp. 453–454, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 125-71-3
- Synonyms: DM; Dextromethorphan hydrobromide; Demorphan; 3-Methoxy-*n*-methylmorphinan; D-Methorphan; Drug store wine; Robowing
- Chemical/Pharmaceutical/Other Class: The methyl ether of the dextrorotatory form of levorphanol, an opiate analgesic
- Chemical Formula: $C_{18}H_{25}NO$
- Chemical Structure:



Uses

Dextromethorphan is used as an antitussive and cough suppressant. It is also used as a drug of abuse.

Exposure Routes and Pathways

Dextromethorphan preparations are administered orally in tablet or liquid form. Ingestion is the most common route of accidental and intentional exposure to dextromethorphan.

Toxicokinetics

Dextromethorphan is rapidly and well absorbed from the gastrointestinal tract. Erratic and slower absorption may occur with high-dose and sustained-release products. In therapeutic doses, peak plasma levels occur at 2.5 h with conventional dosage forms and at 6 h with sustained-release preparations. Dextromethorphan is quickly converted in the liver to an *o*-demethylated product, dextrorphan (3-hydroxy-*N*-methylmorphinan). Dextromethorphan undergoes polymorphic metabolism, depending on variations in cytochrome P450 enzyme phenotype. A total of 5–10% of the Caucasian population are poor metabolizers. The serum half-life of the parent drug is greatly increased in these individuals. Only minor amounts of active metabolites are formed in poor metabolizers. The volume of distribution of dextromethorphan is 5–6.4 l kg^{-1} in dogs. Dextromethorphan and the glucuronide and sulfate ester conjugate, together with (+)-3-hydroxy-*N*-morphinan and traces of unmetabolized dextromethorphan, are excreted in the urine. The plasma half-life is about 2–4 h with conventional dosage forms.

Mechanism of Toxicity

Dextromethorphan is the methylated dextro-isomer of levorphanol. Unlike the L-isomer, it has no analgesic properties. Dextromethorphan acts on the central nervous system (CNS) to elevate the cough threshold. It retains only the antitussive activity of other morphine derivatives. Administration of dextromethorphan may be associated with histamine release. Dextromethorphan is often present in multisymptom products with a combination of ingredients. Toxic effects of concurrent agents such as antihistamines, decongestants, analgesics, and/or alcohol may be exhibited.

Acute and Short-Term Toxicity (or Exposure)

Human

Life-threatening dosage of dextromethorphan is low. CNS depression may result, with amounts of $10\,mg\,kg^{-1}$ or more, in children. Adults may tolerate up to $14\,mg\,kg^{-1}$ with only minor effects. Ingestion of large amounts of dextromethorphan may result in lethargy, respiratory depression, nystagmus, psychosis (euphoria, hallucinations, paranoia, disorientation), and coma. Following a large ingestion of a long-acting preparation, symptoms may persist for 7 or 8 h. Hemodynamic compromise and other severe symptomatology may result from concurrent ingredients in multisymptom cold products.

Chronic Toxicity (or Exposure)

Human

Chronic abuse of dextromethorphan has resulted in psychotic behavior and hallucinations.

In Vitro Toxicity Data

Studies of rat mesencephalic neuron–glia cultures demonstrated decreased glia-induced inflammatory response in the presence of dextromethorphan.

Clinical Management

Basic and advanced life-support measures should be instituted as necessary. Gastric decontamination may be performed as indicated by the patient's symptomatology, the specific product involved, and the history of the ingestion. Activated charcoal may be useful to adsorb dextromethorphan and concurrent ingestants. Naloxone may be helpful in reversing the CNS and respiratory depressant effects of dextromethorphan. The level of consciousness and respiratory status should be carefully monitored. Management of the toxicological consequences of coingestants should be appropriate to the agent involved. Plasma dextromethorphan levels are not clinically beneficial to management of the overdose but may be useful in determining the metabolizer phenotype.

See also: Gastrointestinal System.

Further Reading

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Diabetes, Effect of Toxicity

Kartik Shankar and Harihara M Mehendale

Etiology of Diabetes

Diabetes 'mellitus' is a metabolic disorder characterized by hyperglycemia. Two principal forms of diabetes exist: type 1 diabetes (insulin-dependent diabetes mellitus) and type 2 diabetes (noninsulin-dependent diabetes mellitus). Type 1 diabetes also called as early-onset diabetes occurs due to insufficient insulin secretion by the pancreas, leading to hyperglycemia. This type of diabetes requires treatment with extraneous insulin. The second and more prevalent form of diabetes (90–95% cases of diabetics suffer from type 2 diabetes) is of a more complex etiology. Type 2 diabetes occurs primarily due to peripheral insulin resistance and is (at least in its early stages) accompanied with hyperinsulinemia. Both type 1 and type 2 forms of diabetes have been shown to have some genetic basis. Diet and obesity

significantly affect predisposition of an individual to type 2 diabetes.

Impact of Diabetes

Currently, diabetes afflicts an estimated 194 million individuals worldwide, and this number is expected to rise to at least 300 million by 2025. Sixteen million Americans suffer from diabetes with nearly a million new cases diagnosed every year. Not only is diabetes the sixth leading cause of death in the United States but also the numbers of diabetes-related deaths have gone up a striking 30% in the last decade alone. It is estimated that the total cost of diabetes including medical care and expenses as a result of lost productivity is a staggering US$ 132 billion. The quality of life of diabetics is seriously compromised due to single or in many cases multiorgan complications. Adults with diabetes have two to four times higher chances of heart disease, stroke, and high blood pressure. Diabetes is also the leading cause of blindness and end-stage renal disease among adults. In addition, diabetics are susceptible to nerve disease contributing to lower-extremity amputations.

Diabetes and Idiosyncratic Liver Toxicity

Since the above complications are serious and often life-threatening they have received attention leading to much basic research being done in these areas. However, important and potentially life-threatening complications of the liver in the diabetic condition have escaped attention. In a recent study, the prevalence of drug-induced liver disease among 40 190 type 2 diabetic patients was estimated. Surveys indicated that liver toxicity purely attributable to drug-induced causes were at the rate of 5.0/10 000 person-years, and overall liver disease incidence rate is 53.2/10 000 person-years among type 2 diabetic patients, suggesting a high background rate of liver disease in diabetics. Consistent with these data, a recent epidemiological study of 150 000 diabetic patients indicates that diabetics are twice as likely to suffer from hepatic failure as nondiabetic individuals. In one study, the incidence of acute liver injury in 34 328 patients with type 2 diabetes was evaluated from 1994 to 1998, and it was found that the overall annual incidence was 14.2 and 8.8/100 000 patient-years in the diabetes and general populations, respectively. Neither the mechanisms nor the predisposing factors underlying these liver injuries are clearly understood.

The field of hepatotoxicity in diabetes suddenly came to life when the promising new oral antidiabetic drug, troglitazone, led to severe and, in some cases, fatal hepatotoxicity in diabetics. Fatal episodes of hepatotoxicity resulted in withdrawal of the drug from the US markets. This incident prompted a renewed interest in the area and a need to understand the mechanisms that caused hepatotoxicity. The other members of class of thiazolidienediones, pioglitazone, and rosiglitazone, were also put under scrutiny for overt hepatotoxicity. Till date no major class effect of the thiazolidinediones has been found. So the question, does diabetes increase the sensitivity to potential hepatotoxicants, still lingers. Other incidents of idiosyncratic hepatotoxicity have been observed in diabetic patients on drug therapy with methotrexate, acarbose, and metformin.

Liver Toxicity in Animal Models of Diabetes

Studies using experimental diabetic animal models have indicated that xenobiotic-induced hepatotoxicity is modulated in diabetes. Hepatotoxicity of several structurally and mechanistically diverse chemicals, such as chloroform, thioacetamide, menadione, nitrosoamines, bromobenzene, and CCl_4, is significantly increased in type 1 diabetic rats. It was reported that thioacetamide-induced hepatotoxicity was potentiated in alloxan- or streptozotocin-diabetic rats. Recent studies have confirmed the potentiation of thioacetamide hepatotoxicity in streptozotocin-diabetic rats. Several studies have shown that hepatotoxicity of CCl_4 is potentiated in alloxan- or streptozotocin-induced type 1 diabetic rats.

Almost all of the work examining modulation of xenobiotic-induced hepatotoxicity in diabetes has been done using type 1 diabetic models. Recently, a study reported a novel high-fat diet-induced type 2 diabetic rat model and tested the susceptibility of these diabetic rats to several classical hepatotoxicants. On treatment with nonlethal or sublethal doses of allyl alcohol, CCl_4, or thioacetamide, it was found that hepatotoxicity of all these toxicants is significantly increased in the type 2 diabetic rats.

Chemical-induced liver injury is differentially modified by diabetes in murine type 1 diabetic models. Contrary to the enhanced hepatotoxicity in diabetic rats, diabetes in mice tends to protect animals from severe hepatotoxicity. It has been reported that the induction of diabetes in Swiss mice did not increase the susceptibility of mice to CCl_4 hepatotoxicity as occurs in rats. Development of diabetes also protected mice from acetaminophen toxicity. Further studies also showed that streptozotocin-induced diabetic mice were substantially resistant to lethal doses

of bromobenzene, CCl_4, and thioacetamide. Further, protection against acetaminophen was evident in three strains of diabetic mice. Studies examining the changes in compensatory liver cell division following acetaminophen showed that diabetic mice showed greater cell division compared to control mice. Treatment of diabetic mice with colchicine (an antimitotic agent, known to block cell division) after acetaminophen administration resulted in increased mortality in the diabetic suggesting a critical role of cell division in mediating the diabetic-induced resistance.

Mechanisms Involved in Hepatotoxic Predisposition in Diabetes

Mechanistic studies to examine the altered hepatotoxicity have traditionally focused on bioactivation/detoxification of xenobiotics in diabetic state. Type 1 diabetes, at least in the streptozotocin-induced diabetic rat, increases several phase I drug metabolizing enzymes. However, changes in drug metabolizing enzymes differ depending on the species and type of diabetes. A second and perhaps more determining factor in the ultimate outcome of hepatotoxic challenge is compensatory tissue repair. The liver has an extraordinary ability to regenerate in response to cell loss by physical damage, toxic injury, or infections. In diabetic rats (both type 1 and type 2 diabetes) where hepatotoxicity is potentiated, liver tissue repair is significantly inhibited. On the other hand, diabetic mice, which are resistant to hepatotoxicity, show greater compensatory tissue repair. More mechanistic investigations to decipher the underlying mechanisms that predispose diabetics to hepatotoxicity are underway.

See also: Common Mechanism of Toxicity; Liver; Tissue Repair.

Further Reading

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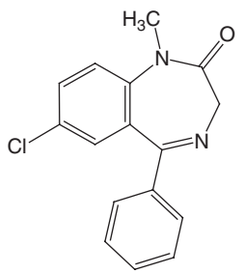
Diazepam

Teresa Dodd-Butera and Molly Broderick

- Chemical Name: 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one
- Chemical Abstracts Service Registry Number: CAS 439-14-5
- Synonyms: Methyl diazepinone; Valium (brand name)
- Chemical/Pharmaceutical/Other Class: Benzodiazepines; Psychoactive; Sedative hypnotics
- Chemical Structure:



Uses

Diazepam is a member of a class of drugs, known as benzodiazepines, introduced in the 1960s. They are relatively safe drugs, in comparison with other types of drugs used to treat anxiety. Diazepam is widely available and has a high therapeutic index; however, the drug also has the potential for abuse.

Diazepam is used primarily in the treatment of mental anxiety. In addition, it acts as a muscle relaxant for a variety of medical conditions. It may also be used as a sedative–hypnotic and anticonvulsant (e.g., for status epilepticus and drug-induced seizures). Diazepam may also be used to alleviate some of the symptoms associated with the following: cholinesterase poisoning, substance abuse withdrawal, antihistamine overdose, Black Widow spider envenomation, and chloroquine overdose. As an anesthetic, diazepam may be used alone or in combination with other drugs for conscious sedation.

Exposure Routes and Pathways

The oral route is a frequent pathway of exposure for therapeutic, accidental, and intentional overdoses.

Intravenous administration may be used to treat seizures. However, rectal suppositories have been used on occasion, if there is no parenteral access. The dermal and inhalational routes have been used in animal models, but are not conventionally used in clinical situations.

Toxicokinetics

Diazepam is rapidly absorbed following oral and parenteral administration, which may contribute to the potential for abuse. It is minimally enterohepatically recirculated, and peaks and declines are seen in serum levels after distribution into the tissue. The volume of distribution has ranged in reports from approximately 1 to 3 l kg^{-1}. Plasma protein binding is greater than 95%, which limits the effectiveness of dialysis after acute overdoses. Elimination half-life is a consideration for both therapeutic and toxic conditions. The range may be 24 h to more than 2 days. Small amounts of the unchanged drug are eliminated in the urine, and the active metabolite is desmethyldiazepam. The *N*-demethylation of diazepam involves CYP2C19 and CYP3A4.

Mechanism of Toxicity

The toxic and therapeutic effects of this drug have been attributed, in large part, to the potentiation of γ-aminobutyric acid (GABA) in the central nervous system (CNS). GABA is a neurotransmitter which mediates pre- and postsynaptic inhibition. Diazepam influences GABA activity by binding to the benzodiazepine receptor complex, thus resulting in increased CNS inhibition.

Acute and Short-Term Toxicity (or Exposure)

Animal

Reports of the lethal dose (50%) in animal studies are as follows: LD_{50} (oral) rat 1200 mg kg^{-1}; LD_{50} (oral) dog 1000 mg kg^{-1}; LD_{50} (oral) mice 700 mg kg^{-1}.

Human

Overdose of diazepam with oral and parenteral administration has the potential to cause impairment of consciousness and judgment, hypotension, bradycardia, coma, and respiratory failure. In addition, deaths have been reported when diazepam is used in combination with other CNS depressants. Sedation and somnolence are common adverse effects from the drug.

The very old and the very young are most susceptible to toxicity. Intravenous exposure results in a more rapid manifestation of symptoms, and toxicity is often iatrogenic. Even in therapeutic doses, parenteral administration may cause apnea and hypotension, especially with rapid administration of the drug.

Chronic Toxicity (or Exposure)

Animal

Carcinogenicity has been suggested, but not confirmed in animal models.

Human

Toxic effects may occur with chronic administration, and patients taking diazepam need medical monitoring. In addition, dependence may develop with regular use of benzodiazepines, and withdrawal symptoms may occur with cessation.

Pregnancy and Breastfeeding Due to its high lipid solubility, diazepam crosses the blood–brain barrier and is present in breast milk. In addition, it crosses the placental barrier. Therefore, it is not recommended during pregnancy and breastfeeding.

In Vitro Toxicity Data

Mutagenic activity of diazepam has been reported in the Ames test (TA 100). In addition, a micronucleus test using mouse bone marrow was found to be genotoxic with diazepam.

Clinical Management

Frequently, diazepam overdoses are adequately managed with clinical observation and supportive care. However, coingestion of ethanol and other CNS depressants which may exacerbate toxicity are common and warrant investigation in the patient history. Flumazenil, a benzodiazepine antagonist, effectively reverses symptoms of CNS toxicity, but is hazardous with the coingestion of other substances such as antidepressants. Therefore, it should not be used routinely.

Withdrawal symptoms may be delayed due to the long half-life and active metabolites of diazepam.

See also: Benzodiazepines.

Further Reading

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Relevant Website

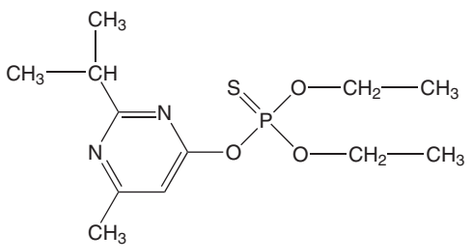
http://www.nlm.nih.gov – NIH Medline plus: Diazepam.

Diazinon

Subramanya Karanth

This article is a revision of the previous print edition article by Thuc Pham, volume 1, pp. 454–455, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 333-41-5
- Synonyms: Basudin; Dazzel; Diazitol; Gardentox; Dipofene; Knox Out; Nucidol; Spectracide
- Chemical/Pharmaceutical/Other Class: Organophosphorus (phosphorothioate) insecticide
- Chemical Structure:



Uses

Diazinon has been commonly used to control household insects such as cockroaches and ants. Household uses were eliminated in the United States in 2002, and lawn and garden uses were cancelled in 2003. Certifications in various agricultural practices were also canceled.

Exposure Routes and Pathways

Dermal and inhalation routes are the primary exposure routes for diazinon.

Toxicokinetics

Diazinon is readily absorbed through the skin, lungs, and gastrointestinal tract. Like other phosphorothioate organophosphorus insecticides, diazinon is bioactivated to diazoxon by microsomal enzymes. Other major metabolites in rats and cows include diethyl thiophosphate and diethyl phosphate. Excretion of diazinon is rapid in laboratory rats, with a half-life of $\sim$12 h. It is mainly excreted in urine (80%) and $\sim$20% is excreted in feces.

Mechanism of Toxicity

Like other organophosphorus insecticides, the active metabolite, diazoxon, elicits toxicity by inhibiting the enzyme acetylcholinesterase in the cholinergic synapse. Acetylcholinesterase inhibition leads to accumulation of the neurotransmitter acetylcholine resulting in neurotoxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} for diazinon was 300–400 mg kg^{-1} in rats. The inhalation LC_{50} (4 h) in rats was 3.5 mg l^{-1}. The dermal LD_{50} in rabbits was 3.6 g kg^{-1}. Very low doses (1 mg kg^{-1}) can produce toxicity in calves.

Human

Acute exposure to diazinon may result in acetylcholinesterase inhibition in the central and peripheral nervous system. Severity of poisoning varies with different formulations. Typical signs of poisoning include weakness, headaches, tightness in the chest, blurred vision, salivation, sweating, nausea, vomiting, diarrhea, and abdominal cramps. Diazinon has been associated with the intermediate syndrome.

Chronic Toxicity (or Exposure)

Animal

Studies in laboratory animals have shown that body weight gain is affected following chronic exposure to

diazinon. The no-observed-adverse-effect level (NOAEL) for chronic dietary exposure in rats is $0.02\,mg\,kg^{-1}$. Diazinon is not carcinogenic to laboratory animals and its mutagenic potential is not clearly understood.

Human

Repeated exposure to diazinon can cause accumulation of acetylcholinesterase inhibition and lower the threshold for subsequent exposures. Chronic exposure has been reported to lower neurobehavioral scores in farm workers. NOAEL for humans is $0.025\,mg\,kg^{-1}\,day^{-1}$.

Clinical Management

General decontamination procedures should be initiated in case of diazinon exposure. For skin decontamination, the exposed area is washed with plenty of water using soap and shampoo. In case of eye exposure, the eyes are flushed with water repeatedly for several minutes. Contaminated clothing is removed and the airway cleared. In case of ingestion, gastric decontamination should be performed immediately (within 30 min). Atropine treatment should be initiated immediately to counteract muscarinic effects. Atropine (adults and children >12 years: 2–4 mg; children <12 years: 0.05–0.1 mg) treatment should be repeated every 15 min until oral and bronchial secretions are controlled and atropinization is achieved. The duration and dosage of atropine treatment is slowly reduced as the condition of the patient improves. Pralidoxime should be administered slowly at the recommended dosage (adults and children >12 years: 1–2 g; children <12 years: 20–50 mg by intravenous infusion in 100 ml saline at $\sim 0.2\,g\,min^{-1}$). This dosage can be repeated at 1–2 h intervals initially and at 10–12 h intervals later depending on the condition of the patient. Repeated atropine treatment over several injections may be necessary for effective control of cholinergic signs.

Environmental Fate

Mobility of diazinon in soil is generally poor and under certain soil conditions it can effectively contaminate groundwater. It has a half-life of 2–4 weeks in soil. It is degraded easily in acidic water and persists for a long time at neutral pH.

Ecotoxicology

Bird kills with diazinon have been reported at all times of the year. Birds are markedly more susceptible to diazinon toxicity than other terrestrial vertebrates. LD_{50} values for birds range from 2.75 to $40.8\,mg\,kg^{-1}$. Diazinon is also very highly toxic to fish and bees.

Exposure Standards and Guidelines

The reference dose for diazinon is $0.0007\,mg\,kg^{-1}\,day^{-1}$, the acceptable daily intake is $0.002\,mg\,kg^{-1}\,day^{-1}$, and the threshold limit value (8 h) is $0.1\,mg\,m^{-3}$.

See also: Acetylcholine; Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates; Pesticides.

Further Reading

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Ibrahim NA and El-Gamal BA (2003) Effect of diazinon, an organophosphate insecticide, on plasma lipid constituents in experimental animals. *Journal of Biochemistry, Molecular Biology and Biophysics* 36(5): 499–504.

Relevant Websites

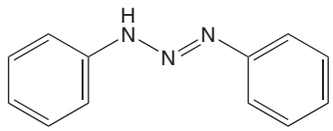
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

http://www.epa.gov – US Environmental Protection Agency.

Diazoaminobenzene

Ruth Custance and Cathy Villaroman

- Chemical Abstracts Service Registry Number: CAS 136-35-6
- Synonyms: Anilinoazobenzene; Benzeneazoanilide; Benzeneazoaniline; DAAB; Alpha-Diazoamidobenzol; *p*-Diazoaminobenzene; 1,3-Diphenyltriazene; 1,3-Diphenyl-1-triazene; DPT; *N*-(Phenylazo) aniline; Diazobenzeneanilide; *p*-Diazoaminobenzene
- Chemical Formula: $C_{12}H_{11}N_3$
- Chemical Structure:



Uses

Diazoaminobenzene (DAAB) is used as a chemical intermediate, a complexing agent, and as a polymer additive. DAAB has been used to promote adhesion of natural rubber to steel tire cords. It has also been used as a blowing agent in the production of a foamed polymeric material. In addition, DAAB is used in the manufacture of dyes and insecticides. DAAB is present in cosmetics, pharmaceuticals, and food products, as a dye contaminant in D&C Red No. 33, FD&C Yellow No. 5, and FD&C Yellow No. 6.

Exposure Routes and Pathways

The presence of DAAB as a dye contaminant in cosmetics and food products could result in low level exposures via the oral and dermal routes. Occupational exposure may occur through inhalation and dermal contact where these chemicals are produced or used as a chemical intermediate and polymer additive. DAAB is harmful to the respiratory tract, skin, and eyes. Most exposures to the general population are typically through consumption of food and use of cosmetics containing DAAB impurities.

Toxicokinetics

DAAB is well absorbed from the gastrointestinal tract but is only minimally absorbed via the dermal route. Regardless of the route of administration, the absorbed portion of the dose is rapidly metabolized and primarily excreted in urine. Within 24 h, ~60% of an oral dose of DAAB was accounted for in the urine of rats and mice as metabolites of benzene, a known human and animal carcinogen, or aniline, a rat carcinogen.

Mechanism of Toxicity

DAAB is a respiratory tract, skin, and eye irritant. DAAB yields benzene and aniline as metabolites. The proposed metabolic pathway for DAAB is that it is cleaved reductively by liver enzymes or gut flora to form aniline, benzene, and nitrogen. DAAB metabolism also results in the formation of a reactive phenyl radical, which could account for an additional risk of toxicity or carcinogenicity. The erythrocyte and lymphoid systems are major targets of DAAB toxicity. Induction of lymphoid atrophy of the thymus and other lymphoid tissues were observed, as well as methemoglobin formation, accompanying anemia, increased spleen weights, and regenerative hematopoiesis.

Acute and Short-Term Toxicity (or Exposure)

DAAB is considered explosive and is harmful to the respiratory tract, skin, and eyes via dermal contact and inhalation. Results from short-term animal toxicity studies suggest that DAAB has toxic effects similar to that from exposures to benzene and aniline. However, DAAB was observed to be more toxic at the application site than benzene or aniline. The mechanism that accounts for the greater acute toxicity of DAAB has not been determined. However, it may be attributable to properties of the parent molecule or to free radicals formed in its metabolism.

Animal

Following dermal application of DAAB in a 16 day study, animals exhibited symptoms that were similar to those from exposures to benzene and aniline. These symptoms included dose-related decreases in thymus weights in rats and mice and increases in the heart weights of rats and mice, liver and spleen in rats, and kidney in male rats and female mice. DAAB also induced hematologic effects in rats and mice, including Heinz-body formation and chemical-related methemoglobinemia. Induction of lymphoid atrophy of the thymus and other lymphoid tissues characteristic of benzene toxicity were also observed. Nonneoplastic lesions were observed in both rats and

mice, which included hyperplasia and inflammation of the skin, and hematopoietic cell proliferation in the spleen. Nonneoplastic lesions in the heart, kidney, and liver were also observed in mice. DAAB also induced toxicity not observed for aniline or benzene, including skin lesions at the application site.

Chronic Toxicity (or Exposure)

DAAB induced a greater number of micronuclei than did a combination of equimolar doses of benzene and aniline. The carcinogenicity of DAAB, specifically, has not been determined. However, the carcinogenicity of its two metabolites, benzene and aniline, has been evaluated. DAAB is considered carcinogenic since benzene is classified as a known human carcinogen.

Animal

In the late 1940s, carcinogenicity studies revealed that dermal exposure to DAAB resulted in skin and lung tumors in some mice. DAAB is metabolized to the known carcinogens aniline and benzene, both of which are carcinogenic in laboratory animals. Oral exposure to benzene induced multiple tumors at multiple sites in rats and mice of both sexes. Rats exposed to aniline in the diet developed sarcomas of the spleen and other body organs. In addition, transgenic mice developed skin tumors and leukemia following dermal exposure to benzene. Therefore, DAAB is considered to be carcinogenic in animals, based on its metabolism to benzene and aniline.

Human

No human studies specifically on DAAB exposure were identified. DAAB is predicted to be a carcinogen because one of its main metabolites, benzene, is classified as a known human carcinogen. A causal relationship between benzene exposure and leukemia has been reported in numerous epidemiological studies. The other main DAAB metabolite, aniline, is not classified as a human carcinogen based on its limited evidence of carcinogenicity in animals and inadequate evidence in humans.

In Vitro Toxicity Data

DAAB was shown to be mutagenic in *Salmonella typhimurium* strains when testing occurred in the presence of induced rat or hamster liver S9 enzymes. No additional genetic toxicity data have been reported for DAAB, but literature exists for benzene and aniline, the two main metabolites. Although benzene and aniline are not mutagenic in the *Salmonella* assay, they are active in other assays, such as in those that detect chromosomal damage.

Environmental Fate

DAAB melts at 98°C, decomposes at 130°C, and explodes at its boiling point of 150°C. The decomposition products of DAAB include benzene, *o*- and *p*-aminodiphenyl, diphenylamine, and azobenzene. DAAB is insoluble in water but soluble in ethyl alcohol, ethyl ether, benzene, pyridine, and hexane.

Exposure Standards and Guidelines

No DAAB-specific regulations have been found. The FDA regulates FD&C Yellow No. 5 and FD&C Yellow No. 6 for use as color additives in foods, drugs, and cosmetics and D&C Red No. 33 for use as a color additive in drugs and cosmetics. The American Conference of Governmental Industrial Hygienists, the National Institute for Occupational Safety and Health, and the Occupational Safety and Health Administration have not established occupational exposure limits for DAAB to date.

See also: Blood; Carcinogenesis; Dyes; Food and Drug Administration, US; Skin.

Further Reading

NTP (2002) *NTP Report on the Metabolism, Toxicity, and Predicted Carcinogenicity of Diazoaminobenzene (CAS 136-35-6),* TR-073. Research Triangle Park: National Toxicology Program.

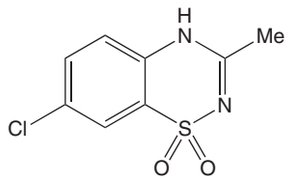
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Diazoaminobenzene.

Diazoxide

William S Utley

- Chemical Abstracts Service Registry Number: CAS 364-98-7
- Synonyms: 2*H*-1,2,4-Benzothiadiazine, 7-chloro-3-methyl-, 1,1-dioxide; Hyperstat; Mutabase proglicem
- Chemical/Pharmaceutical/Other Class: Therapeutic hypoglycemic agent
- Chemical Formula: $C_8H_7ClN_2O_2S$
- Chemical Structure:



Uses

Diazoxide is administered intravenously for the treatment of hypertension and administered orally for the treatment of hypoglycemia.

Exposure Routes and Pathways

Diazoxide is therapeutically administered either intravenously or orally.

Toxicokinetics

Diazoxide is well absorbed orally. It is distributed to the plasma where it is highly bound (>90%) to the plasma proteins. It readily crosses both the placental and blood brain barriers. The volume of distribution is 180 ml kg^{-1}. The half-life of elimination for diazoxide is 15–30 h. Approximately 20–50% of diazoxide is eliminated unchanged by the kidney, with the remainder being metabolized by the liver to 3-carboxy and 3-hydroxymethyl derivatives.

Mechanism of Toxicity

Diazoxide acts as an antihypertensive by relaxing the arteriole smooth muscle. The cardiac output and renin secretion is increased. The result is retention of salt and water and elevated levels of angiotensin II that eventually counteract the hypotensive action of the drug.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute exposures include alterations of neonatal glucose levels, fetal bradycardia, and interference with labor in women treated intravenously with diazoxide. The two most frequently cited side effects are salt and water retention and hyperglycemia.

Chronic Toxicity (or Exposure)

Human

Chronic exposure can lead to hypertrichosis. Diazoxide exhibits the ability to relax smooth muscle and therefore may be contraindicated in late pregnancy. Commonly observed side effects include decreased urination, swelling of feet or lower legs, and rapid weight gain. Occasionally, increased tachycardia may be observed and on rare occasions fever, skin rash, stiffness of arms or legs, trembling and shaking of hands and fingers, unusual bleeding or bruising, may be observed.

Exposure Standards and Guidelines

Diazoxide is regulated in the state of California as a Proposition 65 reproductive toxin.

See also: Hypoglycemics, Oral.

Further Reading

Silvani P, Camporesi A, Mandelli A, Wolfler A, and Salvo I (2004) A case of severe diazoxide toxicity. *Paediatric Anaesthesia* 14(7): 607–609.

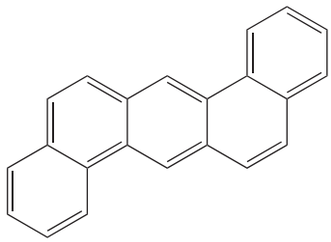
Relevant Website

http://www.nlm.nih.gov – Medline Plus: Diazoxide (Oral).

Dibenz[*a*,*h*]anthracene

William S Utley

- Chemical Abstracts Service Registry Number: CAS 53-70-3
- Synonyms: 1,2,5,6-Dubenzanthracene; DB[*a*,*h*]A; DBA
- Chemical Formula: $C_{22}H_{14}$
- Chemical Structure:



Uses

There are no reported industrial uses for dibenz[*a*,*h*]anthracene. It is used as a research tool. Dibenz[*a*,*h*]anthracene is a by-product of incomplete combustion and therefore is a fairly ubiquitous compound, generally strongly bound to the sediment.

Exposure Routes and Pathways

The primary route of exposure to dibenz[*a*,*h*]anthracene is via the skin, from petroleum-based products. An additional significant route of exposure is through inhalation of cigarette smoke. While ingestion is a route of exposure, the significance of such exposure is debatable. Dibenz[*a*,*h*]anthracene is found in many food items (cereals, fruits, and vegetables) in the low parts per billion (ppb) level. It is found in cigarette smoke at 100–150 ppb and in used motor oil at around 14 000 ppb. It is found in petroleum products such as coal tar, mineral oil, and petroleum waxes.

Toxicokinetics

Dibenz[*a*,*h*]anthracene has an octanol to water partition coefficient ($\log K_{ow}$) of 6.5 and will bioconcentrate in lower organism with less efficient mixed function oxidase systems. Dibenzo[*a*,*h*]anthracene is poorly absorbed via the gastrointestinal track, being excreted primarily unchanged with the feces. The majority of absorbed portion will distribute to the kidney and liver where it is oxidized to the dihydrodiol by mixed function oxidases. Epoxidation of the 3,4-dihydrodiol may lead to the formation of a diol-epoxide, the purported metabolite responsible for its carcinogenicity. In man, dibenz[*a*,*h*]anthracene can be oxidized to the active metabolite where it is quickly eliminated, or can react with target compounds such as the DNA. Dibenz[*a*,*h*]anthracene has not been isolated in the fat tissues, reflecting the effectiveness of the metabolizing enzyme system.

Mechanism of Toxicity

Dibenz[*a*,*h*]anthracene is metabolically activated by the mixed function oxidase (MFO) system of the liver (P448) to form an epoxide that subsequently covalently binds to the DNA. This interaction with the DNA is believed to result in the carcinogenicity of the material. The particular area of the compound oxidized by the MFO system will result in epoxides of varying carcinogenic potency.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, dibenz[*a*,*h*]anthracene has been shown to cause fetal deaths when given at 5 mg kg^{-1} daily for the first days of pregnancy. Tumors in the forestomach were produced in mice given 9–19 mg over a 5–7 month period.

Human

Like many organic compounds, excessive acute exposure to dibenz[*a*,*h*]anthracene can lead to dizziness, nausea, and general central nervous system disturbances that resemble intoxication. Previous exposure to polyaromatic hydrocarbons or genetic predisposition can increase the MFO system that activates dibenz[*a*,*h*]anthracene to the reactive epoxide. In addition, personal habits such as smoking can significantly increase a person's exposure to these compounds.

Chronic Toxicity (or Exposure)

Animal

Dibenz[*a*,*h*]anthracene is a confirmed animal carcinogen. It produced carcinomas in mice both via dermal and oral routes of administration. Skin painting studies have produced mammary tumors. Lung

tumors have been induced in rats receiving intratracheal administration of dibenz[*a*,*h*]anthracene.

Human

Dibenz[*a*,*h*]anthracene is classified as a probable human carcinogen and therefore chronically may produce cancer. In addition, carcinogenic polyaromatic hydrocarbons have been implicated in immunosuppressive activity.

In Vitro Toxicity Data

Dibenz[*a*,*h*]anthracene has been reported to induce DNA damage and gene mutations in bacteria.

Environmental Fate

High K_{oc} values (ranging from 5.7×10^5 to 3.0×10^6) indicate that dibenz[*a*,*h*]anthracene will tend to remain bound to the soil column and not migrate. Neither vitalization nor biodegradation are expected to be significant factors. Reported biodegradation values in nonacclimated soil are in excess of 240–750 days. Acclimated sludge has been reported to metabolize dibenz[*a*,*h*]anthracene in 36 days and may account for a large percentage of its degradation in the environment.

Ecotoxicology

Reported values for *Daphnia magna* range from 0.4 to $0.8\,\mathrm{mg\,l^{-1}}$. Based on the substance's high K_{ow} and modeling, it is predicted to be highly toxic to aquatic life ($LC_{50} < 1\,\mathrm{mg\,l^{-1}}$).

Exposure Standards and Guidelines

The acceptable level in drinking water is $13.3\,\mathrm{ng\,l^{-1}}$. Dibenz[*a*,*h*]anthracene is an resource conservation and recovery act (RCRA) hazardous waste (U063). Dibenz[*a*,*h*]anthracene is classified as a B2 probable human carcinogen, based on sufficient animal data and no human data.

An oral slope factor of $7.3\,\mathrm{mg^{-1}\,kg^{-1}\,day^{-1}}$ has been calculated for benzo[*a*]pyrene based on the incidence of stomach tumors in mice treated with benzo[*a*]pyrene.

The US Environmental Protection Agency (EPA) has calculated the drinking water unit risk as $2.1 \times 10^{-4}\,\mathrm{g^{-1}\,l^{-1}}$. The EPA has calculated an inhalation unit risk of $1.7 \times 10^{-3}\,\mathrm{g^{-1}\,m^{-3}}$.

Dibenz[*a*,*h*]anthracene is listed as an International Agency for Research on Cancer 2A confirmed animal carcinogen and is listed as a California Proposition 65 carcinogen.

Dibenz[*a*,*h*]anthracene is listed in section 112 of the Clean Air Act; listed under sections 304 and 307 of the Clean Water Act; listed as an RCRA hazardous waste U063. Dibenz[*a*,*h*]anthracene is regulated under comprehensive environmental response, compensation, and liability act (CERCLA) with a reportable quantity (RQ) of 1 pound. It is listed as an emergency planning and community right-to-know act (EPCRA) superfund amendments reauthorization act (SARA) 313 reportable substance.

In the European Union, dibenz[*a*,*h*]anthracene is classified as T, N: R-45, R-50/53 (toxic, may cause cancer, and dangerous to the environment).

See also: Benz[*a*]anthracene; Benzo(*a*)pyrene; Clean Water Act (CWA), US; Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

Petry T, Schmid P, and Schlatter C (1996) The use of toxic equivalency factors in assessing occupational and environmental health science associated with exposure to air borne mixtures of polycyclic aromatic hydrocarbons. *Chemosphere* 32: 639–648.

Relevant Website

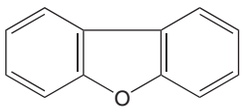
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dibenz[*a*,*h*]anthracene.

Dibenzofuran

Kashyap N Thakore and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 132-64-9
- Synonyms: 2,2′-Biphenylene oxide; 2,2′-biphenylylene oxide; dibenzo(*B*,*D*)furan; diphenylene oxide
- Chemical/Pharmaceutical/Other Class: Strong oxidizing agent; Antiestrogen
- Chemical Formula: $C_{12}H_8O$

- Chemical Structure:



Uses

Dibenzofuran is an industrial chemical or by-product.

Exposure Routes and Pathways

Inhalation is the most common route of exposure. Dibenzofuran is present in cigarette ash and is a by-product of processes in the pharmaceutical industry. When heated to decompose, it emits acrid smoke and irritating fumes. Exposure can also occur by ingestion of contaminated food.

Toxicokinetics

Dibenzofuran may be rapidly absorbed by various routes including oral, nasal mucosal, inhalation, and dermal routes. 2,2′,3-Trihydroxy biphenyl dioxygenase is a key enzyme responsible for meta-cleavage of the first aromatic ring in the degradation pathway. After intravenous or oral administration to rats, most of the compound is quickly distributed to the liver, muscle, skin, and adipose tissue and metabolized. Its metabolites may remain in the adipose tissue for a relatively long period of time. Polychlorinated dibenzofuran is highly lipophilic and is accumulated in adipose and liver tissues at a higher level and in muscle, kidneys, spleen, lungs, brain, and blood at a lower level. The metabolites are rapidly excreted mainly in bile, urine, and feces. They can also be excreted through milk.

Mechanism of Toxicity

Dibenzofuran induces hepatic, skin, and lung cytochrome P450 1A1, 1A2, and aryl hydrocarbon hydroxylase in rats. Thus, toxicity results from aryl hydrocarbon receptor signal transduction pathway. Bioactivation of many polycyclic hydrocarbon carcinogens is mediated by these enzymes.

Acute and Short-Term Toxicity (or Exposure)

Animal

2,3,7,8-Tetrachlorodibenzofuran (TCDF) causes wasting syndrome, thymic atrophy, and immune suppression in rodents, hair and fingernail loss in monkeys, chloracne formation in the rabbit ear, and hyperpigmentation in the rhesus monkey. It is hepatotoxic and has profound effects on both steroid and growth factor receptor systems. Significant species variability is seen in the toxicity of this compound. The LD_{50} for TCDF is 5–10 $\mu g\,kg^{-1}$ body weight in guinea pigs, $\geq 6000\,\mu g\,kg^{-1}$ body weight in mice, and $1000\,\mu g\,kg^{-1}$ body weight in monkeys.

Human

Dibenzofuran may be harmful by inhalation, ingestion, or skin absorption and may cause irritation. It is globally distributed, is persistent in the environment, and tends to accumulate in human tissues. The major primary sources of exposure for the general population are combustion (municipal waste incineration and automobile exhaust), carbon electrode processes (smelters), chemical manufacturing wastes (chlorophenols), open-use agricultural and industrial chemicals (chlorophenols and chlorophenoxy herbicides), polychlorinted biphenyls, and aqueous chlorination (sewage sludge and kraft pulp mills). Exposure can occur through contaminated food (fish, meat, and dairy products; breast milk in the case of infants). All the chlorinated compounds have the potential to cause dermal, hepatic, and gastrointestinal toxicities. The half-life in humans is relatively long.

Chronic Toxicity (or Exposure)

Animal

It is a liver tumor promoter, teratogenic, and immunotoxic affecting natural killer cells.

Human

It is not classifiable as to human carcinogenicity.

Clinical Management

Due to long biological half-life and lipid solubility of dibenzofurans, blood analysis may serve as an index of past cumulative occupational exposure and a means of assessing a person's exposure situation. In case of contact, the eyes and skin should be flushed immediately with water for at least 15 min. If inhaled, the victim should be removed to fresh air. If the person is not breathing, artificial respiration should be given; if breathing with difficulty, oxygen should be given. If the patient is in cardiac arrest, cardiopulmonary resuscitation should be given. In case of ingestion, the mouth should be washed out with water provided the person is conscious. These life-supporting measures should be continued until medical assistance has arrived. Liquids should not be

administered to and vomiting should not be induced in an unconscious or convulsing person.

In the workplace, technical measures should prevent any contact with the skin and mucous membranes. Workers potentially exposed to this compound should wear personal protective equipment and their work should be carried out only in restricted and ventilated areas. After use, clothing and equipment should be placed in an impervious container for decontamination or disposal. Preemployment and periodic medical examination should focus on liver function.

Environmental Fate

It is expected to have very low to no mobility in soil and significantly degrade in soil. It dissolves in water and also volatilizes. It exists primarily in the gas-phase in the atmosphere and reacts with photochemically-produced hydroxy radicals. It is biodegraded at contaminated sites where populations of adapted microorganisms are present; otherwise biodegradation may be slow. Biodegradation is also slow when oxygen is limited. It adsorbs very strongly to sediment and particulate matter in the water column. It has a potential for bioconcentration in aquatic organisms.

Other Hazards

It is not flammable.

Exposure Standards and Guidelines

The US Environmental Protection Agency is required to establish and phase in specific performance based standards for all air emission sources that emit dibenzofuran as one of the listed pollutants.

See also: Chlorophenols; Combustion Toxicology; Polychlorinated Biphenyls (PCBs); Polycyclic Aromatic Hydrocarbons (PAHs); TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin).

Relevant Websites

http://ntp.niehs.nih.gov – National Toxicology Program (NTP) (2004) Summary of Data of Chemical Selection: Dibenzofuran.

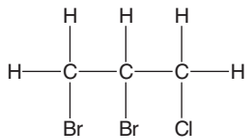
http://www.epa.gov – Environmental Protection Agency (US EPA) (2003) Substance Registry System, Washington DC.

Dibromochloropropane

Mark L Winter

This article is a revision of the previous print edition article by Thuc Pham, volume 1, pp. 458–459, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 96-12-8
- Synonyms: 1,2-Dibromo-3-chloropropane; DBCP; Nemafume; Nemanax; Nemaset; Nemagon; Femafume; Fumazone
- Chemical/Pharmaceutical/Other Class: Halogenated alkane
- Chemical Structure:



Uses

Dibromochloropropane (DBCP) was used in the United States as a soil fumigant and nematocide. The use of dibromochloropropane has been banned in the United States but it is still used in some other countries.

Exposure Routes and Pathways

Respiratory and dermal routes of exposure were most common.

Toxicokinetics

DBCP is well absorbed by any route of exposure. Absorption is almost complete following oral exposure. Microsomal transformation leads to the formation of reactive metabolites. Metabolites undergo conjugation with glutathione. DBCP induces microsomal enzymes in the testes, liver, and kidneys. Covalently bound metabolites accumulate in the liver and kidneys. Urinary excretion is the major route of elimination.

Mechanism of Toxicity

At high exposures, DBCP stabilizes neuronal membranes and leads to nervous system depression.

Reactive metabolites of DBCP (e.g., epoxides) bind to cellular macromolecules. With testicular toxicity, DBCP may act by preventing differentiation of spermatogonia into mature sperm.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral LD_{50} of DBCP in male rats and guinea pigs is $\sim$150–300 mg kg^{-1}. The dermal LD_{50} is >1 g kg^{-1}. DBCP acts as a central nervous system depressant at high vapor concentrations.

Chronic Toxicity (or Exposure)

Animal

Early animal studies demonstrated reduced testicular weights, with testicular atrophy at higher exposure levels. Several studies reported decreased sperm count and infertility with long-term exposure to DBCP. It is an experimental carcinogen, capable of increasing tumor incidence in a variety of tissues.

Human

The most marked chronic toxicity in humans from DBCP exposure is male infertility. Occupational exposure to DBCP has been associated with reduced sperm count and elevated levels of follicle-stimulating hormone and luteinizing hormone in humans. From a group of studies on occupational exposure to DBCP, it was estimated that $\sim$15% of exposed workers were azoospermic. The effects on sperm production last years after exposure has ended, in particular with men exposed for a period of more than 4 years. Upon testicular biopsy, the seminiferous tubules were devoid of spermatogenic cells, with only Sertoli cells remaining. Some authors have concluded that paternal exposure to DBCP, severe enough to cause azoospermia or oligospermia, did not increase the rate of congenital malformations or of impaired health status of offspring conceived during or after exposure.

In Vitro Toxicity Data

DBCP was mutagenic in several bacterial assays.

Clinical Management

Acute exposure to DBCP vapors requires removal from the source and symptomatic treatment. There is no treatment for testicular toxicity.

Exposure Standards and Guidelines

The reference concentration for inhalation is 2×10^{-4} mg m^{-3}. The maximum contaminant level (MCL) is 0.0002 mg l^{-1}.

See also: Pollution, Water; Reproductive System, Male.

Further Reading

Gehring PJ, Nolan RJ, Watanabe PG, and Schumann AM (1991) *Solvents, fumigants, and related compounds Handbook in Pesticide Toxicology*, Vol. 2, pp. 637–730. San Diego: Academic Press.

Hoyer PB (2001) Reproductive toxicology: Current and future directions. *Biochemical Pharmacology* 62: 1557–1564.

Meistrich ML, Wilson G, Shuttlesworth GA, and Porter KL (2003) Dibromochloropropane inhibits spermatogonial development in rats. *Reproductive Toxicology* 17: 263–271.

Potashnik G and Phillip M (1988) Lack of birth defects among offspring conceived during or after paternal exposure to dibromochloropropane (DBCP). *Andrologia* 20(1): 90–94.

Thomas MJ and Thomas JA (2001) Toxic responses of the reproductive system. In: *Casarett and Doull's Toxicology*, 6th edn., pp. 673–709. New York: McGraw-Hill.

Dibutyl Ether *See* Diethyl Ether.