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TOXICOLOGY HANDBOOK

Principals Related to Hazardous Waste Site Investigations

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FOREWORD

This handbook was prepared by ICAIR, Life Systems, Inc., under U.S. Environmental Protection Agency (EPA) Contract 68-01-7037 during the period February, 1985 to June, 1985. The program was directed by Mr. Timothy E. Tyburski. The handbook was compiled by Mr. Jeffrey Heaton with technical support from Dr. William Brattin, Dr. Carol Maczka, Mr. Kevin Gleason, Ms. Yvonne Hales, Mr. Steve Lavenhar, Ms. Betty Neustadter, Ms. Lee Ann Smith and Ms. Jo Ann Duchene. Advice about the topics and the appropriate level of detail was obtained from four toxicologists experienced in giving expert witness testimony in hazardous waste litigation actions: Dr. Herbert Cornish, Dr. Curtis Klaassen, Dr. Andrew Reeves and Dr. James Selkirk.

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DISCLAIMER

This document has not undergone final review within EPA and is for internal Agency use/distribution only.

There has been an EPA workgroup review of the development of this handbook prior to this draft. This final draft is being distributed to EPA personnel for a six-month review period, after which changes will be made based on the comments received.

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LIST OF ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists
ADI	Acceptable Daily Intake
AL	Acceptable Level
ALA	Delta-Aminolevulinic Acid
ALA-D	Delta-Aminolevulinic Acid Dehydratase
CAS	Chemical Abstract Service
CDC	Centers for Disease Control
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CHO	Chinese Hamster Ovary
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
EC	Effective Concentration
ED	Effective Dose
EEG	Electroencephalogram
EL	Exposure Level
EPA	United States Environmental Protection Agency
FDA	U.S. Food and Drug Administration
FEL	Frank Effect Level
FR	Federal Register
IARC	International Agency for Research on Cancer
ICAIR	Interdisciplinary Planning and Information Research
HI	Hazard Index
IQ	Intelligence Quotient
IRCP	International Commission on Radiological Protection
LC	Lethal Concentration
LD	Lethal Dose
LOAEL	Lowest Observed Adverse Effect Level
LOEL	Lowest Observed Effect Level
MATC	Maximum Allowable Toxicant Concentration
MCL	Maximum Contaminant Level
MTD	Maximum Tolerated Dose
NAS	National Academy of Science
NCI	National Cancer Institute
NCV	Nerve Conduction Velocity
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute of Occupational Safety and Health
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level

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List of Acronyms - continued

NTP	National Toxicology Program
OERR	Office of Emergency and Remedial Response
OSW	Office of Solid Waste
OWPE	Office of Waste Programs Enforcement
PCDF	Polychlorinated-dibenzofuran
PND	Post Natal Day
QA	Quality Assurance
QC	Quality Control
RCRA	Resource Conservation and Recovery Act of 1976
SD	Standard Deviation
SEM	Standard Error of the Means
SNARL	Suggested No Adverse Response Level
STEL	Short-Term Exposure Limit
SW	Slow Wave
TI	Therapeutic Index
TC	Toxic Concentration
TCDD	Tetrachlorodibenzo-p-dioxin
TCDF	Tetrachlorodibenzo-furan
TD	Toxic Dose
TLV	Threshold Limit Value
VSD	Virtually Safe Dose
WHO	World Health Organization

GLOSSARY

Abiotic - Nonliving, especially the nonliving elements in ecological systems.

Absorbed dose - The amount of a chemical that enters the body of an exposed organism.

Absorption - The uptake of water or dissolved chemicals by a cell or an organism.

Absorption factor - The fraction of a chemical making contact with an organism that is absorbed by the organism.

Active transport - An energy-expending mechansim by which a cell moves a chemical across the cell membrane from a point of lower concentration to a point of higher concentration, against the diffusion gradient.

Acute - Occurring over a short period of time; used to describe brief exposures and effects which appear promptly after exposure.

Additive Effect - Combined effect of two or more chemicals equal to the sum of their individual effects.

Adsorption - The process by which chemicals are held on the surface of a mineral or soil particle. Compare with absorption.

Ambient - Environmental or surrounding conditions.

Animal studies - Investigations using animals as surrogates for humans, on the expectation that results in animals are pertinent to humans.

Antagonism - Interference or inhibition of the effect of one chemical by the action of another chemical.

Assay - A test for a particular chemical or effect.

Background level - Normal ambient environmental concentration of a chemical.

Bias - An inadequacy in experimental design that leads to results or conclusions not representative of the population under study.

Bioaccumulation - The retention and concentration of a substance by an organism.

Bioassay - Test which determines the effect of a chemical on a living organism.

Bioconcentration - The accumulation of a chemical in tissues of an organism (such as fish) to levels that are greater than the level in the medium (such as water) in which the organism resides (see bioaccumulation).

Biodegradation - Decomposition of a substance into more elementary compounds by the action of microorganisms such as bacteria.

Biomagnification - The serial accumulation of a chemical by organisms in the food chain, with higher concentrations of the substance in each succeeding trophic level.

Biotransformation - Conversion of a substance into other compounds by organisms; includes biodegradation.

Cancer - A disease characterized by the rapid and uncontrolled growth of aberrent cells into malignant tumors.

Carcinogen - A chemical which causes or induces cancer.

CAS registration number - A number assigned by the Chemical Abstracts Service to identify a chemical.

Central nervous system - Portion of the nervous system which consists of the brain and spinal cord; CNS.

Chromosome - Rodlike structure in the nucleus of a cell that forms during mitosis; composed of DNA and protein; chromosomes contain the genes responsible for heredity.

Chronic - Occurring over a long period of time, either continuously or intermittently; used to describe ongoing exposures and effects that develop only after a long exposure.

Chronic exposure - Long-term, low level exposure to a toxic chemical.

Clinical studies - Studies of humans suffering from symptoms induced by chemical exposure.

Confounding factors - Variables other than chemical exposure level which can affect the incidence or degree of a parameter being measured.

Cost/benefit analysis - A quantitative evaluation of the costs which would be incurred versus the overall benefits to society of a proposed action such as the establishment of an acceptable dose of a toxic chemical.

Cumulative exposure - The summation of exposures of an organism to a chemical over a period of time.

Degradation - Chemical or biological breakdown of a complex compound into simpler compounds.

Demography - The study of the characteristics of human populations such as size, growth, density, distribution and vital statistics.

Dermal - Of the skin; through or by the skin.

Dermal exposure - Contact between a chemical and the skin.

Diffusion - The movement of suspended or dissolved particles from a more concentrated to a less concentrated region as a result of the random movement of individual particles; the process tends to distribute them uniformly throughout the available volume.

Dose - The quantity of a chemical to which an organism is exposed. (See absorbed dose.)

Dose-response - A quantitative relationship between the dose of a chemical and an effect caused by the chemical.

Dose-response curve - A graphical presentation of the relationship between degree of exposure to a chemical (dose) and observed biological effect or response.

Ecology - The study of the interrelationships between living organisms and their environment, both physical and biological.

Ecosystem - The interacting system of a biological community and its nonliving environment.

Ecotoxicological studies - Measurement of effects of environmental toxicants on indigenous populations of organisms.

Endangerment assessment - A site-specific risk assessment of the actual or potential danger to human health or welfare and the environment from the release of hazardous substances or waste. The endangerment assessment document is prepared in support of enforcement actions under CERCLA or RCRA.

Endpoint - A biological effect used as an index of the effect of a chemical on an organism.

Environmental fate - The destiny of a chemical after release to the environment; involves considerations such as transport through air, soil and water, bioconcentration, degradation, etc.

Enzyme - A protein, synthesized by a cell, that acts as a catalyst in a specific chemical reaction.

Epidemiological studies - Investigation of elements contributing to disease or toxic effects in human populations.

Exposure - Contact with a chemical or physical agent.

Exposure assessment - The determination or estimation (qualitative or quantitative) of the magnitude, frequency, duration, route, and extent (number of people) of exposure to a chemical.

Exposure coefficient - Term which combines information on the frequency, mode, and magnitude of contact with contaminated medium to yield a quantitative value of the amount of contaminated medium contacted per day.

Exposure level, chemical - The amount (concentration) of a chemical at the absorptive surfaces of an organism.

Exposure scenario - A set of conditions or assumptions about sources, exposure pathways, concentrations of toxic chemicals and populations (numbers, characteristics and habits) which aid the investigator in evaluating and quantifying exposure in a given situation.

Extrapolation - Estimation of unknown values by extending or projecting from known values.

Half-life - The length of time required for the mass, concentration, or activity of a chemical or physical agent to be reduced by one-half.

Hematopoiesis - The production of blood and blood cells; hemopoiesis.

Hepatic - Pertaining to the liver.

Hepatoma - A malignant tumor occurring in the liver.

Histology - The study of the structure of cells and tissues; usually involves microscopic examination of tissue slices.

Homeostasis - Maintenance of a constant internal environment in an organism.

Hormone - A chemical substance secreted in one part of an organism and transported to another part of that organism where is has a specific effect.

Human equivalent dose - A dose which, when administered to humans, produces an effect equal to that produced by a dose in animals.

Hydrology - The study of the properties, distribution, behavior and effects of water on the earth's surface, in the soil and underlying rocks and in the atmosphere.

Hydrolysis - The breakdown of a chemical into two parts concomitant with addition of the elements of water (H- and OH-) to the products.

Hypoxia - A deficiency of oxygen.

Intake - Amount of material inhaled, ingested, or absorbed dermally during a specified period of time.

Integrated exposure assessment - A summation over time, in all media, of the magnitude of exposure to a toxic chemical.

In vitro studies - Studies of chemical effects conducted in tissues, cells or subcellular extracts from an organism (i.e., not in the living organism).

In vivo studies - Studies of chemical effects conducted in intact living organisms.

Irreversible effect - Effect characterized by the inability of the body to partially or fully repair injury caused by a toxic agent.

Latency - Time from the first exposure to a chemical until the appearance of a toxic effect.

LC_{50} - The concentration of a chemical in air or water which is expected to cause death in 50 percent of test animals living in that air or water.

LD_{50} - The dose of a chemical taken by mouth or absorbed by the skin which is expected to cause death in 50 percent of the test animals so treated.

LOAEL - Lowest-Observed-Adverse-Effect Level; the lowest dose in an experiment which produced an observable adverse effect.

Materials balance - An accounting of the mass flow of a substance from sources of production, through distribution and use, to disposal or distribution, and including any releases to the environment.

Metabolism - The sum of the chemical reactions occurring within a cell or a whole organism; includs the energy-releasing breakdown of molecules (catabolism) and the synthesis of new molecules (anabolism).

Metabolite - Any product of metabolism, especially a transformed chemical.

Modeling - Use of mathematical equations to simulate and predict real events and processes.

Monitoring - Measuring concentrations of substances in environmental media or in human or other biological tissues.

Mutagen - An agent that causes a permanent genetic change in a cell other than that which occurs during normal genetic recombination.

Mutagenicity - The capacity of a chemical or physical agent to cause permanent alteration of the genetic material within living cells.

Necrosis - Death of cells or tissue.

NOAEL - No-Observed-Adverse-Effect Level; the highest dose in an experiment which did not produce an observable adverse effect.

Oncology - Study of cancer.

Oral - Of the mouth; through or by the mouth.

Pathogen - Any disease-causing agent, usually applied to living agents.

Pathogenic - Causing or capable of causing disease.

Pathology - The study of disease.

Permissible dose - The dose of a chemical that may be received by an individual without the expectation of significantly harmful result.

Pharmacokinetics - The dynamic behavior of chemicals inside biological systems; it includes the processes of uptake, distribution, metabolism, and excretion.

Population at risk - A population subgroup that is more likely to be exposed to a chemical, or is more sensitive to a chemical, than is the general population.

Potentiation - The effect of one chemical to increase the effect of another chemical.

Prevalence study - An epidemiological study which examines the relationships between diseases and exposures as they exist in a defined population at a particular point in time.

Prospective study - An epidemiological study which examines the development of disease in a group of persons determined to be presently free of the disease.

Qualitative - Descriptive of kind, type or direction, as opposed to size, magnitude or degree.

Quantitative - Descriptive of size, magnitude or degree.

Receptor - (1) In biochemistry: a specialized molecule in a cell that binds a specific chemical with high specificity and high affinity; (2) In exposure assessment: an organism that receives, may receive, or has received environmental exposure to a chemical.

Renal - Pertaining to the kidney.

Reservoir - A tissue in an organism or a place in the environment where a chemical accumulates, from which it may be released at a later time.

Retrospective study - An epidemiological study which compares diseased persons with non-diseased persons and works back in time to determine exposures.

Reversible effect - An effect which is not permanent, especially adverse effects which diminish when exposure to a toxic chemical is ceased.

Risk - The potential for realization of unwanted negative consequences or events.

Risk assessment - A qualitative or quantitative evaluation of the environmental and/or health risk resulting from exposure to a chemical or physical agent (pollutant); combines exposure assessment results with toxicity assessment results to estimate risk.

Risk estimate - A description of the probability that organisms exposed to a specified dose of chemical will develop an adverse response (e.g., cancer).

Risk factor - Characteristic (e.g., race, sex, age, obesity) or variable (e.g., smoking, occupational exposure level) associated with increased probability of a toxic effect.

Risk specific dose - The dose associated with a specified risk level.

Route of exposure - The avenue by which a chemical comes into contact with an organism (e.g., inhalation, ingestion, dermal contact, injection).

Sink - A place in the environment where a compound or material collects (see reservoir).

Sorption - A surface phenomenon which may be either absorption or adsorption, or a combination of the two; often used when the specific mechanism is not known.

Stochastic - Based on the assumption that the actions of a chemical substance result from probabilistic events.

Stratification - (1) The division of a population into subpopulations for sampling purposes; (2) the separation of environmental media into layers, as in lakes.

Subchronic - Of intermediate duration, usually used to describe studies or levels of exposure between five and 90 days.

Synergism - An interaction of two or more chemicals that results in an effect that is greater than the sum of their effects taken independently.

Systemic - Relating to whole body, rather than its individual parts.

Teratogenesis - The induction of structural or functional development abnormalities by exogenous factors acting during gestation; interference with normal embryonic development.

Teratogenicity - The capacity of a physical or chemical agent to cause non-hereditary congenital malformations (birth defects) in offspring.

Therapeutic Index - The ratio of the dose required to produce toxic or lethal effect to dose required to produce non-adverse or therapeutic response.

Threshold - The lowest dose of a chemical at which a specified measurable effect is observed and below which it is not observed.

Time-Weighted Average - The average value of a parameter (e.g., concentration of a chemical in air) that varies over time.

Tissue - A group of similar cells.

Toxicant - A harmful substance or agent that may injure an exposed organism.

Toxicity - The quality or degree of being poisonous or harmful to plant, animal or human life.

Toxicity Assessment - Characterization of the toxicological properties and effects of a chemical, including all aspects of its absorption, metabolism, excretion and mechanism of action, with special emphasis on establishment of dose-response characteristics.

Transformation - Acquisition by a cell of the property of uncontrolled growth.

Uncertainty Factor - A number (equal to or greater than one) used to divide NOAEL or LOAEL values derived from measurements in animals or small groups of humans, in order to estimate a NOAEL value for the whole human population.

EXECUTIVE SUMMARY

1.0 INTRODUCTION

Risk assessment is the process of characterizing the risk either to human health or to the environment as the result of chemical releases into the environment from some specific site (such as a factory or waste storage facility). The process of collecting and interpreting the information needed to perform a risk assessment consists of two main branches: toxicity assessment (characterization of the inherent toxicity of a chemical) and exposure assessment (determination of how much of the chemical is coming into contact with humans or other species). Figure ES-1 is a generalized scheme that provides an overview of the risk assessment process and illustrates some of the major elements involved.

Examination of Figure ES-1 makes clear that risk assessment requires the interaction and cooperation of scientists from a variety of disciplines, including geology, hydrology, meteorology, ecology, biochemistry, chemistry and toxicology. This handbook is intended to provide a description of the toxicity assessment process in a way that is useful to non-toxicologists.

2.0 FUNDAMENTAL CONCEPTS IN TOXICOLOGY

Living organisms are composed of cells, and all cells must carry out a large number of chemical reactions in order to maintain themselves and perform their functions. Introduction of a foreign chemical into a cell may interfere with one or more of these cellular reactions, leading to impaired cell function or viability.

Toxicology is the study of how specific chemicals cause injury to living cells and whole organisms. Studies are performed to determine how easily the chemical enters the organism, how it behaves inside the organism, how rapidly it is removed from the organism, what cells are affected by the chemical and what cell functions are impaired. With respect to the risk assessment process, the ultimate goal is usually to derive a reliable estimate of the amount of chemical exposure which is considered acceptable for humans or other organisms. It is important to recognize that, for many chemicals, current toxicological knowledge is insufficient to answer this question with assurance.

3.0 DOSE-RESPONSE RELATIONSHIPS

The relationship between degree of exposure to a chemical (dose) and the magnitude of chemical-induced effects (response) is described by a dose-response curve. In general, dose-response curves fall into two groups: those in which no response is observed until some minimum (threshold) dose is reached, and those in which no threshold is apparent.

A hypothetical dose-response curve with a threshold is shown in Figure ES-2. The most important part of this curve is the dose at which significant effects first begin to occur. The highest dose which does not produce an observable adverse effect is the No-Observed-Adverse-Effect-Level (NOAEL), and the lowest

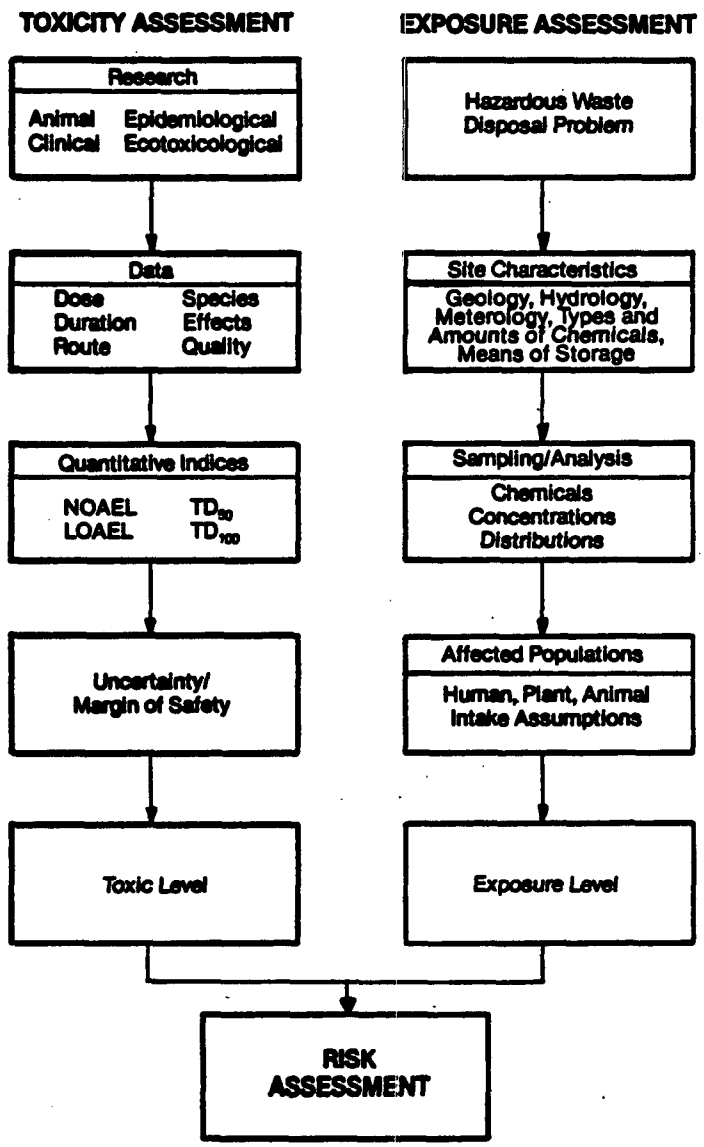


FIGURE ES-1 OVERVIEW OF THE RISK ASSESSMENT PROCESS APPLIED TO A HAZARDOUS WASTE SITE

dose which produces an observable adverse effect is the Lowest-Observed-Adverse-Effect-Level (LOAEL). With respect to the toxicity assessment process, identification of these points is a primary objective of the toxicologist. Other useful points are the TD_{50} (the dose which produces 50% of the maximum toxic effect or produces the toxic effect in 50% of the exposed organisms) and the TD_{100} (the lowest dose which produces the maximum toxic effect or produces the toxic effect in all exposed organisms).

An example of a dose-response curve that does not have a threshold is shown in Figure ES-3. For chemicals of this sort, there is no dose that is free of risk, but as the dose decreases to low levels, so does the probability that the effect will occur. Cancer is an adverse response that is believed to have no observable threshold. For these types of chemicals, selection of a "safe" exposure limit is made on the basis of what risk level is acceptable to society.

4.0 IMPORTANT PARAMETERS IN TOXICITY ASSESSMENT

Even under controlled laboratory conditions, it is not always simple to obtain reliable and useful dose-response data. There are a number of important variables which determine the characteristics of dose-response curves and must be considered in performing toxicity tests and interpreting toxicity data. The most important of these variables are discussed below.

4.1 Route of Exposure

The toxicity of some chemicals depends on whether the route of exposure is by ingestion, inhalation or dermal contact. In addition, there may be local responses at the absorption site (gastrointestinal tract, lungs, skin), since the concentration of the chemical is highest at that location. The route of exposure employed in experimental animal studies is normally selected based on the anticipated route of exposure of humans to the specific chemical.

4.2 Duration/Frequency of Exposure

The toxicity of many chemicals depends not only on dose (the amount of chemical contacted or absorbed each day) but also on the length of exposure (number of days, weeks or years). This is especially true for chemicals which produce irreversible injuries to cells or tissues. Thus, brief exposure to a low dose of such a chemical may produce so little damage that no significant injury occurs, but continued exposure will result in an accumulation of damage that eventually becomes apparent as a significant injury. For this reason, a full toxicological evaluation of any chemical must include consideration of the time-dependence of any adverse effects that occur. Typically, studies focus on acute (one-day), subchronic (roughly 5 to 90 days) and chronic (lifetime) exposures.

4.3 Test Species

For obvious reasons, laboratory investigations of chemical toxicity employ animals as test species. Unfortunately, not all animal species are equally

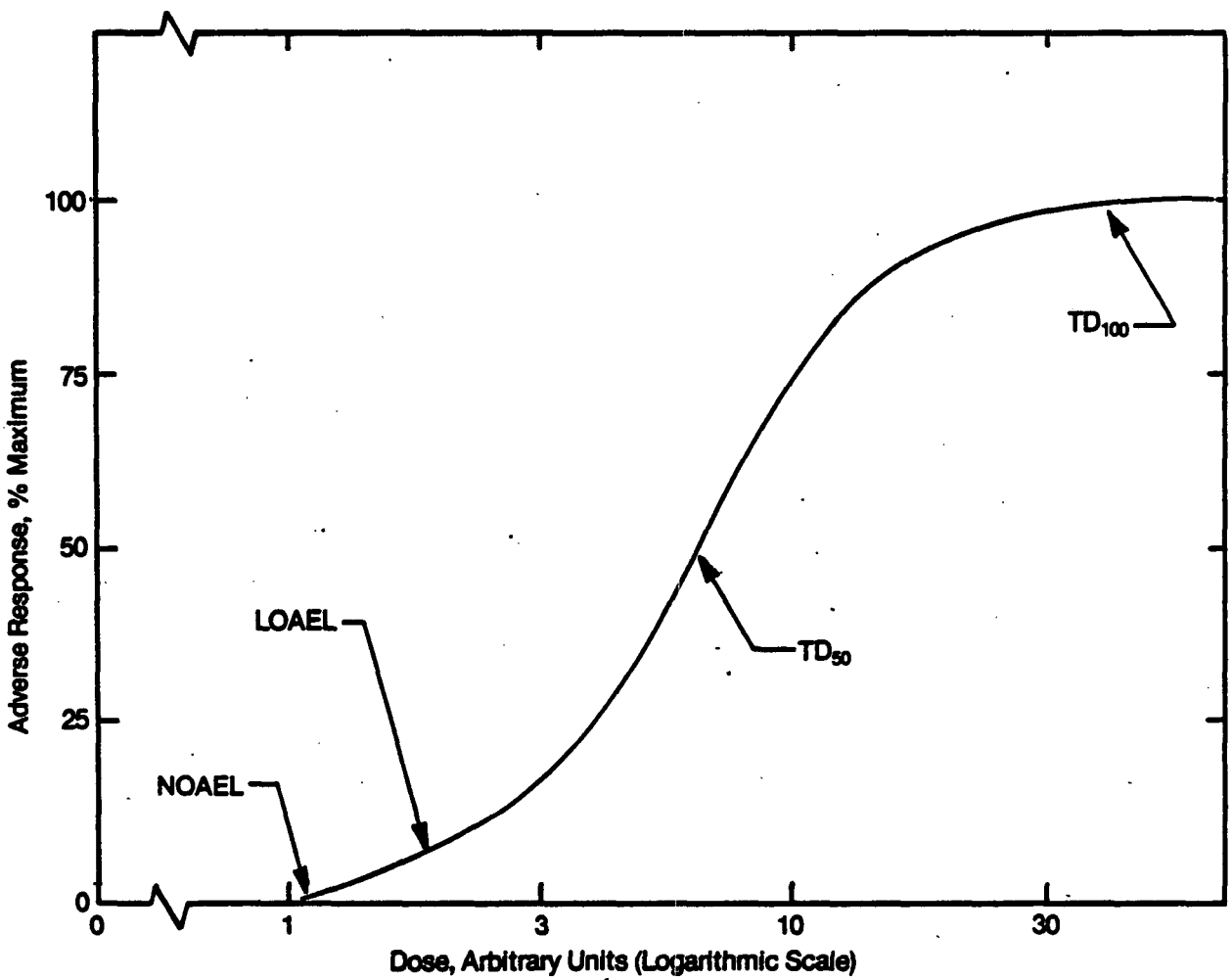


FIGURE ES-2 HYPOTHETICAL DOSE-RESPONSE CURVE WITH A THRESHOLD

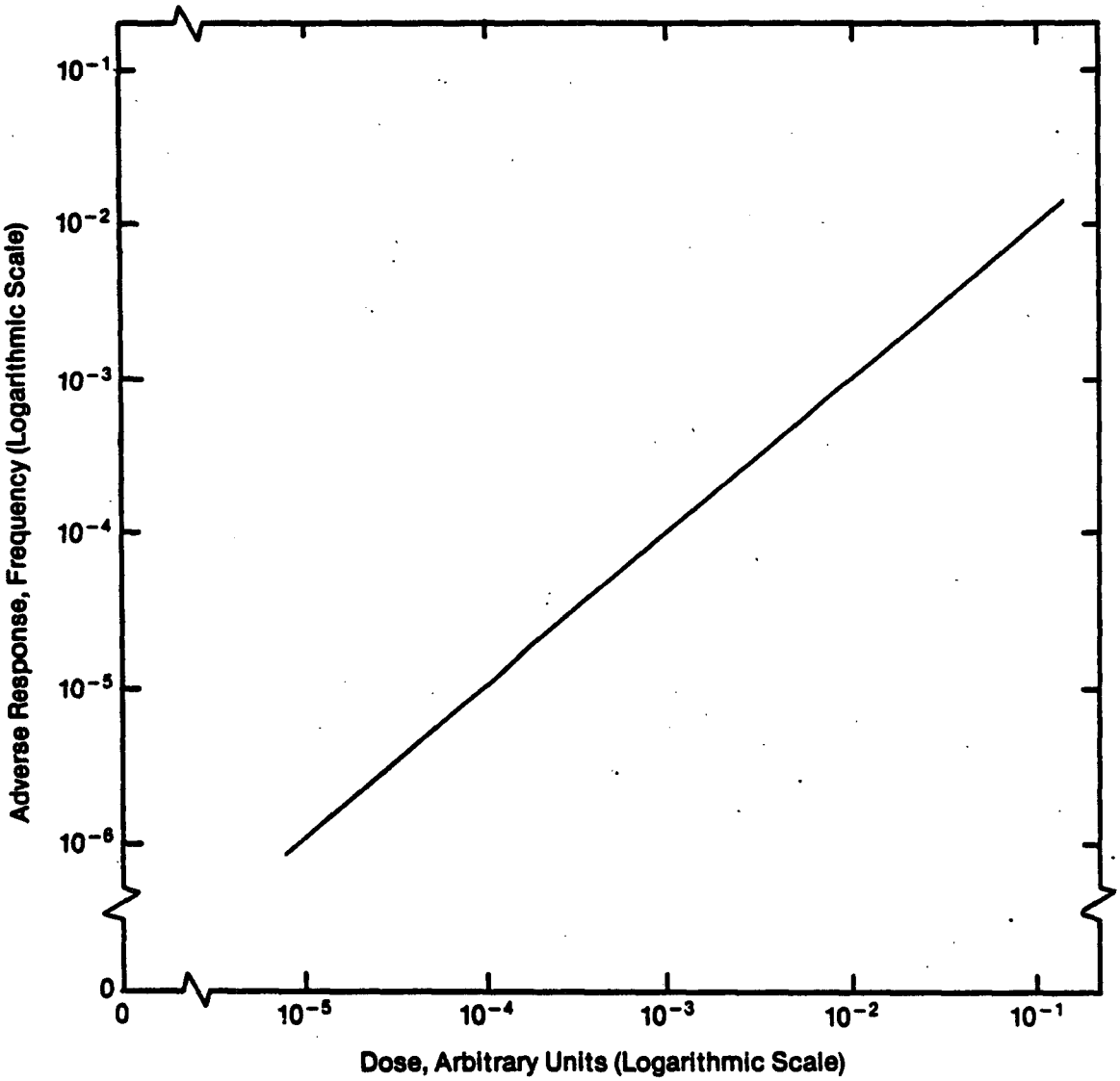


FIGURE ES-3 HYPOTHETICAL DOSE-RESPONSE CURVE WITH NO THRESHOLD

sensitive to the toxic effects of chemicals, and information which is obtained in some animals may not be directly relevant to humans. This is often a result of differences among species with respect to absorption, excretion or metabolism of a chemical, although other factors (e.g., genetic susceptibility) may also be involved. It is for this reason that thorough toxicity assessments involve studies with several species of animals.

4.4 Individual Characteristics

Individual members of a population (especially humans) are not identical and usually do not respond identically to equal exposures to a chemical. This variation has two elements: variation among subgroups of the population as a function of age, race or sex, and variation between individuals within a subgroup (e.g., white males aged 20 to 30 years). Whether the reasons for these variations are understood or not, it is important to identify any subgroups that may have greater sensitivity to a chemical than the general population.

4.5 Toxicological Endpoints

When a chemical is given to a test animal, it is often possible to detect and measure a number of changes in the animal. Those changes which are used by the toxicologist as an index of the chemical's toxicity are called "endpoints." Some commonly measured endpoints are carcinogenicity, hepatotoxicity, mutagenicity, neurotoxicity, renal toxicity, reproductive and fetal toxicity and teratogenicity. One of the most important parts of any toxicity study is selection of the best endpoint to monitor. Usually the endpoint which is most sensitive (i.e., the parameter in which a measurable change first occurs as dose levels increase) is judged to be most appropriate as an index of toxicity. For example, a study measuring a sensitive biochemical indicator of early liver injury is more valuable than a study measuring only gross injury (necrosis or cirrhosis) to the liver.

5.0 TYPICAL PROTOCOLS USED IN TOXICOLOGICAL STUDIES

A typical experiment designed to determine the dose-response curve for a chemical might involve giving six groups of animals a series of increasing doses of the chemical (e.g., 0, 1, 3, 10, 30 or 100 mg/kg) and measuring the magnitude or frequency of the response at each dose level.

A well-performed experiment of this sort may be sufficient to describe the shape of the dose-response curve for a particular test species. However, as discussed above, results in one species are not always directly applicable to humans or other species. Thus, any data which help to define a dose-response curve directly in humans are especially valuable. One source of human data is clinical experience with patients seeking medical attention for health problems resulting from exposure to chemicals. While this is useful in identifying toxic effects in humans, quantitative information on the amount of chemical causing the effects is usually not known. Moreover, even when the exposure is known, it is generally high on the dose-response curve and is not of direct help in defining the NOAEL in humans.

A second source of data in humans is epidemiological studies. An epidemiological study usually seeks to determine whether a particular health effect is associated with exposure to a certain chemical. Typically, incidence of the health effect is compared between two groups of humans, one group known to be exposed to the chemical at a higher level than the other. These studies are often difficult to analyze due to interference by confounding variables such as age, weight, diet, smoking, etc., which may also be related to the health effect under study. Even when meaningful associations are established, epidemiological studies rarely supply dose-response information. It is precisely because of the difficulty in obtaining useful dose-response data in humans that experiments in animals are so important in toxicity assessments.

Contamination of the environment with a toxic chemical may threaten not only humans but wildlife and plants as well. Assessment of the toxic effects of chemicals on fish, terrestrial animals and plants is performed in much the same way as for laboratory animals. Representative species are exposed to various doses or exposure levels of the chemical for varying lengths of time, and signs of adverse effects are noted. From data of this sort it is possible to correlate concentrations of the chemical in the environment to specific levels of risk to indigenous flora and fauna.

6.0 EXTRAPOLATION OF TOXICOLOGICAL DATA FROM ANIMALS TO HUMANS

Since most toxicological data on a chemical are obtained from studies in animals, it is frequently necessary to extrapolate from results in animals to predicted results in humans in order to derive appropriate guidelines or standards regarding acceptable human exposure limits.

6.1 Study Selection

The initial step in this process is selection of the most appropriate animal studies which have been performed. Criteria for selection of studies include the use of appropriate endpoints, optimal analytical methods, proper experimental design and correct data analysis. Studies which provide a clear picture of dose-response relationships are especially valuable in estimating no-effect dose levels.

6.2 Conversions and Corrections

Once suitable studies have been identified, it is necessary to convert the doses administered to the animals to equivalent doses in humans. Since doses are normally described in terms of amount per unit weight (e.g., mg/kg), this would be simple if all animals were equally sensitive on this basis. However, it appears that a better correlation among different species exists when dose is expressed as amount per unit surface area (mg/m^2), and so it is necessary to make this correction. Another correction which is desirable (if sufficient data are available) is for differences between animals and humans in absorption, metabolism or excretion rates of chemicals.

6.3 High-Dose to Low-Dose Extrapolation

One of the most difficult problems in extrapolating data from animal studies to expected results in humans concerns dose levels. Most laboratory experiments are performed at dose levels of the chemicals that produce clear, easily measured responses in the test animals. However, exposure to chemicals in the environment often occurs at dose levels low enough that adverse effects are not immediate or obvious. Thus, it is necessary to extrapolate results obtained at high doses to results expected at low doses. This is especially difficult in the case of a carcinogen. In this case, extrapolation must span a very large change in dose levels (four to six orders of magnitude). Extrapolations are done mathematically by application of equations (models) thought to describe the dose-response curve, but unfortunately, the shape of the dose-response curve at low doses is not known with certainty. The result is that different models yield predictions about risk at low doses that may differ by several orders of magnitude.

6.4 Dealing with Uncertainty

There are many sources of uncertainty inherent in biological research. With respect to the toxicity assessment process, the problems associated with high-dose to low-dose extrapolation, inter-species extrapolation and variation in the sensitivity of sub-groups and individuals have been mentioned above. Because of the uncertainty created by these and other problems, it is generally necessary and appropriate to include a "margin of safety" when establishing a toxicity level. For chemicals that appear to exhibit thresholds in the dose-response curve (i.e., noncarcinogens), this is accomplished by dividing the dose for which no adverse effect has been observed (the NOAEL) by an appropriate uncertainty factor. The basic philosophy behind the selection of uncertainty factors is that when information is lacking and estimates must be made, it is better to err on the low side (i.e., be conservative) than to run the risk of setting too high a value. A similar philosophy underlies the means of dealing with uncertainty in estimating risk from chemicals considered to be without thresholds (e.g., carcinogens). A conservative model is used to make dose extrapolations, and then the upper 95% confidence limit of the extrapolation is employed. Taken together, this ensures that these risk estimates will be highly conservative (i.e., they may be very low, but will not be too high).

7.0 EXPOSURE ASSESSMENT

The second portion of the risk assessment process is the collection and evaluation of data on the actual and potential exposure of humans and other species to chemicals on or near the site. As opposed to toxicity assessment, which is performed primarily in the laboratory, exposure assessment involves a detailed study of the specific site. Specifically, the toxicologist needs to know what chemicals are being released from the site into the environment, what the pattern of chemical distribution is in and around the site and the expected pattern of chemical distribution in the future.

7.1 Collection of Occurrence Data

The first step in answering these questions is collection of data regarding the identities and concentrations of chemicals which occur in and around

the site. Consideration of the history of the site (identity, amount and means of storage of chemicals) often helps to focus concern on one or two specific chemicals known to be present. However, since information of this sort is sometimes incomplete or unreliable, it may be necessary to test for a variety of pollutants. In order to provide an adequate description of the distribution of chemicals in the environment, samples of air, soil, sediment, surface water, groundwater and biota (e.g., fish, shellfish) should be collected at the site and at a series of distances and directions from the site. In some cases, mathematical models of chemical transport in air, water or soil are used to calculate environmental concentrations of chemicals in locations where they have not been or cannot be measured directly.

Since the results of the chemical analysis of environmental samples are usually an essential part of any legal proceedings regarding the site, it is very important to follow strict scientific and legal procedures during collection and analysis of the samples.

7.2 Identification of Exposed Organisms

No matter how toxic a chemical may be or how concentrated it may be in the environment, no injury can occur to an organism if the chemical does not come into contact with the organism. For this reason, an essential part of the exposure assessment process is identification of organisms (humans and other species) that live in or periodically enter the contaminated area. Additional important information includes a description of how the organisms come in contact with the chemical (inhalation, ingestion in water, dermal contact, etc.) and how long they are exposed.

7.3 Calculation of Exposure

Given quantitative data on the concentration and distribution of chemicals in and around the site, it is necessary to calculate the degree of exposure of humans and of environmental organisms that come into contact with the chemical. For human exposure to a chemical in water, for example, this calculation involves finding the product of the concentration in the water at the location where the exposure occurs times the intake of water per day by the exposed humans at that location. If the absorption factor is known, the absorbed dose can be calculated. Similar calculations may be made for other routes of exposure, and the total dose from all routes of exposure is found by addition.

7.4 Estimation of Past and Future Exposure

A thorough risk assessment may require knowledge not only of present exposure, but of past and future exposure as well. Past exposure to some chemicals can be estimated from measurements of the levels of the chemical in the body. This is useful only for chemicals which tend to accumulate in the body. Estimates of past or future exposure levels must usually be calculated, based on the expected movement and stability of the chemical in the environment.

Movement of a chemical is determined by the properties of the chemical and the geologic and climatic characteristics of the site. For example, if a

chemical is readily soluble and is found to occur primarily in groundwater, the rate and direction of groundwater flow will be the principal controlling force in chemical movement. Conversely, if a chemical is strongly adsorbed to surface soil, weather conditions controlling soil erosion (such as annual rainfall and prevailing wind patterns) would be of chief concern.

Stability of a chemical in the environment is determined by the rate at which it undergoes reactions. Such reactions include microbial degradation, photolysis, hydroloysis and reduction or oxidation. Usually these reactions decrease the amount and the toxicity of the chemicals, but some reactions actually increase toxicity.

8.0 RISK ASSESSMENT

The final step in the risk assessment process is integration of the results of the toxicity assessment process and the exposure assessment process. In simplest terms, what is required is a comparison of the exposure levels with the levels believed to pose health risks. For example, if the groundwater being tapped by wells used for human consumption contains 50 µg/L of trichloroethylene, and a concentration of 5 µg/L of trichloroethylene is believed to pose a risk of cancer ($1/10^6$), then the level of risk to humans is $1/10^5$. Conversely, if air levels of chloroform are 1 ppm, and levels of 10 ppm in air are considered to be safe, then no present risk exists.

In real life, risk assessment may not be quite so simple. For example, there may be significant (and legitimate) differences of opinion between scientists regarding interpretation of key data on toxicity, predictions of chemical fate or transport, etc. In addition, when data is lacking and judgments must be made, it is not uncommon that dissimilar conclusions may be reached by different scientists, depending on the assumptions which are employed.

Thus, risk assessment involves a blend of scientific fact, judgment and common sense. Clearly, however, the keystone to a sound risk assessment is collection of a sound, reliable and complete set of exposure data along with a thorough toxicity assessment.

9.0 TOXICOLOGICAL INFORMATION SUMMARIES

The last three chapters of the handbook present summaries of the current understanding of selected chemicals which occur at many hazardous waste sites: dioxins and furans, trichloroethylene and lead. These summaries contain a greater level of technical detail, similar to that which a practicing toxicologist would prepare. For the nontoxicologist, they present both specific information on frequently occurring chemicals and an opportunity to apply the understanding obtained from the earlier chapters of the handbook.

1.0 INTRODUCTION

The U.S. Environmental Protection Agency (EPA) is responsible for investigating hazardous waste disposal sites under the Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA) and the Resource Conservation and Recovery Act of 1976 (RCRA). The activities under CERCLA are directed by the EPA Office of Emergency and Remedial Response (OERR). The activities under RCRA are directed by the EPA Office of Solid Waste (OSW). The EPA Office of Waste Programs Enforcement (OWPE) provides program management for EPA solid waste and emergency/remedial response enforcement activities. This handbook was prepared under the direction of OWPE. All three offices (OWPE, OERR and OSW) are directed by the EPA Assistant Administrator for Solid Waste and Emergency Response.

1.1 Purpose and Scope of the Toxicology Handbook

Enforcement actions at hazardous waste sites require regional EPA personnel to understand general toxicological principles and to be able to effectively interact with toxicologists. The purpose of this handbook is to explain to non-toxicology-trained personnel those principles of toxicology relevant to hazardous waste site investigations. This handbook is not highly technical and is intended to provide a working familiarity with relevant toxicological principles. It is not intended to teach a nontoxicologist how to perform hazard, exposure or risk assessments. Sources of more detailed information are provided in the handbook. By understanding the principles of toxicity, exposure, risk and endangerment assessment processes and the relevant toxicology of the chemicals of high concern presented in this handbook, regional personnel will be better able to conduct the hazardous waste site investigations. Better informed hazardous waste site investigations will result in fewer problems in subsequent Federal activities such as litigation and remedial actions.

1.2 Handbook Organization

The Toxicology Handbook is divided into eleven chapters in addition to an executive summary and glossary in the front and an index at the back. A description of the contents of each chapter appears in section 2.0. At the conclusion of chapters four through eight, key guidance and implementation documents are provided to direct the user to additional technical information. Background references used in preparing chapters are listed at the end of each chapter.

2.0 FUNDAMENTAL CONCEPTS IN TOXICOLOGY

Toxicology is the study of how chemical substances, either natural or man-made, cause undesirable effects in living organisms. Knowledge derived from toxicological studies has many applications, including the provision of recommendations to public officials charged with the protection of human health and the environment from hazardous substances.

Risk to human health and the environment from toxic chemicals is a matter of grave concern to modern society. Large amounts of hazardous substances have been inadequately or improperly disposed of, and reports of toxic chemicals entering air, water and food occur with alarming regularity. However, simple detection of a hazardous chemical in the environment is not necessarily cause for alarm. Indeed, modern chemical detection techniques are so sensitive that it is possible to detect contaminants at very low levels. Additionally, nearly any chemical (including such common items as coffee, alcohol, salt, even water) can produce adverse effects when consumed in high enough amounts. Clearly, determination of the problems posed by a chemical detected in the environment must involve an evaluation of how much of a chemical is present, judged in terms of how toxic that chemical is.

The process of characterizing the inherent toxicity of a chemical is termed toxicity assessment. This process involves determining what adverse effects the chemical causes in exposed organisms, and how much of the chemical is required to produce these effects. The important concepts involved in toxicity assessment are introduced in sections 2.1 through 2.3 of this chapter and are explained in more detail in chapters 3.0 through 6.0 of this handbook.

The process of determining how much of a chemical is in the environment and may come into contact with humans or other organisms is termed exposure assessment. The important concepts in this process are introduced in section 2.4 of this chapter and described in more detail in chapter 7.0.

The process of integrating available information on the inherent toxicity of a chemical with information on how much of a chemical may make contact with exposed organisms is termed risk assessment. A description of the main elements in this process, especially as they are performed at hazardous waste sites, is contained in chapter 8.0. Figure 2-1 provides an overview of the risk assessment process.

Chapters 9.0 through 11.0 provide detailed summaries of toxicological information on chemicals of common concern at hazardous waste sites: dioxin (and furan), trichloroethylene and lead.

2.1 Mechanisms of Chemical Toxicity

All living organisms are composed of cells. In humans and other higher organisms, nearly all cells are specialized to perform some specific function to benefit the entire organism. For example, muscle cells are specialized to perform motion, retinal cells are specialized to detect light and red blood cells are specialized to carry oxygen. In addition to these specialized

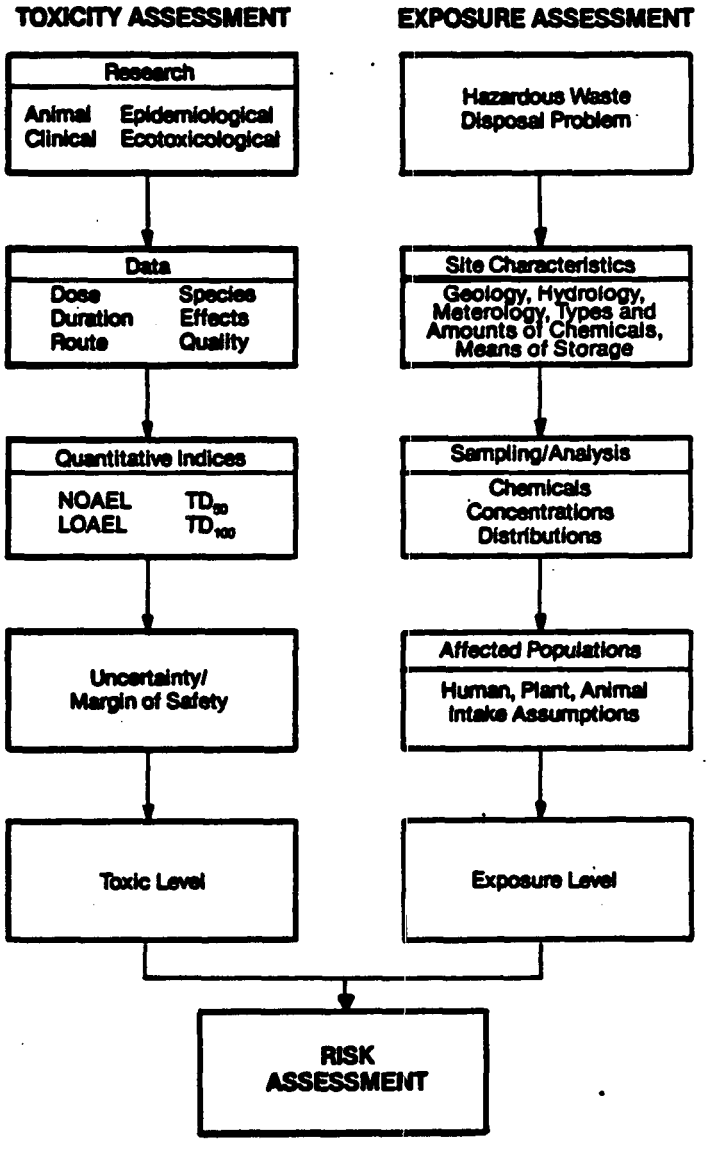


FIGURE 2-1 OVERVIEW OF THE RISK ASSESSMENT PROCESS APPLIED TO A HAZARDOUS WASTE SITE

functions, all cells must perform certain basic functions, such as generating the energy they need and maintaining and repairing themselves. All of these specialized and basic functions are the direct result of chemical reactions which occur in the cells. It is essential to the proper functioning of a cell that all of the chemical reactions occurring within it proceed at the proper rate; any significant increase or decrease in even one key reaction may lead to failure of the cell.

In view of the large number of chemical reactions that are occurring in all cells at all times, and the importance that these reactions not be disturbed, it is perhaps not surprising that introduction of a foreign chemical into a cell may result in adverse effects. Different types of chemicals may disturb cellular chemical reactions by different means. Some chemicals may react with key cellular molecules, thereby changing their properties, damaging them or rendering them ineffective. Other chemicals may substitute for normal body chemicals, leading to formation of unusual new products, which may be more or less toxic than the original chemicals, and preventing formation of normal products.

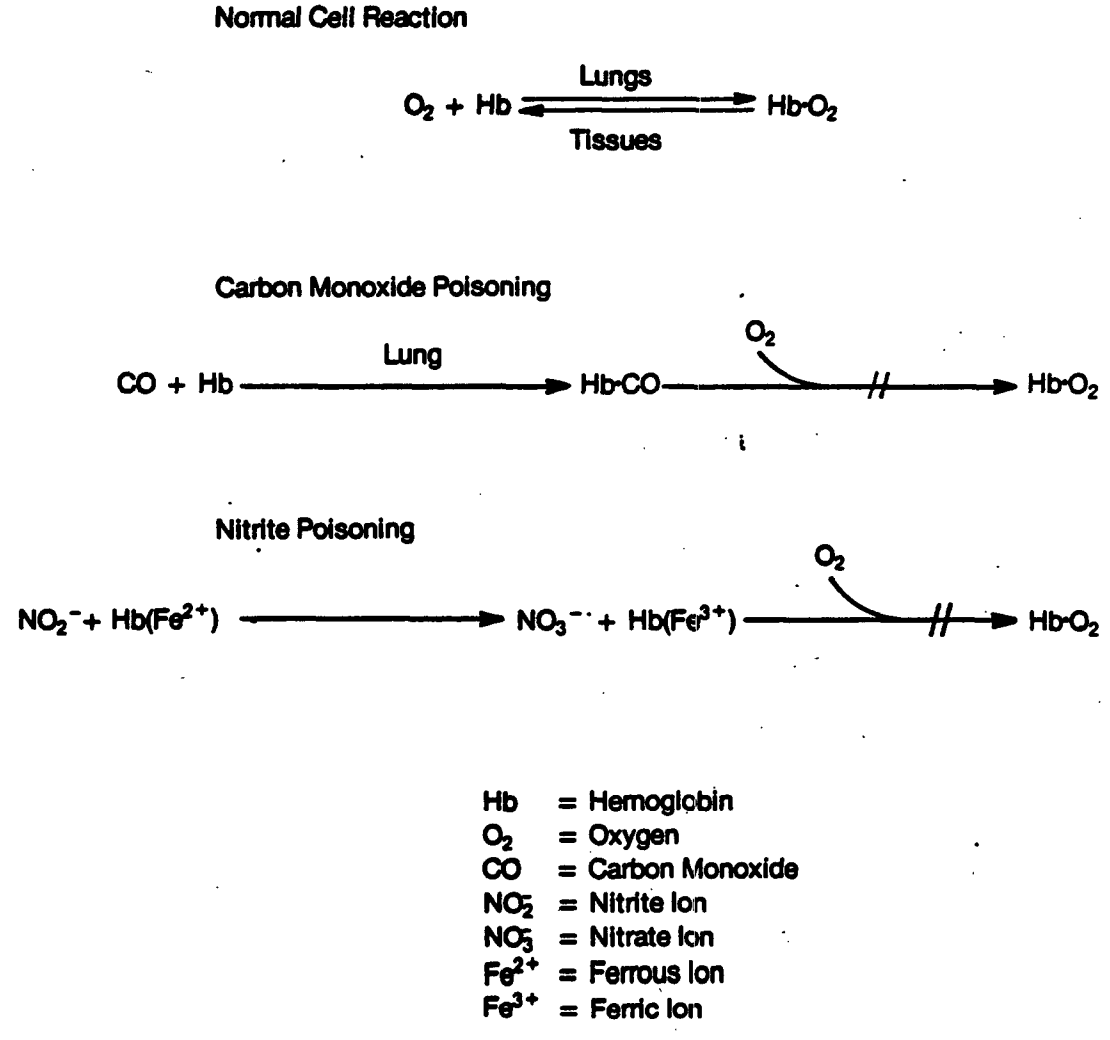
Figure 2-2 summarizes two simple examples of these sorts of toxic mechanisms. Red blood cells contain hemoglobin (Hb), a chemical specialized for the binding and release of oxygen molecules (O_2). Carbon monoxide (CO) is a chemical that is sufficiently similar to oxygen that it may substitute for oxygen and bind with hemoglobin. Hemoglobin molecules which bind a molecule of carbon monoxide are thus rendered unable to carry their normal product (oxygen), and death may result from oxygen starvation of the cells. Nitrite (NO_2^-) is a chemical that has a similar result, but involves a different mechanism. Hemoglobin contains one atom of iron, and this is in a particular form (termed ferrous ion, written as Fe^{+2}). Nitrite reacts with this ferrous ion, changing it to ferric ion (Fe^{+3}). This change completely destroys the ability of hemoglobin to carry oxygen and, as with carbon monoxide, death may ensue from oxygen starvation at the cellular level.

Ultimately, the toxic effects of any chemical must be due to its interference with some important cellular reaction, but the details will vary with each individual chemical.

2.2 Essential Toxicological Information

A full understanding of the toxicological effects of a chemical requires detailed investigation of a number of aspects of how a chemical behaves in an organism, including:

- Absorption - How readily does the chemical enter the organism through the skin, stomach or lungs?
- Distribution - What cells of the organism does the chemical enter? Do some cells take up more than others?
- Excretion - How rapidly does the organism get rid of the chemical? Does it tend to accumulate in the organism over long periods?



Hb = Hemoglobin
O_2 = Oxygen
CO = Carbon Monoxide
NO_2^- = Nitrite Ion
NO_3^- = Nitrate Ion
Fe^{2+} = Ferrous Ion
Fe^{3+} = Ferric Ion

FIGURE 2-2 EXAMPLES OF MECHANISMS BY WHICH TWO CHEMICALS ALTER A CELLULAR FUNCTION

- Toxic Effects - What are the toxic effects that occur in an organism exposed to the chemical? What cells are injured? Are the effects permanent?

- Sensitive Populations - Does the toxicity of the chemical depend on age, sex or race? If so, why? What is the most sensitive sub-group?

- Mechanism - What chemical reactions are altered by the chemical? How does the chemical do this?

While all of these questions must ultimately be answered in order to fully describe the toxicity of a chemical, it is possible (and often necessary) to make regulatory decisions regarding a chemical with a more limited amount of knowledge. Specifically, the key information that is required is an estimate of the highest amount (dose) of a chemical that does not produce any undesirable or unacceptable effects in the exposed organism (plant, animal, human).

For some chemicals, there may be no dose that is without risk of causing adverse effects. This is suspected to be true for chemicals which cause cancer (carcinogens). In this case, even a single molecule of the chemical might react within a cell causing a change in the system controlling cell growth that cannot be reversed, leading (in the worst case) to a fatal tumor.

For other chemicals, there may be a certain minimum level of exposure (a threshold value) below which no significant or observable effects occur. This is due to one or both of two reasons: (1) there is considerable "reserve capacity" in many tissues, such that limited damage (e.g., a 5% loss of hemoglobin) does not cause any decrease in function; and (2) most cells have at least a limited capacity to repair or compensate for cellular damage. Of course, both the reserve and the repair capacity of a tissue can be overwhelmed by too much of a harmful chemical and it is at that point that the organism as a whole begins to suffer adverse effects.

Thus, the essential toxicological questions that must be answered in order to make informed regulatory decisions regarding a chemical are:

- Does the chemical cause effects that do not have a dose-response threshold (e.g., cancer)?

- For effects that do have a dose-response threshold, what is that threshold value?

A generalized description of the means of answering these questions through toxicological research is presented in chapters 3.0, 4.0, 5.0 and 6.0, of this handbook.

2.3 Limitations to Toxicological Knowledge

Existing toxicological knowledge cannot provide answers to all the questions of concern to regulatory agencies. One reason is simply the large number of

chemicals of potential or known concern that are in use today. Toxicological investigation and characterization of a chemical is a slow and expensive process and evaluation of all existing chemicals would greatly exceed the capacity of the toxicological community. Additionally, new chemicals are being developed and proposed for use at a rate that further overloads the capacity to perform additional toxicological studies.

When it becomes necessary to make a decision on a chemical in the absence of full and detailed toxicological knowledge, the only solution is to make reasonable assumptions. Often there is partial information on the chemical or knowledge of a similar chemical. In these cases, assumptions may be made by extension or extrapolation of existing knowledge to achieve answers to questions that have not been studied directly.

Such extensions and extrapolations of information are never certain, and it is possible for knowledgeable and reasonable scientists to differ considerably in the extrapolations which they feel are appropriate.

The optimal solution to this problem is to choose defensible and well reasoned assumptions to yield a range of potential risk including that which is most conservative (i.e., provides for the greatest margin of safety). When the requirements for human health concern are addressed in terms of cost and feasibility, it is important to remember that present toxicological knowledge is insufficient in many cases to provide definitive answers about a chemical and it is, therefore, important to characterize this uncertainty.

2.4 Exposure to Toxic Chemicals

No matter how toxic a chemical may be, it cannot cause an effect on a living organism unless it comes into contact with that organism. This contact between chemical and organism is termed exposure. There are two key aspects of exposure that are important determinants of the effect on the organism. First is the site of contact between the chemical and the organism (skin, eyes, lungs, gastrointestinal tract). This is important since the amount of chemical actually entering the body (absorbed) depends on the ease with which it can cross these body surfaces. The second aspect is the amount of chemical making contact with the organism. This amount is usually described as a concentration in each of the relevant environmental media (air, water, soil, food). From these environmental concentrations it is possible to calculate the amount of chemical contacting the organism, based on the rate of contact between the organism and the media (e.g., liters of air breathed per day, liters of water consumed per day, etc.).

From these considerations, it is clear that an evaluation of the exposure to humans or other organisms by a chemical in the environment requires answers to the following questions:

- In what environmental media (air, water, soil, food) is the chemical present?
- How much of the chemical is or is likely to be present in these media?

- How many and which organisms are or will likely become exposed to the chemical?
- By what routes may the chemical make contact with exposed organisms?
- What is the frequency and duration of contact?

Collection of this information constitutes the exposure assessment process. A more detailed description of the issues and requirements of this process is presented in chapter 7.0.

3.0 DOSE-RESPONSE RELATIONSHIPS

As discussed in chapter 2.0, complete assessment of the toxicity of a chemical requires detailed study of a number of areas relating to how the chemical behaves in the body. However, as input to regulatory decision making, the major objective of a toxicity assessment is the determination of the maximum dose which produces no significant adverse effect (response) in exposed organisms. This information is obtained by deriving the dose-response curve for the chemical.

3.1 Identification of No-Effect Levels From Dose-Response Curves

The dose-response relationship is the most fundamental concept in toxicology. The dose-response curve describes the relationship that exists between degree of exposure to a chemical (dose) and the magnitude of the effect (response) in the exposed organism. By definition, no response is seen in the absence of chemical. As the amount of chemical exposure increases, the response becomes apparent and increases. Depending on the mechanism by which the chemical acts, the curve may rise with or without a threshold (Figure 3-1). In both cases, the response normally reaches a maximum after which the dose-response curve becomes flat.

3.1.1 Chemicals with Thresholds

Many chemicals produce responses that display a threshold value (an exposure below which no responses can be detected). One example of this is shown in Figure 3-2. Nitrite (NO_2^-) is a chemical which produces injury to red blood cells by oxidizing the iron atom in hemoglobin from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) form. Within the red blood cell is a system designed to protect the cell against this kind of damage. It involves providing a "sacrificial" chemical, glutathione (GSH), that is oxidized by the nitrite instead of the iron. Only when the amount of nitrite exceeds the ability of the cell to supply glutathione is the iron of hemoglobin oxidized.

A second reason that a threshold may occur in a dose-response curve can also be understood by consideration of nitrite toxicity. There is a considerable excess of oxygen-carrying capacity in normal blood, and even when some hemoglobin becomes oxidized (and hence non-functional), the amount of oxygen supplied to tissues is still in excess of the tissues' needs. Only when a large amount of hemoglobin has been oxidized will there be an adverse effect in tissues consuming oxygen (Figure 3-3).

3.1.2 Determination of Threshold Values

Regardless of the precise reason for the existence of a threshold, it is important to determine the threshold value with some accuracy. This value is often called the No-Observed-Adverse-Effect Level (NOAEL). The lowest value where a significant adverse effect is first seen is the Lowest-Observed-Adverse-Effect Level (LOAEL). A typical experiment designed to define the shape of the dose-response curve and provide an estimate of the NOAEL and the LOAEL involves exposing groups of experimental subjects (e.g., mice or rats) to a

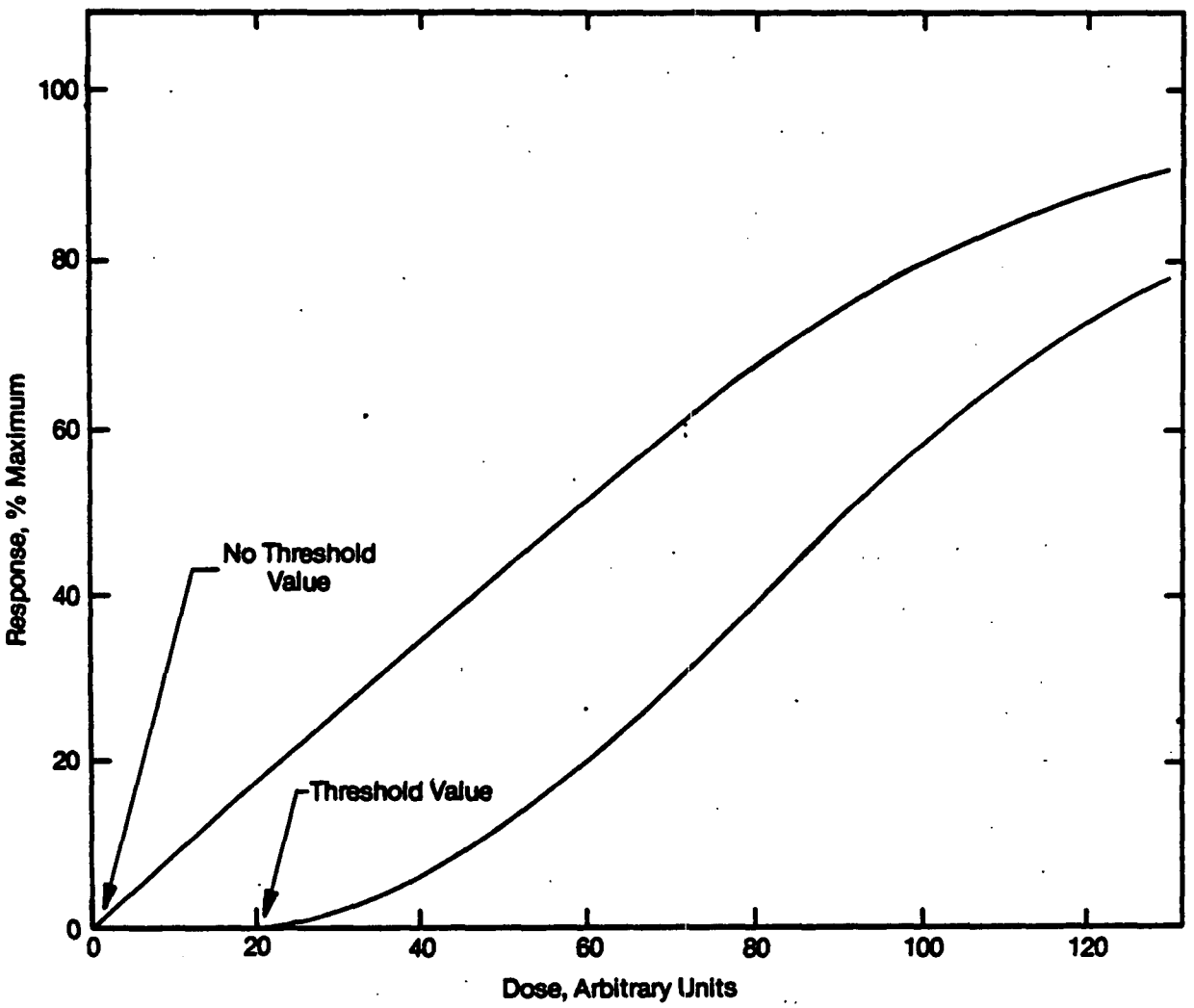


FIGURE 3-1 HYPOTHETICAL DOSE-RESPONSE CURVES

The dose-response curve on the left illustrates a no threshold effect; there is a response at all doses greater than zero. The dose-response curve on the right illustrates an effect with a threshold; no response occurs until some minimum (threshold) dose is exceeded.

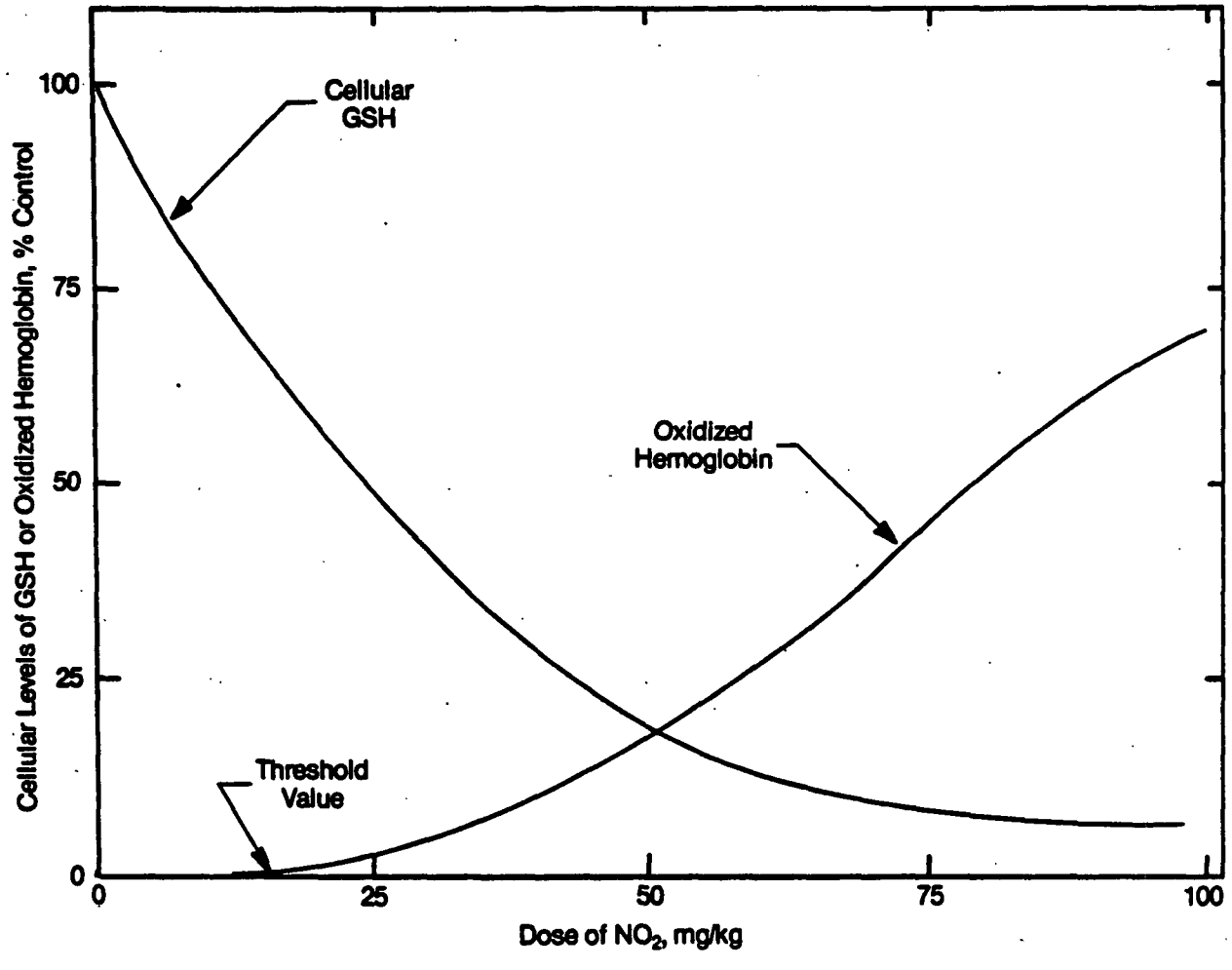


FIGURE 3-2 CELLULAR DEFENSE MECHANISMS AS A BASIS FOR THE THRESHOLD PHENOMENON

This figure illustrates the red blood cell's ability to counteract nitrite's harmful oxidation of hemoglobin. The cell produces a chemical (GSH) which preferentially reacts with NO_2^-, protecting hemoglobin. Only when the dose of nitrite exceeds the cell's ability to produce GSH is hemoglobin oxidized. The curves shown are hypothetical.

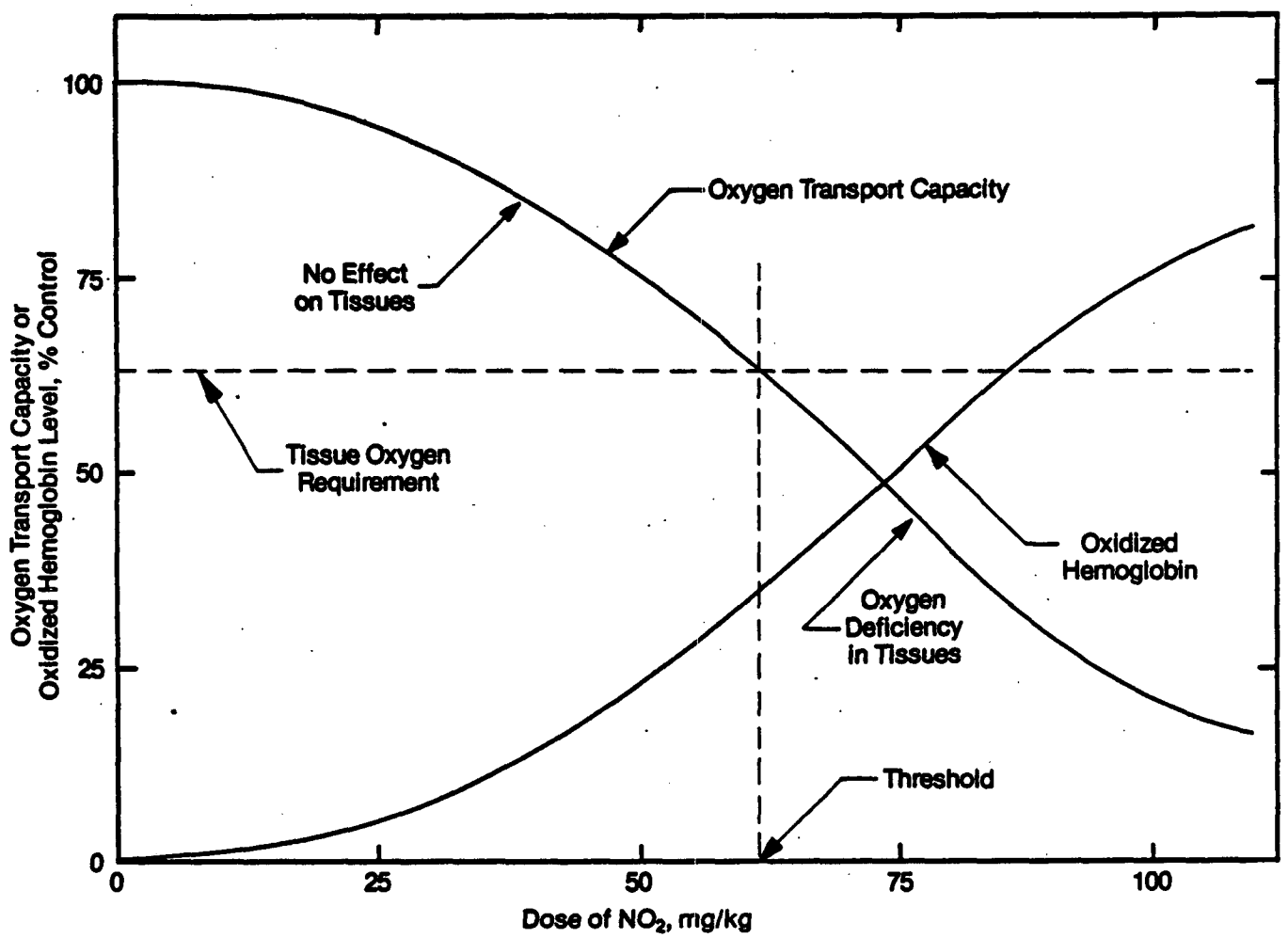


FIGURE 3-3 TISSUE RESERVE AS A BASIS FOR THE THRESHOLD PHENOMENON

This figure illustrates that oxidation of hemoglobin by nitrite can reduce the oxygen transport capacity of blood significantly prior to the occurrence of oxygen starvation in tissues. The curves shown are hypothetical.

series of doses of the chemical. An example of the results of such an experiment is shown in Table 3-1. There are several useful ways in which these data may be displayed graphically. In prior examples (Figures 3-1, 3-2 and 3-3), the dose was plotted on a linear scale. It is usually more convenient to plot the doses on a logarithmic scale. This type of plot expands the low-dose region of greatest interest, and compresses the high-dose region. The data shown in Table 3-1 are plotted in Figure 3-4 using a logarithmic dose scale. Based on the smooth curve drawn through the data points in Figure 3-4, the NOAEL value is about 0.3 mg/kg, and the LOAEL value is between 1 and 3 mg/kg. Usually NOAEL and LOAEL values are not derived by extrapolation between doses, but are selected from the doses actually administered. In this case, the NOAEL (the highest dose which did not produce a statistically significant effect) is 1.0 mg/kg, and the LOAEL (the lowest dose which did produce a statistically significant effect) is 3.0 mg/kg. (See Section 6.1 for discussion of statistical significance.)

When a dose-response experiment is well-designed, it will span a series of doses on both sides of the NOAEL and the LOAEL, permitting accurate identification of each. Poorly designed experiments may only test doses that are too high or too low (not spanning the NOAEL/LOAEL range). It is difficult or impossible to derive reasonable estimates of the NOAEL or the LOAEL from this sort of data.

A very important point which must always be remembered is that NOAEL and LOAEL values depend on the effect (endpoint) being measured. For example, selecting lethality, hemoglobin oxidation or reduction of red blood cell glutathione levels as endpoints, NOAEL values might be 30, 3 or 0.3 mg/kg, respectively. Choosing the most appropriate endpoint is not always simple and is discussed in more detail in chapter 4.0.

3.1.3 Chemicals Without Thresholds

Some chemicals produce adverse effects that are characterized by a dose-response curve with no threshold (see Figure 3-1). This is usually because the cells that are affected have little or no "defense" against the chemical and have little or no ability to repair or compensate for damage that is done. For example, recent research suggests that there may be no threshold for the effects of lead on the nervous system in infants and children. Chemicals that produce cancer (carcinogens) are also considered to belong to this group.

For chemicals with no threshold, any exposure is associated with some degree of risk. A hypothetical relationship between dose and risk of cancer is shown in Figure 3-5. The risk of cancer is expressed as a frequency, where 10^{-6} for example, means that if one million (10^6) people were exposed for their lifetime, on average one cancer case ($1/10^6 = 10^{-6}$) would occur. Risk decreases as dose decreases, but never reaches zero for chemicals without thresholds. Figure 3-5 assumes a linear relationship exists between risk and dose at low dose levels, but this is not known with certainty. This matter is discussed more fully in section 6.4.

TABLE 3-1 HYPOTHETICAL RESULTS OF AN EXPERIMENT TO DEFINE A DOSE-RESPONSE CURVE FOR NITRITE-INDUCED HEMOGLOBIN OXIDATION

Group	Number of rats	Dose of Nitrite, mg/kg	Oxidized Hemoglobin, % (mean ± SD)
1	10	0	3 ± 3
2	10	0.1	4 ± 2
3	10	0.3	4 ± 4
4	10	1	6 ± 3
5	10	3	12 ± 5[(a)]
6	10	10	24 ± 9[(a)]
7	10	30	62 ± 5[(a)]
8	10	60	80 ± 12[(a)]

This table shows the results of a hypothetical experiment in which eighty rats were divided in eight groups of ten and administered varying doses of nitrite by stomach tube. After one hour, the level of oxidized hemoglobin in the blood of each rat was measured. Results presented are the mean and standard deviation (SD) for each dose group.

(a) Statistically different ($P<0.05$) from control (group 1).

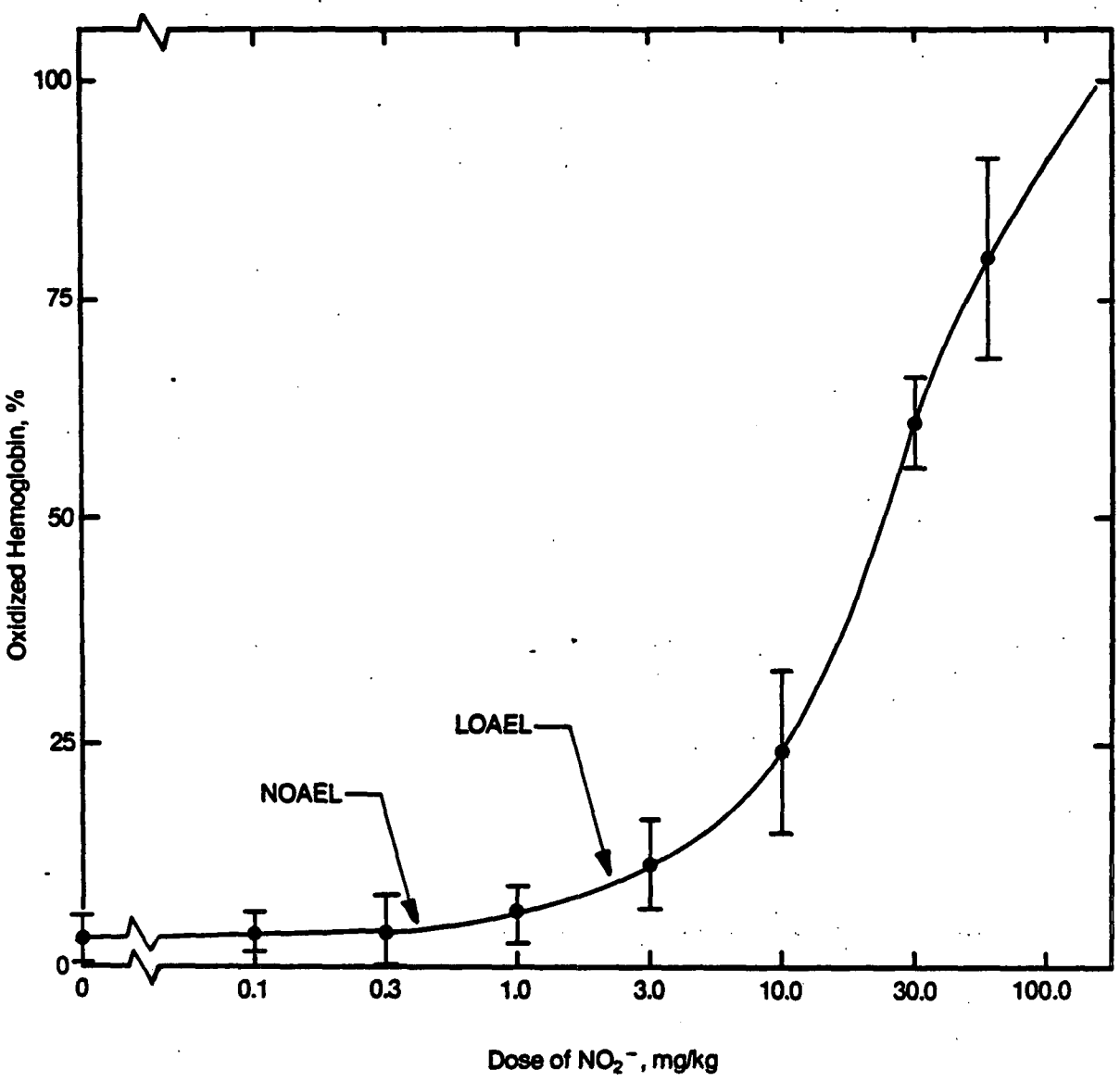


FIGURE 3-4 DOSE-RESPONSE CURVE (LOGARITHMIC SCALE) FOR NITRITE-INDUCED HEMOGLOBIN OXIDATION

This figure is a plot of the hypothetical data shown in Table 3-1. The logarithmic scale magnifies the low-dose region of the curve, making it easier to identify NOAEL and LOAEL values. The mean data values are shown by the dots, and the standard deviations are shown by the vertical bars.

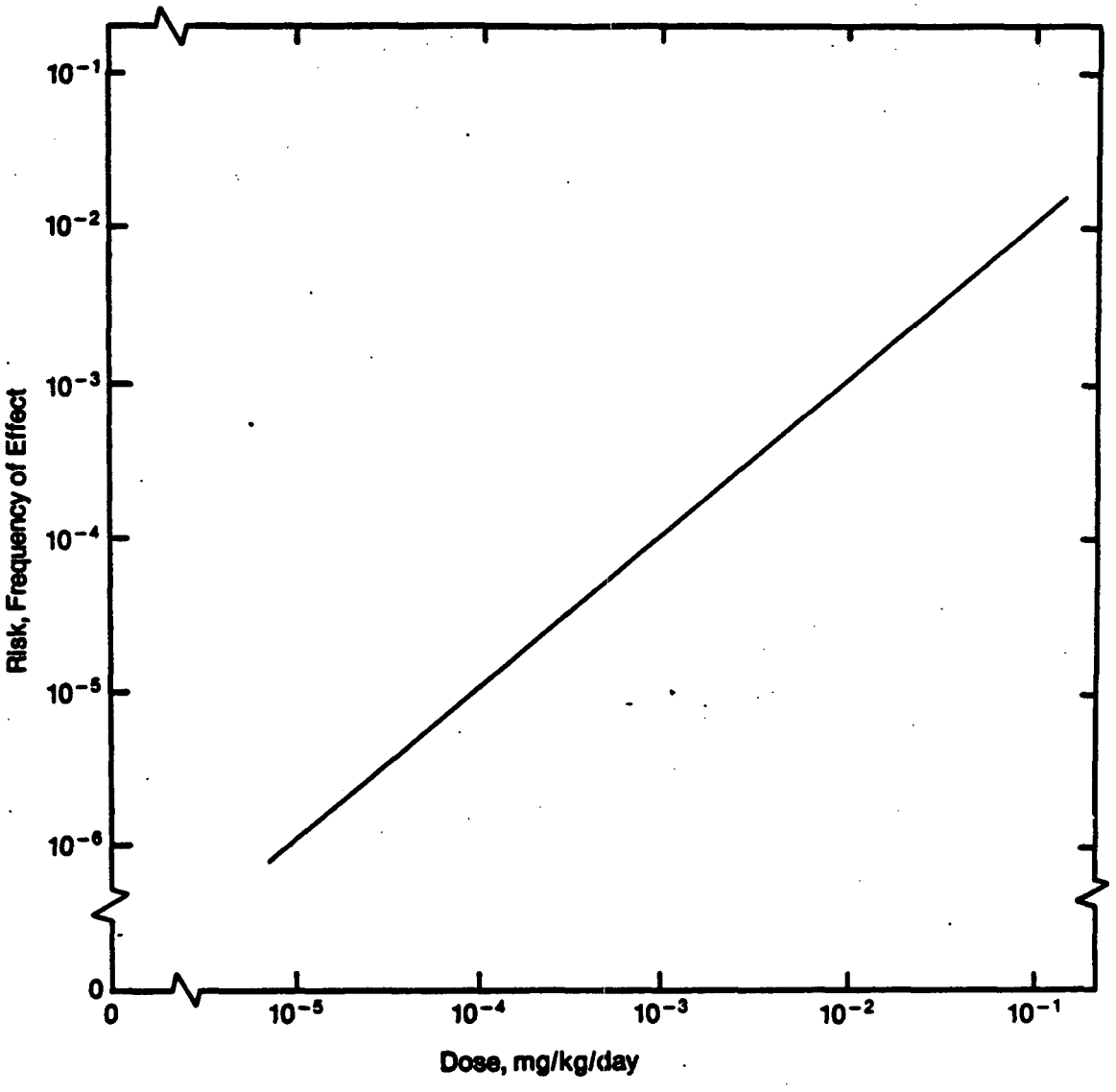


FIGURE 3-5 HYPOTHETICAL RELATIONSHIP BETWEEN RISK AND DOSE FOR CHEMICALS WITH NO THRESHOLD

This figure illustrates that for chemicals with no threshold, as dose decreases so does risk (frequency of the effect), but the frequency never reaches zero unless the dose is zero. For example, a dose of 10^{-3} mg/kg/day (1 µg/kg/day) would cause an effect in about 1/10,000 (10^{-4}) exposed organisms.

While it would be ideal from the health effects perspective to set zero exposure (and zero effect) as a goal, it is usually not technically or economically feasible. Therefore, a judgement must be made as to what risk level (greater than zero) is "acceptable." This choice is basically a cost-benefit judgement made by public officials and by society, assessing the magnitude of the risk from the chemical in comparison to risks from other sources (accident, crime, flood, tornado, etc.), taking the cost and feasibility of achieving the selected acceptable level into consideration.

Once a risk level is established (e.g., 10^{-6}), an acceptable exposure limit can simply be determined from a curve similar to the one shown in Figure 3-5. Unfortunately, it is not possible to make direct experimental measurements of dose-response relationships at such low response rates (this would require testing many millions of animals at each dose). Therefore, curves such as shown in Figure 3-5 must be extrapolated from measurements made at high doses (involving hundreds of animals at each dose). Performance of such extrapolations is discussed in chapter 6.0.

3.2 Other Uses of Dose-Response Curves

In addition to providing a convenient means of determining no-effect levels for chemicals, dose-response curves also help characterize the toxic properties of a chemical and are useful in comparing the toxicity of several chemicals. In characterizing or comparing dose-response curves, a number of quantitative indices are commonly employed to aid in the description of the shape and location of a dose-response curve. Table 3-2 lists some of these parameters and describes their meaning and usefulness.

It is common to select the mid-point on the dose-response curve for comparison of the toxicity of two chemicals. This is simply because it is usually easier to determine the mid-point accurately than to make an accurate estimate of the NOAEL. In general terms, the mid-point is referred to as the ED_{50} (the dose which produces 50% of the effect). When a toxic effect is being measured, the term TD_{50} is used. When the effect being measured is lethality, the term LD_{50} is used. Because lethality is unambiguous and simple to measure, an LD_{50} value is often the first characterization of a chemical's toxicity to be derived. However, lethality is usually too crude an index of adverse effects to be useful in assessing toxicity of a chemical in the environment, and a TD_{50} or ED_{50} based on a more sensitive endpoint should be used whenever possible in evaluating or comparing chemical toxicity.

The slope of a dose-response curve is another important variable to be considered in assessing the toxicity of a chemical. Figure 3-6 shows two dose-response curves, where chemical B is more toxic than chemical A at low doses, even though the TD_{50} for chemical B is higher (less toxic) than for chemical A. The reasons why the slopes of dose-response curves differ for different chemicals involve the particular cellular mechanisms and metabolic functions which they affect. While the details of these mechanisms and functions are of concern to the toxicologist in understanding chemical toxicity, understanding is not necessary for evaluating the degree of chemical toxicity. The important concept to grasp is that chemicals with steep dose-response curves need to be treated with caution since there may be only a small difference between a dose producing no effect and a dose producing serious outcome.

TABLE 3-2 QUANTITATIVE INDICES DERIVED FROM DOSE-RESPONSE CURVES

Index	Definition/Use
ED	Effective dose; may be used to mean any effect, but usually is reserved to describe non-toxic or beneficial effects.
TD	Toxic dose
LD	Lethal dose
ED_{50}	Dose which produces 50% of an effect (either 50% of the maximum change or a significant change in 50% of an exposed population).
TD_{50}	Dose which produces 50% of a toxic effect.
LD_{50}	Dose which causes death in 50% of an exposed population.
EC,TC, LC	Analogous to ED, TD, LD, but refers to the concentration of chemical in water, and is used in studies of aquatic or marine organisms.
TI	Therapeutic Index, a ratio describing the margin of safety between beneficial and adverse effects.

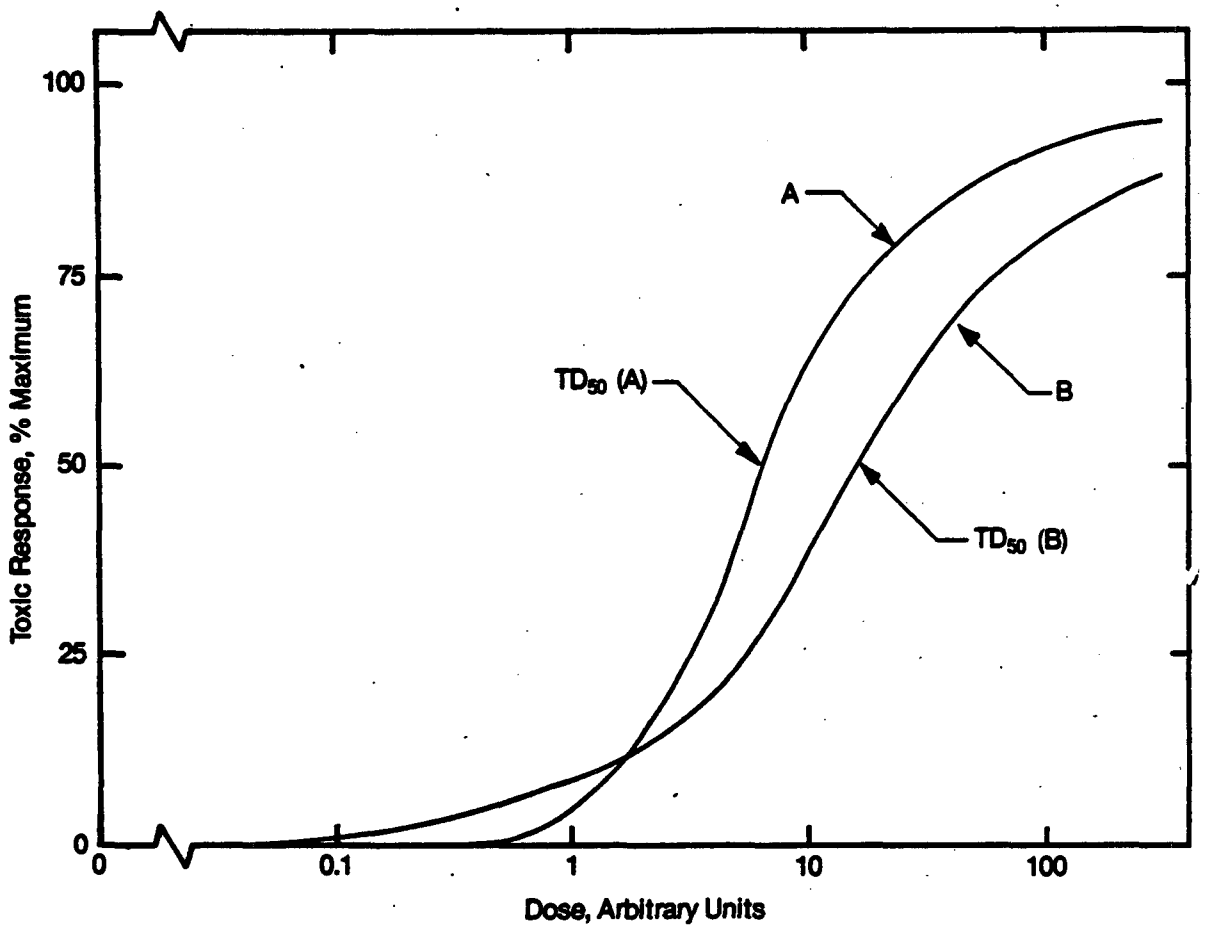


FIGURE 3-6 HYPOTHETICAL DOSE-RESPONSE CURVE OF TWO CHEMICALS, A AND B

This figure illustrates that the shape of the dose-response curve, as well as particular points along it, must be considered in making comparisons and evaluating toxicity. In this example, chemical B is more toxic at lower doses than chemical A, even though chemical A reaches the TD_{50} dose at a lower level than chemical B.

A special category of chemicals is one with beneficial as well as adverse effects. Fluoride ion is an example. Low levels of fluoride provide protection to teeth from decay, but higher levels cause tooth disfigurement and bone damage. Figure 3-7 shows hypothetical dose-response curves for two chemicals (A and B) with both beneficial and adverse effects. When the curves do not overlap significantly (Figure 3-7, Chemical A), it is relatively easy to designate a dose that yields beneficial effects without adverse effects. When the curves are close together (Figure 3-7, Chemical B), the maximum beneficial level cannot be reached without producing adverse effects. The closeness of the beneficial and the adverse dose response curves is described by the Therapeutic Index (TI), which is the ratio of the mid-points of the two curves:

$$TI = (TD_{50})/(ED_{50})$$

When this index has a value of 10 or higher the two curves are well separated and doses for beneficial purposes are reasonably safe. Lower values of TI indicate that the curves are close to each other and that doses for beneficial purposes may result in adverse effects as well.

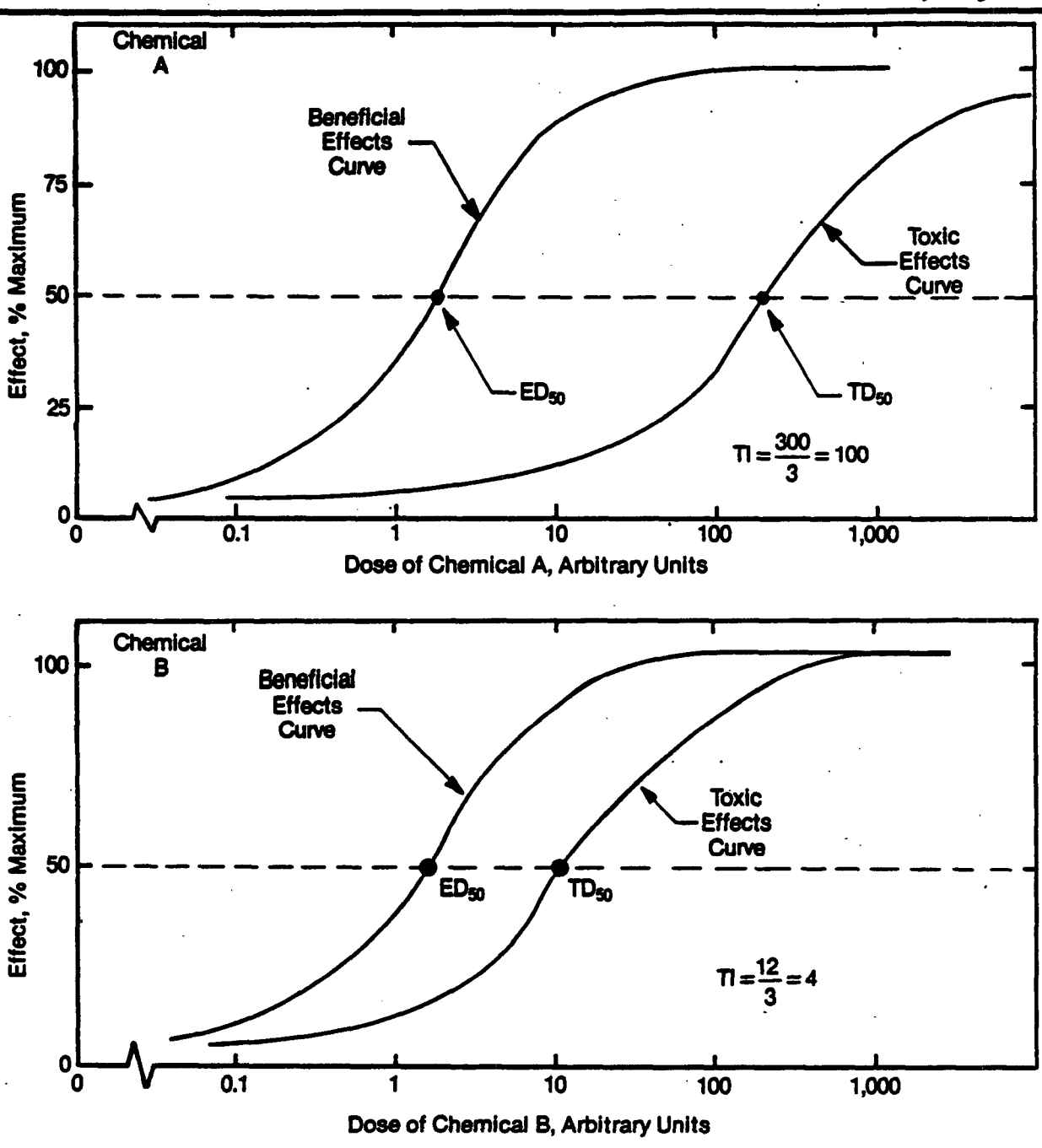


FIGURE 3-7 HYPOTHETICAL DOSE-RESPONSE CURVES FOR CHEMICALS WITH BENEFICIAL AND ADVERSE EFFECTS

When a chemical has both beneficial and adverse effects, the two usually do not follow the same dose-response relationship. When beneficial effects are produced at doses much lower than are required for adverse effects, the chemical is relatively safe and has a high therapeutic index (TI). When the beneficial and toxic effects curves are close to each other, the chemical is less safe and has a lower TI.

4.0 IMPORTANT PARAMETERS IN TOXICITY ASSESSMENTS

As discussed in section 2.2, the essential question to answer in assessing the toxicity of a chemical is: what is the maximum exposure to the chemical that is safe? The answer to this question often depends on a number of factors, including route of exposure, length of exposure, species and individual characteristics of the exposed organism and the nature or endpoint of the toxic effect being measured (Figure 4-1). This chapter describes why the toxicity of a chemical usually depends on these factors and how these factors are investigated and characterized in the toxicity assessment process.

4.1 Route of Exposure

For a chemical to exert a toxic effect on an organism, it must first gain access to the cells and tissues of that organism. In humans, the major routes by which toxic chemicals enter the body are through ingestion, inhalation and dermal absorption. The absorptive surfaces of the tissues involved in these three routes of exposure (gastrointestinal tract, lungs, skin) differ from each other with respect to the rate with which chemicals move across them. A summary of the factors which influence absorption of chemicals through these three routes of exposure is presented below.

4.1.1 Ingestion

Ingestion brings chemicals into contact with the tissues of the gastrointestinal tract. The normal function of the gastrointestinal tract is absorption of foods and fluids that are ingested. The gastrointestinal tract is also effective in absorbing toxic chemicals that are contained in the food or water. The degree of absorption generally depends on whether the chemical is hydrophilic (easily soluble in water) on lipophilic (easily soluble in organic solvents or fats). Lipophilic compounds (e.g., organic solvents) are usually well absorbed, since the chemical can easily diffuse across the membranes of the cells which line the gastrointestinal tract. Hydrophilic compounds (e.g., metal ions) cannot cross the cell lining in this way, and must be "carried" across by transport systems in the cells. The extent of the transport depends on how efficient the transport system is and how closely the chemical resembles the normal compound for which the transport system is intended.

If a chemical is a weak organic acid or base, it will tend to be absorbed by diffusion in the part of the gastrointestinal tract in which it exists in its most lipid-soluble (least ionized or polar) form. Since gastric juice in the stomach is acidic and the intestinal contents are nearly neutral, the polarity of a chemical can differ markedly in these two areas of the gastrointestinal tract. A weak organic acid is in its least polar form while in the stomach and, therefore, tends to be absorbed through the stomach. A weak organic base is in it least polar form while in the intestine and, therefore, tends to be absorbed through the intestine.

Another important determinant of absorption from the gastrointestinal tract is the interaction of the chemical with gastric or intestinal contents. Many chemicals tend to bind to food, and so a chemical ingested in food is often not absorbed as efficiently as when it is ingested in water. Additionally,

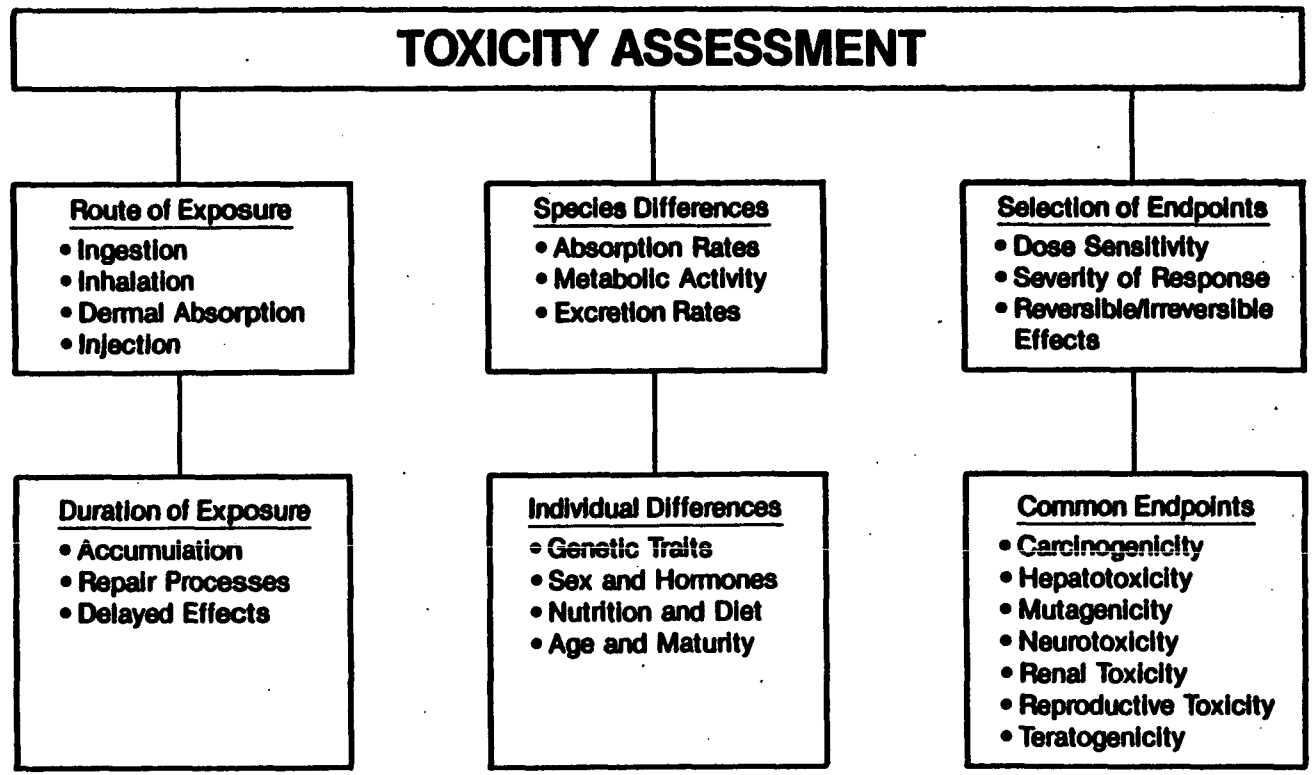


FIGURE 4-1 FACTORS CONSIDERED DURING TOXICITY ASSESSMENTS

some chemicals may not be stable in the strongly acidic environment of the stomach and others may be altered by digestive enzymes or intestinal flora (bacteria which reside in the intestines) to yield different chemicals with altered toxicological properties. For example, intestinal flora can reduce aromatic nitro groups to aromatic amines, which may be carcinogenic.

4.1.2 Inhalation

Inhalation brings chemicals into contact with the lung. Most inhaled chemicals are gases (e.g., carbon monoxide) or vapors of volatile liquids (e.g., trichloroethylene). Absorption in the lung is usually high because the surface area is large and blood vessels are in close proximity to the exposed surface area. Gases cross the lung via simple diffusion, with the rate of absorption depending on the solubility of the toxic agent in blood. If the gas has a low solubility (e.g., ethylene), the rate of absorption is limited by the rate of blood flow through the lung, whereas the absorption of readily soluble gases (e.g., chloroform) is limited only by the rate and depth of respiration.

Chemicals may also be inhaled in solid or liquid form as dusts or aerosols. Liquid aerosols, if lipid-soluble, will readily cross the cell membranes by passive diffusion. The absorption of solid particulate matter is highly dependent upon the size and chemical nature of the particles. The rate of absorption of particulates from the alveoli is determined by the compound's solubility in lung fluids, with poorly soluble compounds being absorbed at a slower rate than readily soluble compounds. Certain small insoluble particles may remain in the alveoli indefinitely. Larger particles (2 to 5 microns) are deposited in the tracheobronchiolar regions of the lungs where they are cleared by coughing and sneezing or they are swallowed and deposited in the gastrointestinal tract. Particles of five microns or larger are usually deposited in the nasopharyngeal region where they are subsequently either expelled or swallowed.

4.1.3 Dermal Absorption

Absorption of toxicants through the epidermal layer of the skin is hindered by the densely packed layer of horny, keratinized epidermal cells. Absorption of chemicals occurs much more readily through scratched or broken skin. There are significant differences in skin structure from one region of the body to another (palms of hands versus facial skin), and these differences further influence dermal absorption.

Absorption of chemicals by the skin is roughly proportional to their lipid solubility, and can be enhanced by application of the chemical in an oily vehicle and rubbing the resulting preparation into the skin. Some lipid-soluble compounds can be absorbed by the skin in quantities sufficient to produce systemic effects. For example, carbon tetrachloride can be absorbed by the skin in amounts large enough to produce liver injury.

4.1.4 Exposure by Injection

In toxicity assessment, the route of exposure employed in experimental animal studies is normally chosen to be the same as the anticipated route of exposure of humans to the specific chemical. However, in studying chemicals toxicologists frequently administer chemicals to laboratory animals by injection (parenterally), the most common routes being subcutaneous (s.c.), intraperitoneal (i.p.), intramuscular (i.m.) and intravenous (i.v.). These routes are employed because they are often more convenient and yield more reproducible results than oral, dermal or inhalation routes of exposure. However, results from studies of this sort must be interpreted with caution, since parenteral administration bypasses the normal absorptive processes, and a parenteral dose may be more toxic than the same dose given by ingestion, inhalation or dermal application.

4.2 Duration/Frequency of Exposure

The toxicity of many chemicals depends on the length of time over which exposure has occurred. There are several reasons for this dependency. First, some chemicals are not readily eliminated from the body, so that continued exposure to low doses (each too small to produce an effect) may lead to accumulation of the chemical in the body at levels which are high enough to produce adverse results. For example, cadmium is strongly retained in the body and tends to accumulate in the kidney. When levels become high enough (usually after many years), kidney dysfunction begins to occur.

A second reason why toxic effects may depend on duration of exposure is related to the ability of cells to repair themselves. When an injury to a cell cannot be quickly reversed by repair processes, there is a tendency for the injury to accumulate in the cell as a function of increasing exposure duration. Thus, a dose of a chemical that causes a small, but irreversible, injury may have no immediately apparent effect, but a clear adverse response may develop with continued exposure.

Finally, some adverse effects simply require an extended period of time to develop, even though they might be the result of exposure months or years earlier. Lead exposure, for example, may impair the development of the nervous system in young children, but this effect requires an extended period of exposure, and does not become apparent for several years. Similarly, the development of tumors following exposure to a carcinogen may take months or years to occur.

4.3 Species of Exposed Organism

It is generally true that if a chemical is found to be toxic in one species of organism (e.g., rat) it will also be toxic in similar organisms (e.g., other mammals, including humans). However, there are often significant differences in the sensitivity of different species to a chemical, and sometimes there are qualitative differences in the types of effects that occur. The reason for these differences among species is usually related to differences in the absorption or metabolism of the chemical or to differences in anatomic function.

4.3.1 Absorption Differences Among Species

The rate of absorption of chemicals across the skin, lungs or gastrointestinal tract is determined primarily by the properties of the cells at the surfaces of these tissues, and there are some significant differences in these cells among species. For example, the skin of the rat and rabbit are more permeable, the skin of the cat is less permeable, and the skin of the pig, guinea pig, and monkey are similar in permeability characteristics to those observed in humans. Additionally, physical and chemical conditions which influence gastrointestinal absorption may also differ among species. For example, gastrointestinal transit time, surface area to volume ratio and pH in various parts of the gastrointestinal tract often differ among species. Finally, the bacterial populations in the gastrointestinal tract vary among species. Some bacteria may convert one chemical into another one that is more or less absorbable and thus alter the apparent toxicity of the chemical, or they may convert a nontoxic chemical into a toxic one.

4.3.2 Differences in Chemical Metabolism Among Species

Metabolism is the name applied to any chemical reaction which a chemical may undergo while in the body. The liver and kidney are especially active in these reactions, but metabolism of a chemical may occur in any tissue. Nearly all chemicals are modified by one or more reactions, but the nature and extent of these reactions may vary widely among different organisms. The rate of metabolism of chemicals is often the limiting step in detoxification and/or excretion of chemicals, so differences in metabolic activity can markedly influence how long a toxic chemical endures in the body. In addition, metabolism of a chemical may sometimes generate a more toxic chemical. For example, pyridine is extensively methylated in cats, gerbils, guinea pigs and hamsters, while it is poorly methylated in mice, rabbits, rats and humans. Since methylation (addition of a methyl group, CH_3) may increase the toxicity of pyridine, the effects produced by equal doses in these two groups of animals may be more adverse in the animals which methylate pyridine efficiently.

4.4 Individual Characteristics of Exposed Organisms

Just as there are significant differences among species with respect to toxicity of some chemicals, so there may also be significant differences among subgroups of a population (as a function of sex, race or age) and among individuals in a population. The principal factors which underlie these variations in sensitivity are outlined below.

4.4.1 Genetic Traits

The genetic makeup of an individual is expressed by the presence or absence of key enzymes in cells, and differences in these enzymes underlie much of the variation in susceptibility of individual members of a particular strain or population. For example, the variation in the susceptibility of some rabbits to the toxic effects of atropine is explained by the presence of an enzyme, atropine esterase, in the blood of the resistant animal.

It should be recognized that laboratory animals used in most experimental studies of chemical toxicity are highly inbred in order to achieve a very uniform genetic composition, so variations in chemical sensitivity between individual animals of the same species are usually small. Conversely, humans are genetically highly heterogeneous and variations between individuals, even of the same age, sex and race, can be significant. For example, two sub-groups have been identified in the human population with the respect to the ability to acetylate certain chemicals; slow and rapid acetylators. Acetylation (addition of the acetyl group, CH_3CO-) is significant because it can lead to detoxification of some chemicals.[3] Slow acetylators have less N-acetyltransferase in their livers than rapid acetylators, and this is the enzyme that catalyzes the acetylation process. Therefore, slow acetylators are more likely to develop toxic effects to certain chemicals.

4.4.2 Sex and Hormonal Status

Differences in toxicity between sexes have been demonstrated in studies on the effects of chloroform, benzene and some organophosphate insecticides. For example, female mice show little response to chloroform exposures that are lethal for males. The difference has been shown to be under direct endocrine (hormonal) control. As another example, female rats and rabbits are more susceptible to the toxic effects of parathion and benzene, respectively, than are male rats and rabbits. These sex-related effects become reversed after castration and administration of hormones. Pregnancy, with its increased hormonal activity, has been shown to markedly increase the susceptibility of mice to some types of pesticides, and similar effects have been reported for a lactating animal exposed to heavy metals. Hyperthyroidism (excessive secretion of thyroid hormone) and hyperinsulinism (excessive secretion of insulin) may also alter the susceptibility of animals, including humans, to toxic chemicals.

4.4.3 Nutritional Status and Dietary Factors

Humans are able to achieve large adjustments in the absorption and metabolism of foods and minerals to compensate for fluctuations in dietary intake levels. These metabolic adaptations frequently influence the absorption and/or metabolism of toxic chemicals as well. For example, long-term ingestion of a diet low in essential minerals (iron, calcium, zinc) leads the body to increase absorption and retention of these minerals. However, along with this adaptation to retain essential minerals, the absorption of toxic metals (cadmium, barium) also increases. Generally, low calorie or protein-deficient diets result in increased sensitivity to a number of toxic chemicals.

4.4.4 Age and Maturity

Some chemicals are more toxic to one age group than another (usually being more toxic to infants and children than adults). In some cases this is only because infants and children drink and eat proportionately larger amounts than do adults, and thereby ingest proportionately larger doses. However, infants and children may be inherently more sensitive to chemicals for reasons related to the development process. For example, lead ingestion has much more severe effects on the nervous system of infants and children then it does on adults.

Additionally, the ability of the young to metabolize and detoxify chemicals is usually less than for adults. Elderly humans may be more sensitive to some chemicals, since the detoxifying capacity of liver and the excretory capacity of kidney tend to decrease in old age.

4.5 Toxicological Endpoints

Exposure of an organism to a chemical often results in multiple effects. For example, long-term exposures to dioxin results in hepatotoxicity (liver toxicity), genotoxicity (chromosomal damage), teratogenicity (structural/functional abnormality), fetotoxicity (injury to developing fetuses) and carcinogenicity (growth of malignant tumors). Effects which are measured by the toxicologist as an index of a chemical's toxicity are called "endpoints". The criteria for identifying the endpoint most appropriate for use in toxicity assessment include dose sensitivity, the severity of the effect, and whether the effect is reversible or irreversible.

4.5.1 Criteria for Selection of Endpoints

4.5.1.1 Dose Sensitivity

The most appropriate endpoint for use in the toxicity assessment process is usually the one in which a measurable change can first be detected in response to increasing doses. For example, pyridine is toxic to the central nervous system (CNS), the liver and the kidney. However, CNS toxicity can be demonstrated at much lower doses than adverse kidney and liver effects. In studying pyridine, then, CNS effects are appropriate as the most sensitive endpoint.

4.5.1.2 Severity of Response

The selection of a toxicological endpoint is sometimes based on the extent of damage to a particular organ following exposure. A toxic chemical may produce harmful effects in a number of organs, but the severity of the response may be quite different. For example, carbon tetrachloride exposure may result in mild damage to the kidney, but severe damage and loss of function in the liver. In studying carbon tetrachloride, then, effects on the liver are the most appropriate endpoint.

Sometimes a low dose of a chemical may produce an effect that is not in itself clearly adverse. For example, a low dose of acrylamide may cause slowed axonal transport in nerve cells without measurably affecting the ability of the cells to carry nerve impulses.

To distinguish between detectable effects which are adverse and those which are not, the term LOEL (Lowest-Observed-Effect Level) is used, as distinct from LOAEL (Lowest-Observed-Adverse-Effect Level). Similarly, NOEL (No-Observed-Effect Level) implies no detectable effect of any sort, while NOAEL (No-Observed-Adverse-Effect Level) may include some effect which is judged not to be adverse.

The decision as to whether an effect is adverse or not must be judged on the basis of whether the change is an early indication of a more serious consequence or whether the change is not of significant concern.

4.5.1.3 Reversible Versus Irreversible Effects

Some toxic effects are reversible and others are not. In a tissue that has a strong ability to regenerate (e.g., the liver), most injuries are reversible, whereas injury to the CNS is largely irreversible, since specialized cells of the CNS cannot divide or be replaced. Carcinogenic effects of chemicals are also irreversible. Irreversible effects are often chosen as toxicological endpoints since these effects are likely to produce serious consequences following chronic (long-term, low level) exposure to a chemical.

4.5.2 Common Toxicological Endpoints and Measuring Techniques

Table 4-1 lists endpoints that are commonly used to assess the toxic effects of a chemical, along with the experimental means of measuring such effects. More detailed descriptions of these endpoints are given below.

4.5.2.1 Carcinogenicity

Cancer is a complex group of diseases whose causes are not yet fully understood, but there is ample evidence that some chemicals can cause or promote certain types of tumors in animals or humans. The carcinogenic potential of a chemical can be measured with lifetime animal bioassays, short-term carcinogenicity tests (with bacterial or cultured mammalian cells), or limited *in vivo* bioassays. Each of these methods is associated with certain advantages and disadvantages, as discussed below.

Standard lifetime animal bioassays are long-term experiments conducted to measure the effect of a chemical on frequency of tumor occurrence.

Typically, large groups of animals (at least 50 per sex per dose) are exposed to the chemical for their lifetime, and the number and types of tumors occurring in exposed animals are compared to control animals.

These studies are considered to be the most predictive of carcinogenicity screening tests. However, substantial controversy exists over certain standard practices used in the bioassays. For example, to compensate for the relative insensitivity of these studies, the maximum tolerated dose (MTD) is frequently used to maximize the likelihood of detecting carcinogenicity. The use of MTD is controversial because high doses of a chemical may produce physiological conditions that affect the induction and development of tumors. Normal detoxification and repair mechanisms may be overwhelmed by the use of the MTD, or different absorption, distribution, metabolism or excretion may result from the use of the MTD. These events might result in a response at the MTD that may not be indicative of effects at lower exposure levels.

A similar controversy exists over the use of strains of test animals that are very susceptible to carcinogens. The purpose of using these animals is to

TABLE 4-1 MEASUREMENT OF COMMON TOXICOLOGICAL ENDPOINTS

Toxicological Endpoint	Parameters Measured
Behavioral Toxicity	Motor function (motor activity, coordination strength), sensory function (vision, audition), integrative systems (learning and memory).
Carcinogenicity	Tumor frequency in tissues, detected by gross observation or histological examination.
Hematologic Toxicity	Hematocrit, hemoglobin levels, changes in cellular components (erythrocytes, leucocytes, platelets), plasma components, and foreign substances.
Hepatoxicity	Gross and microscopic examination, organ weight, liver function (bile formation, lipid metabolism, protein metabolism, carbohydrate metabolism, metabolism of foreign compounds, serum enzyme activities).
Inhalation Toxicity	Gross anatomy, microscopic and ultrastructural anatomy, changes in function.
Mutagenicity	Chromosome alterations, bacterial mutations, DNA damage.
Neurotoxicity	Gross observation, clinical evaluation, neurological exams, behavioral tests, neurohisto-pathological tests, neurochemical tests.
Renal Toxicity	Urinalysis, function tests (clearance, glomerular filtration rate), gross and microscopic examinations, organ weight.
Reproductive Toxicity	Fertility, litter size and survival, gestation survival, postnatal body weight.
Teratogenicity	Gross abnormalities, skeletal and visceral malformations, microscopic abnormalities, functional/behavioral deviations.

increase the ability to detect carcinogenic potential in chemicals. However, these sensitive strains may have very high spontaneous tumor frequencies, and the meaning and validity of a positive test result in such a sensitive strain is not entirely clear. Because of these uncertainties, it is very desirable to perform lifetime carcinogenicity bioassays using two or more different animal species.

There are two major types of short-term carcinogenicity tests used to indicate carcinogenic potential: mutagenicity tests and transformation tests using cultured mammalian cells. Mutagenicity experiments are often used to evaluate the potential for inducing tumors because of basic similarities in the postulated molecular mechanisms of chemical carcinogenesis and mutagenesis. Some mutagenicity tests, especially the Ames test, have been extensively validated and shown to correlate very well with known carcinogens, but there is still a significant frequency of false-positive or false-negative results (approximately 10% each for the Ames test). Positive results in mutagenicity tests support other experimental findings of carcinogenic potential and are generally considered to provide suggestive evidence of carcinogenic hazard. They do not constitute definite proof of a chemical's carcinogenicity in humans, nor do negative results rule out the possibility of carcinogenic potential.

A major disadvantage of mutagenicity tests using bacterial test systems is the basic biological differences between bacterial cells and human cells, making extrapolations to human health effects somewhat tenuous. Testing in mammalian cells provides a stronger basis for extrapolating to human health effects, but the test methods are not as well developed or validated as those using bacteria. The primary short-term test of this sort is based on mammalian cell transformation. Transformation occurs when cultured cells develop uncontrolled growth, an event analogous to the formation of a tumor in an organism. A number of transformation tests using mammalian cells have been developed in recent years and are in widespread use. The cells are treated with the chemical in question and the transformation frequency is measured. Cell transformation is usually detected by observing changes in the cultured cells, and is confirmed by injecting the transformed cells into animals where they become malignant tumors. A major disadvantage is that the carcinogenic potential of a chemical may depend on its metabolism in the living organism, with one or more metabolic products being more carcinogenic than the original chemical. In a case such as this, a transformation test might yield negative results, while positive results would be obtained in a lifetime animal bioassay.

Limited in vivo bioassays can provide evidence of the tumorogenic potential of a chemical without the great time investment and expense required for a lifetime bioassay. These tests generally yield results in 30 weeks or less and use mice or rats as test animals. Examples of limited in vivo bioassays include skin tumor formation in mice, breast cancer induction in rats, or altered liver foci (an early step in liver tumor formation) in mice or rats. While limited in vivo bioassays are not an adequate substitute for lifetime bioassays, they are more useful as predictors of carcinogenicity than short-term tests, and positive results in well-designed and executed limited in vivo bioassays are additional supportive evidence of carcinogenic hazard.

It is not uncommon that a chemical will yield different results in different tests of carcinogenic potential (positive in some tests, negative in others). In such cases, the probability that the chemical is a human carcinogen is determined by a "weight-of-evidence" approach (USEPA 1984). In general, positive results in animal systems or positive finding in epidemiological studies are required before a chemical is classified as a probable human carcinogen. This may be supported by positive results in bacterial or cell system tests, but positive results in these systems are insufficient alone.

4.5.2.2 Hepatotoxicity

Liver damage is a frequent response to exposures to toxic chemicals. Since the liver is such a vital organ, a variety of procedures have been developed over the years to assess extent of liver damage. Because the liver has considerable reserve capacity, tests that measure its ability to perform its functions may not reveal an effect until the liver is already extensively damaged. A more sensitive test involves measurement of liver enzymes in blood serum. This test is based on the observation that when liver cells are damaged, some of the active enzymes within the cells escape into the blood. This increase in liver enzymes can be measured simply by collecting a sample of blood and measuring enzyme activity. A disadvantage of this test is that liver enzymes do not endure very long in the blood, and so only an on-going injury can be detected. Finally, evidence of liver damage may be detected both during and well after a chemical-induced injury by microscopic examination of the liver for signs of abnormality. Therefore, microscopic examination for histological changes is another excellent endpoint.

4.5.2.3 Mutagenicity

Mutagenesis is the induction of changes in genetic material that are transmitted during cell division. If mutations are present in the genetic material of eggs or sperm, the fertilized ovum may not be viable. A mutation may also result in congenital abnormalities or death of a fetus at a later developmental period. There are a number of powerful tests for mutagenic potential of chemicals, most involving bacteria or other cells in culture. For example, the Ames test measures the frequency of a certain type of mutational event in the bacterial species Salmonella typhimurium. Other valuable tests examine Chinese hamster ovary (CHO) cells for alterations in chromosome structure, or determine whether unscheduled DNA synthesis (a strong indicator of damage to the DNA) is occurring in other cultured mammal cells.

4.5.2.4 Neurotoxicity

The nervous system is of special toxicological concern, since chemical-induced injury to nerve cells is often irreversible and may lead to adverse health effects. There are many means of measuring nervous system functions, including tests of reflexes, coordination, conditioned responses in animals and intelligence (IQ) tests in humans. In addition, there are sophisticated means of analyzing the status of individual nerves by measuring the rate at which they transmit nerve impulses or the rate at which they synthesize and transport cellular materials.

The EPA has published guidelines that focus on delayed neurotoxicity as an endpoint. Delayed neurotoxicity is a syndrome in which damage to the peripheral nervous system and some portions of the CNS may result in paralysis. The domestic hen is typically the species chosen for evaluation of delayed neurotoxicity. In the acute delayed neurotoxicity test, a single dose of the test material is administered orally to groups of adult domestic hens. The hens are observed daily for at least 21 days for behavioral abnormalities, ataxia (inability to coordinate muscles) and paralysis. Selected sections of nervous tissues are also examined histopathologically. In tests for subchronic delayed neurotoxicity, groups of hens are administered the test substance orally for 90 days, followed by an observation period of seven days. As in the acute studies, the hens are observed daily for behavioral abnormalities, ataxia and paralysis, and selected sections of nerve tissue are examined histopathologically.

4.5.2.5 Renal Toxicity

Damage to the kidneys is another common and serious consequence of exposure to a toxic chemical. As with the liver, techniques to assess kidney injury may include functional tests in the intact animal, along with direct histological examination of kidney tissue. Urinalysis offers another convenient and sensitive means of detecting kidney damage. For example, detection of substances not normally present in urine (proteins, cells or cell fragments, glucose) is strong evidence that the kidney has been injured.

4.5.2.6 Reproductive Toxicity

Fertility and reproductive toxicity studies are usually performed in rats or mice at dose levels that produce no overt toxicity in the exposed adults. In a typical study, the male parent is exposed to a chemical for 60 to 80 days and the female for 14 days prior to mating. The percentage of females that become pregnant is determined. The number of stillborn and live offspring, and their weight, growth, survival and general condition during the first three weeks of life are also recorded.

The perinatal (during late pregnancy) and lactational (during nursing) toxicities of chemicals may be measured in a similar fashion. Pregnant female rats are exposed to the chemical from the fifteenth day of gestation to the time of weaning. Parameters measured may include all of those above, as well as analysis of milk for presence of the chemical.

4.5.2.7 Teratogenicity

Teratology is defined as the study of functional or physical defects induced during development of an animal from the time of conception to birth. Teratogenic studies are usually performed in rats and/or rabbits with doses of the test chemical that produce no maternal toxicity. Teratogens are most effective when administered during the period of organogenesis, so pregnant females are usually exposed on days 6 to 15 of gestation. Prior to delivery, some females are sacrificed and examined for the number of fetal implantations in the uterus. Dead and living fetuses are counted, weighed and examined for gross malformations. These fetuses are examined microscopically for more

subtle effects, and some are cleared of soft tissue and examined for skeletal abnormalities. Since teratogens can produce functional as well as morphologic changes, offspring of other females are sometimes monitored after delivery for changes in behavior or development.

4.6 Key Guidance and Implementation Documents

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5.0 TYPICAL PROTOCOLS USED IN TOXICOLOGICAL STUDIES

As discussed in chapters 3.0 and 4.0, assessment of the toxicity of a chemical involves identification of the adverse effects which the chemical causes and systematic study of how these effects depend upon dose, route and duration of exposure and test organism. This information is derived from studies which may be divided into four general categories:

- Studies in laboratory animals evaluate the toxicity of a chemical with special reference to predicting the toxicity in humans.
- Clinical studies are case-by-case investigations of the symptoms and diseases in humans who are exposed to a toxic chemical at doses high enough to require medical attention.
- Epidemiological studies seek to determine whether a correlation exists between chemical exposure and frequency of disease or health problems in large groups of human populations.
- Ecotoxicological studies assess the toxic effects of chemicals on indigenous aquatic and terrestrial plants and animals.

This chapter describes the usual experimental designs (protocols) used in these studies.

5.1 Studies in Laboratory Animals

Table 5-1 summarizes protocols that might be used in testing the toxicity of a chemical in laboratory animals. In order to determine how the effects of a chemical depend on exposure levels, all studies involve administration of a series of doses. To investigate how the effects of a chemical depend on duration of exposure, chemicals are administered for one day or in one dose (acute), for 5 to 90 days (subchronic) and for long periods (2 years to lifetime). To determine how effects may depend on the characteristics of the test organism, the chemical is administered to both sexes of two or more species. To identify the cells and tissues that are affected by the chemical, a broad range of endpoints are evaluated for chemical induced changes.

5.1.1 Acute Studies

In an acute study, animals are given a brief exposure to the chemical (a single oral dose, a 4-hour inhalation exposure or a 24-hour dermal exposure) and are observed for subsequent effects. The chemical is usually tested in two animal species, most often rats, mice, dogs or guinea pigs.

Often the first endpoint to be measured is lethality. Determination of oral LD_{50} values requires the testing of four to six dose levels with five to ten animals/sex/dose level. The test chemical is administered once to each test animal and the number of animals that die, the time of death, and toxic signs observable directly and at necropsy (animal autopsy) are recorded. Acute dermal and inhalation studies are similar, except that emphasis is placed on signs of injury to the skin or lungs.

TABLE 5-1 TYPICAL TESTING PROTOCOLS IN ANIMALS

Type of Study	Test Species	Number of Dose Levels[a]	Dosing Regimen	Approximate No. of Animals of Each Sex Per Dose Level	Observation Period	Typical Observations
ACUTE						
Oral	Rats, mice, guinea pigs	4 - 6	Single dose	5-10	14 days	Survivors, body weight changes (day 14), gross histopathology and toxicities, clinical chemistry (dog only).
	Dogs		Single dose	2-3	14 days	
Dermal	Rabbits	3 - 4	Single application for 24 hours	5	14 days (evaluated 24 hours, 7 days and 14 days)	Survivors, body weight changes (day 14), gross toxicity and histopathology, especially of skin.
Inhalation	Rats	4 - 5	4-Hour exposure	5	14 days	Survivors, body weight changes (day 14), gross toxicity and histopathology, especially of lungs.

-continued-

(a) Includes a dose level of zero (control).

Table 5-1 - continued

Type of Study	Test Species	Number of Dose Levels[a]	Dosing Regimen	Approximate No. of Animals of Each Sex Per Dose Level	Observation Period	Typical Observations
SUBCHRONIC						
Oral	Rats	3 - 4	Daily doses	20	90 days	Survivors, body weight changes, diet consumption, urinalysis, hematology, clinical chemistry, gross and microscopic examination of major tissues and organs.
	Dogs	3 - 4	Daily doses	6	90 days	
Dermal	Rabbits	3 - 4	Daily application	10	90 days	
Inhalation	Rats	3 - 4	Daily doses[b]	10	90 days	
CHRONIC						
Oral	Rats	3 - 4	Daily doses	50	2 years	Survivors, body weight changes, diet consumption, urinalysis, hematology, clinical chemistry, gross and microscopic examination of major tissues and organs.
	Dogs	3 - 4	Daily doses	6	2 years	
Inhalation	Rats	3 - 4	Daily doses[b]	50	2 years	

(b) Minimum of 5 days per week.

Acute toxicity studies are useful in (1) providing a quantitative measure of acute toxicity (LD_{50}) for comparison with other chemicals, (2) identifying the functions or organs most severely affected and (3) defining the appropriate doses to be used in longer-term studies (subchronic, chronic).

5.1.2 Subchronic Studies

Following acute toxicity testing, the chemical is next tested for toxicity following subchronic exposure, usually between five and 90 days. Doses are administered daily to both sexes of at least two species (e.g., rats and dogs) by the expected route(s) of human exposure. At least three dose levels of the test chemical are used. These doses are selected to span the full dose-response range and to define the NOAEL or LOAEL if possible. A variety of parameters are monitored as described in Table 5-1. If one or more unique endpoints are recognized as being especially characteristic of the chemical's effects, more detailed attention is focused on them.

5.1.3 Chronic Studies

Chronic studies are performed similarly to subchronic studies, except that emphasis is placed on searching for evidence of slowly emerging adverse effects (e.g., cancer). Doses are generally selected to be low enough that most animals survive the full exposure period. Larger numbers of animals are employed to obtain statistically significant results in endpoints that naturally vary among individuals (e.g., longevity, tumor frequency).

In summary, testing protocols in animals are designed to identify the principal adverse effects of a chemical as a function of dose, route of exposure, species and sex of test animals and duration of exposure. When carefully performed, these studies will yield NOAEL or LOAEL values for the most sensitive noncarcinogenic endpoint for each route and length of exposure, and, if carcinogenic, a series of exposure levels corresponding to excess cancer risks of 10^{-5}, 10^{-6} and 10^{-7}.

5.2 Clinical Studies in Humans

The medical community often reports detailed descriptions of human diseases and other health problems resulting from exposure to toxic chemicals. Exposures may be accidental (e.g., a farmer applying pesticide without proper protection) or intentional (e.g., suicide or homicide). In view of the difficulties and uncertainties in extrapolating toxicological information from animals to humans, this sort of direct toxicological observation is especially valuable in characterizing toxic responses of clinical significance in humans. Unfortunately, clinical studies are rarely sufficient to provide a complete description of a chemical's toxicity. This is because clinical observations are usually available on only a small number of individuals, and quantitative information on the exposure levels causing the effect are rarely known. This absence of quantitative dose information diminishes the usefulness of clinical studies in estimating a NOAEL or LOAEL in humans. Additionally, even when exposure levels are known, these levels are usually high on the dose-response curve and so are not of direct use in defining the NOAEL in humans. Finally, clinical studies do not account for factors such as age, smoking or previous exposure to other chemicals.

5.3 Epidemiological Studies

Epidemiological studies seek to determine whether or not correlations exist between the frequency or prevalence of a disease or health condition in human populations and some specific factor such as concentration of a toxic chemical in the environment. The major advantages of epidemiological studies are that they are based on large numbers of humans and exposure levels are usually sub-clinical. Thus the data are directly relevant, with no need to extrapolate from data in animals or to make projections from a small number of humans exposed to a high dose of the chemical.

5.3.1 Types of Epidemiological Studies

This section provides a discussion of the three basic types of epidemiological studies and the considerations associated with data derived from such studies.

5.3.1.1 Retrospective Studies

In most instances the most feasible approach in terms of cost, time and statistical power is through a retrospective (case-control) study, which compares diseased persons (cases) with non-diseased persons (controls) and works back in time to determine exposure. The validity of a retrospective study depends upon careful selection of the control group. The control group should be similar to the case group in all respects except exposure for the risk factor under investigation. The distribution for age, sex, race, socioeconomic status, education, emotional status and other potentially confounding factors should be the same for each group.

The design of a typical retrospective study is shown in Figure 5-1.

Weaknesses in retrospective studies include confounding factors and biases. Errors in detecting a cause and effect relationship can stem from failure to account and adjust for all confounding factors related to the disease and risk factor under consideration. This task is complicated in retrospective studies by the lack of accurate historical data. Due to the dependence on recall data, retrospective studies are especially subject to biases. For example, diseased patients may be more likely to recall exposure than non-diseased patients.

5.3.1.2 Prospective Studies

Prospective (cohort) studies examine the development of a disease or condition in a group (cohort) of persons who have been determined to be presently free of the disease or condition. The cohort consists of subgroups who have and have not been exposed to a toxic chemical. The subgroups of the cohort are then followed for several years to observe differences in the rate at which disease develops in relation to exposure to the toxic chemical. The design of a typical prospective study is shown in Figure 5-2.

Compared to retrospective studies, prospective studies are advantageous in that exposure amounts are observable and likely to be more reliable than recalled exposure which may have occurred years before. Often elaborate

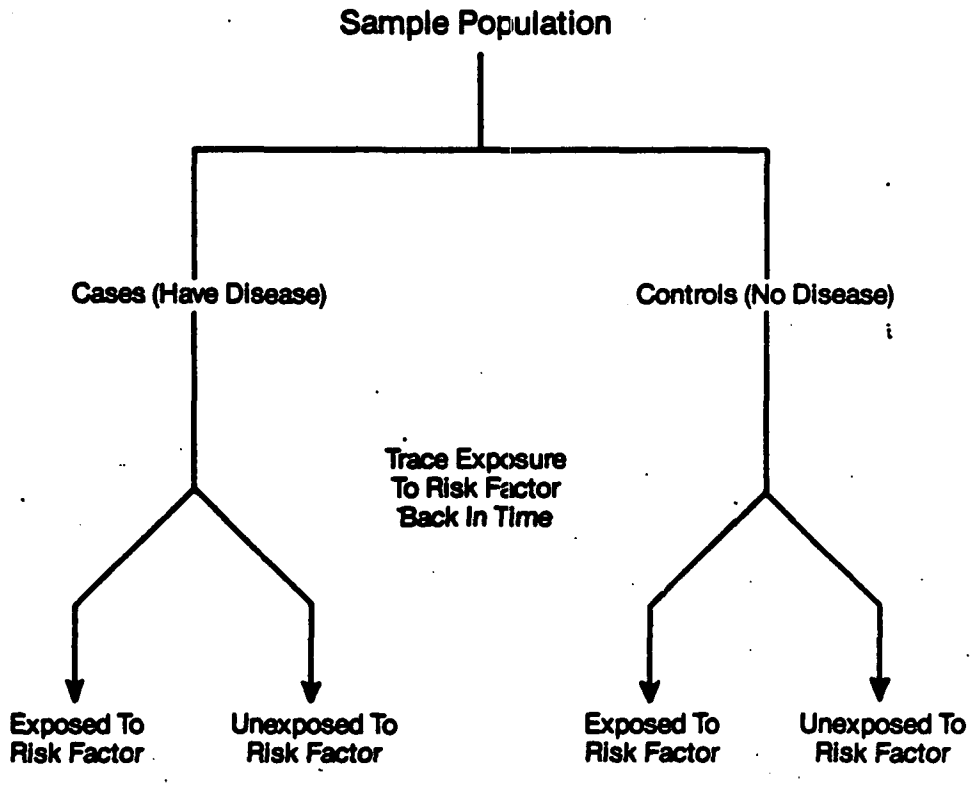

FIGURE 5-1 DESIGN OF TYPICAL RETROSPECTIVE (CASE-CONTROL) STUDY

A group of persons (cases) having an injury, condition or disease (e.g., cancer) is selected and their past history with respect to a risk factor (e.g., exposure to a carcinogen) is compared to a group of persons (controls) who do not have the injury condition or disease. Careful statistical analysis of the data is performed to determine whether there is an association between the specific risk factor and the condition.

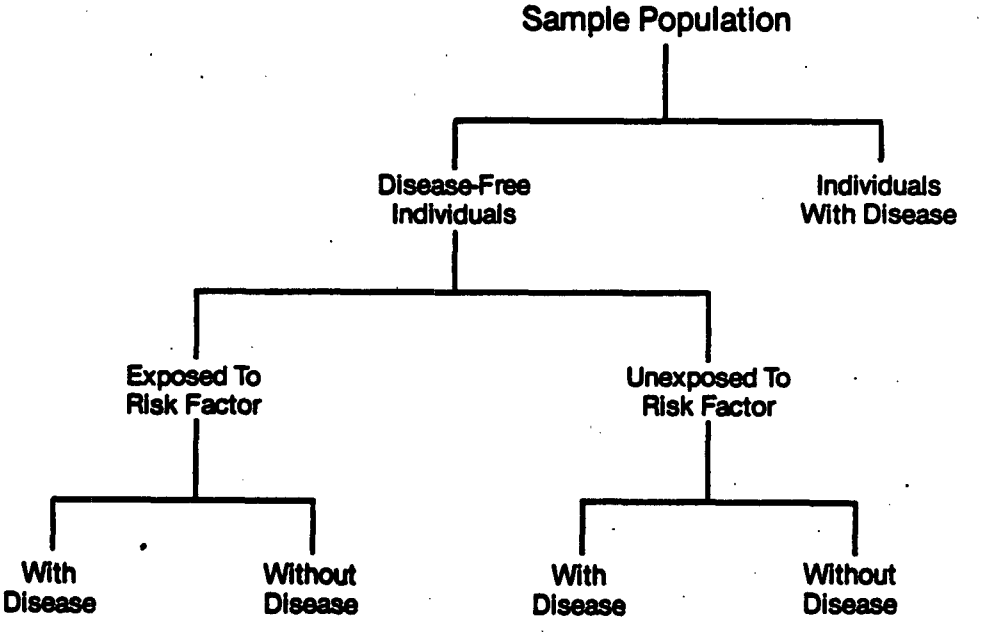

FIGURE 5-2 DESIGN OF TYPICAL PROSPECTIVE (COHORT) STUDY

First, a disease-free group is selected from the general population. This group is then divided into subgroups according to the presence or absence of a risk factor (e.g., exposure to a carcinogen). A toxicological endpoint (e.g., cancer incidence) is measured in the two groups to determine whether a relationship exists between presence of the risk factor and development of a disease (e.g., cancer).

physical examinations and environmental monitoring are part of the prospective study protocol and subjects are followed for many years. This permits observation of and adjustment for confounding factors. Also, moving forward in time allows latency (the time from initial exposure to disease diagnosis) to be more accurately measured. Unfortunately, failure to observe some study variable that later is found to be important can negate the value of a study; thus prospective study protocols specify that many characteristics be observed. Not surprisingly, prospective studies are more expensive and time consuming than retrospective studies.

The most difficult challenge in a prospective study is selecting the two subgroups. For example, it would be difficult to select a comparison subgroup (i.e., not exposed to risk factor) for a subgroup of industrial workers exposed to toxic chemical. Other individuals exposed to similar stresses and living similar lifestyles, but having no exposure to the chemical, may be impossible to identify.

5.3.1.3 Prevalence Studies

Prevalence (cross-sectional) studies examine the relationships between diseases and exposure as they exist in a defined population at one particular time. Analysis of data collected in a prevalence study focuses on the correlation between the incidence of a disease and selected risk factors (e.g., exposure to a carcinogen). Sometimes it is possible to obtain dose-response curves that relate the frequency and/or severity of some biological effect to the intensity of the exposure. The design of a typical prevalence study is summarized in Figure 5-3.

The key limitation to prevalence studies stems from the fact that they represent a "snapshot" in time. They may point out a relationship, but do not describe how such a relationship may have developed. More importantly, since prevalence studies eliminate the time relationship between exposure to an environmental hazard and development of a biological effect, some cause-and-effect relationships may not be detected. One important value of a prevalence study is that it can identify the best source of controls for a retrospective study and is essentially the first step in conducting a prospective study.

5.3.2 Uncertainties and Limitations Associated with Epidemiological Data

The conclusions derived from epidemiological studies can be strengthened when the investigators are aware of and deal with the most common limitations associated with such studies. These include the following:

- Confounding Factors. Confounding factors are variables which the epidemiologist cannot control, but which may influence the parameter being measured. For example, smoking is a confounding variable in measurements of cancer frequency, and age and weight are confounding factors in measuring blood pressure. When confounding factors are recognized, it is sometimes possible to correct for them. However, this is often not possible, and in some cases the nature of all confounding variables are not even known.

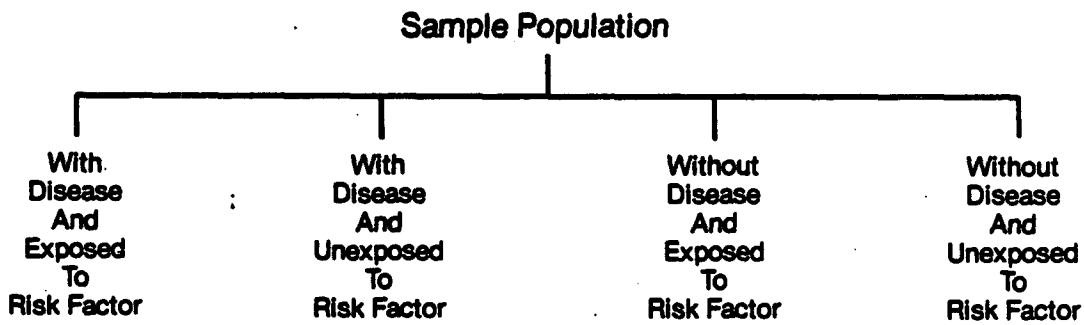

FIGURE 5-3 DESIGN OF TYPICAL PREVALENCE (CROSS-SECTIONAL) STUDY

A group of in individuals is selected and each is carefully investigated to determine (1) the present degree of exposure to a toxic chemical (i.e., at the time of the study) and (2) the present health status of the individual (presence or absence of specific diseases or adverse effects).

- Bias. Bias is the collection of data that is not truly representative of the whole population, but is characteristic of some subgroup. Bias may be introduced, for example, by failing to adjust for age, socio-economic status, smoking, weight or other variables, or by failing to follow all members of the exposed and control groups during prospective studies. This is especially true when effects being measured have a long latency, as is true for cancer.

- Data Analysis. The primary question in any epidemiological study is whether an association or correlation between some risk factor and a biological effect is indicative of a causal relationship or is simply random. A number of statistical techniques, such as multivariate analysis, determine whether a significant association should be accepted as causal, and proper use and interpretation of these statistical methods is essential. In addition, it is important that the data make sense biologically. For example, given the long latency period for cancer, a large increase in cancer incidence in one month does not make sense biologically.

5.4 Ecotoxicological Studies

The presence of toxic chemicals in the environment may adversely affect the abundance, species composition and diversity, stability, productivity and physiological condition of indigenous fish and wildlife populations. Methods used for assessing the effects of toxic chemicals on these populations include the collection, identification and counting of organisms, biomass measurements, measurements of metabolic rates, and measurements of the toxicity, bioaccumulation and biomagnification of toxic chemicals. Testing for these effects may be conducted with representative species of plankton, periphyton, macrophytes, macroinvertebrates, fish, amphibians, reptiles, birds on mammals. These single-species studies are usually very similar in design and objectives to studies using inbred laboratory animals, and many of the key considerations are the same (dose-response relationships, effect of duration, differences in sensitivity between species, etc.). The main difference is that species employed in ecotoxicological tests are selected to be representative of indigenous fish and wildlife, while laboratory animals are intended to serve as models for humans.

This section discusses the types of laboratory experiments (short-term, intermediate and long-term bioassays) which are performed in order to assess the toxicity of chemicals on representative test species, provides an example of a typical single-species experiment and discusses some of the limitations of ecotoxicological data relative to long-term exposure potential.

5.4.1 Bioassays

Bioassays measure the responses of test organisms to a particular chemical. Bioassays are useful in determining the suitability of certain environmental conditions for life, the effects of environmental factors (e.g., pH, temperature) on the toxicity of a chemical and the comparative sensitivity of organisms to a chemical. Bioassays are typically classified according to duration of exposure (short-term, intermediate or long-term).

5.4.1.1 Short-Term Bioassays

Short-term (acute) bioassays are generally used to determine the level of a toxic chemical that produces lethality in a specified percentage of test organisms over a specified period of time. Acute toxicity tests may be categorized as range-finding or definitive tests. Range-finding tests are usually 24-hour tests conducted to determine the concentration to be used in the definitive tests. The test organisms are exposed to a wide range of concentrations to determine the highest concentration that killed no (or few) organisms and the lowest concentration that killed all (or most) organisms. Definitive tests then employ a series of concentrations between those highest and lowest lethal concentrations.

Experimentally, a 50% effect is the most reproducible adverse effect. The most frequently used measure of acute toxicity is the median lethal concentration or dose (LC_{50} or LD_{50}). Acute definitive tests provide an indication of concentrations that should be used in conducting partial or complete life-cycle tests.

5.4.1.2 Intermediate-Term Bioassays

No sharp division exists between short- and intermediate-term bioassays or between intermediate- and long-term bioassays. Generally, tests lasting eight days or less are considered to be short-term; tests lasting 8 to 90 days are considered intermediate-term. Duration of exposure is the only difference in these tests.

5.4.1.3 Long-Term Bioassays

Long-term bioassays measure sublethal effects that occur through chronic exposure to concentrations lower than those causing acute effects. When long-term bioassays are conducted, exposure continues over as much of the life cycle as possible. In life-cycle and partial life-cycle bioassays, the objective is to determine the maximum allowable toxicant concentration (MATC), (the concentration of a toxic chemical that may be present without causing significant harm). Parameters measured by this type of bioassay include growth, reproduction, maturation, spawning, hatching, survival, behavior and bioaccumulation.

The EPA has developed guidelines for conducting a variety of ecotoxicological tests. A list of these guidelines appears in Table 5-2. These guidelines present methodologies for each test. The EPA has also developed support documents that provide the scientific rationale used in the development of the test guidelines.

5.4.2 An Example of an Acute Toxicity Study on Fish

An example of a representative single-species study is the acute toxicity test for fish. A range-finding test is initially conducted to determine the range of concentrations to be used in the definitive test. For the definitive test, a minimum of 20 fish are exposed to each of five or more concentrations in two or more replicate test chambers. Controls are exposed to the same experimental conditions as the test fish, but are not exposed to the test chemical.

TABLE 5-2 ECOTOXICOLOGICAL TESTS FOR WHICH EPA HAS DEVELOPED GUIDELINES

Daphnid Acute Toxicity Test

Daphnid Chronic Toxicity Test

Mysid Shrimp Acute Toxicity Test

Mysid Shrimp Chronic Toxicity Test

Oyster Acute Toxicity TEst

Oyster Bioconcentration Test

Penaeid Shrimp Acute Toxicity Test

Algal Acute Toxicity Test

Fish Acute Toxicity Test

Fish Bioconcentration Test

Fish Early Life Stage Toxicity Test

Seed Germination/Root Elongation Toxicity Test

Early Seedling Growth Toxicity Test

Plant Uptake and Translocation Test

Avian Dietary Test

Bobwhite Reproduction Test

Mallard Reproduction Test

Lemna Acute Toxicity Test

Recommended species for this test include the rainbow trout (Salmo gairdneri), bluegill sunfish (Lepomis macrochirus) and fathead minnow (Pimephales promeleas). These species were selected based on the following factors:

- A large toxicity data base exists for each species
- All species are readily available and easy to maintain
- All species are widely distributed in the aquatic environment
- Economically important species are represented

In conducting this test, juvenile fish of the same age and appearance are used. After a specified period of acclimation, the fish are exposed to the test chemical under either flow-through or static conditions.

Data that should be collected, recorded or derived include:

- Detailed information about the test fish, including: the scientific name, average weight (wet weight), standard length, age, source, history, observed diseases, treatments, mortalities, acclimation procedures and food used.

- Detailed information about the test system, including: number of replicates used, number of organisms per replicate, loading rate, flow rate for flow-through tests, levels of dissolved oxygen, pH and the temperature and lighting regime.

- Information about the test conditions, including: solvent used, the test chemical concentration in the stock solution, the highest solvent concentration in the test solution, and a description of the solubility of the test chemical in water, other solvents used and the concentration of the test chemical in each test chamber just before the start of the test and at all subsequent sampling periods.

- Methods and data records of all chemical analyses of water quality parameters and test substance concentrations, including method validations and reagent blanks.

- The number and percentage of test organisms that died, and the number that showed any abnormal effects in the control and in each test chamber at each observation period.

- The 24-, 48-, 72- and 96-hour LC_{50} values and confidence limits and the methods used to calculate the LC_{50} values and their confidence limits.

- When available, the no-observed-effect level (the highest concentration tested at which there were no mortalities or abnormal behavioral or physiological effects).

- The concentration-response curve at each observation period for which LC_{50} values were calculated.

5.4.3 Limitations of Ecotoxicological Studies

The short-term nature of most studies on representative species from the ecosystem limits their usefulness in assessing the affects of long-term exposure. Specific limitations include the following:

- Bioaccumulation. The concentration of a chemical within an organism may be increased by bioaccumulation. The properties of a chemical which contribute to high bioaccumulation include a high partition coefficient and resistance to degradation. A short-term study may not allow sufficient time for bioaccumulation to play a significant role.

- Effects of latency. For some ecotoxicological endpoints (e.g., carcinogenicity), the observed effect may be delayed from the time of initial exposure. Short-term studies may, therefore, fail to detect the majority of late-occurring effects.

- Interactions and synergisms. It is likely, especially at a hazardous waste site, that many different chemicals escape from the site into the environment. This greatly complicates the task of assessing the hazard involved, since interactions between chemicals can increase or decrease the toxicity of specific chemicals.

- Fluctuations in susceptibility. Susceptibility may vary considerably over the lifetime of the organism (e.g., during rapid growth periods) and, therefore, may not be addressed in a short-term study.

- Sporadic or uneven exposure. Long-term exposure of indigenous species in the environment may include periods of uneven or sporadic exposure. This could be caused, for example, by variations in chemical or biological degradation rates of the chemical, seasonal animal migration patterns and changes in river water level and flow. Test animals, on the other hand, tend to receive constant exposure for a specified period. The effect of such different treatments, even when the total dose is the same, is still unknown.

5.5 Key Guidance and Implementation Documents

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5.6 Background References

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6.0 EXTRAPOLATION OF TOXICOLOGICAL DATA FROM ANIMALS TO HUMANS

If it were possible to obtain detailed toxicological information on a chemical directly from studies in humans, it would not be too difficult to calculate appropriate exposure limits or risk estimates that define the levels at or below which no significant adverse effects would occur in an exposed human population. However, good quantitative toxicity data in humans for a specific toxic chemical are often limited or absent, and derivation of exposure limits that are applicable to humans nearly always requires extrapolation of results obtained in animals. In view of the many potential differences among species with respect to sensitivity to toxic chemicals (see section 4.3), it is not surprising that extrapolations of this sort are rather complicated and sometimes involve considerable uncertainty. This chapter describes the major problems and uncertainties involved in deriving human exposure standards from studies in animals, and the means that are currently used to circumvent these problems and uncertainties.

6.1 Selection of Appropriate Studies

The first step in derivation of an exposure limit or risk estimate is review of existing toxicological data and selection of the most applicable study or studies. The factors which are important in making this selection are:

- Route of Exposure. Because absorption and toxicity of a chemical often depend on the route of exposure (see section 4.1), it is important that an inhalation standard be based on inhalation data, that a water standard be based on ingestion data and so on. Exceptions to this rule should be viewed with caution.

- Duration of Exposure. Because the toxic effects of some chemicals tend to accumulate with time (see section 4.2), it is important to select a study involving long-term exposure for derivation of a standard intended to provide protection from chronic exposure.

- Species of Animal. Since there are sometimes considerable differences in sensitivity to a chemical among species (see section 4.3), it is nearly always desirable to select studies involving humans when sufficient data are available. When human data are sparse, care should be taken not to select a study in an animal that is known to be significantly different from humans in sensitivity or response to the chemical.

- Endpoint. Many chemicals produce multiple effects (see section 4.4). It is important to identify the most appropriate endpoint of a chemical's toxicity and select a study which has determined a NOAEL or LOAEL value on the basis of that endpoint.

- Statistical Significance. Fluctuation in measurements and variations among animals is an inherent aspect of toxicological investigations. It is not always simple to determine whether a chemical has caused an effect or not. Statistical analysis of the data is the most objective means of answering the question: "How certain is it that the chemical

did (or did not) cause an effect?" Table 6-1 summarizes some common statistical terms, and Table 6-2 illustrates the statistical analysis of some sample data. In general, an effect is not considered to be statistically significant unless there is 95% confidence that a change did occur.

- Study Quality. While statistical analysis is very useful in judging the significance (or lack thereof) of experimental results, more subjective analysis of the quality of the study must also be performed. This analysis should consider, for example, possible flaws in the measuring techniques, failure to consider all important variables, biased experimental design and so on. This sort of critiquing of a study requires intimate familiarity with all aspects of the study and can only be done by an experienced toxicologist.

6.2 Conversion of Dose Levels

When the study or studies selected as most appropriate involve animals, the doses administered to the animals must be converted to an equivalent dose in humans. When an animal is exposed to a chemical in a laboratory study involving oral exposure, it is customary to describe the amount of chemical the animal ingests in units of mg chemical per kg body weight (mg/kg). If humans and animals were equally sensitive to the chemical on this basis (weight equivalence), then a dose without effect in the animal would also be without effect in humans. However, a considerable body of laboratory data in animals and clinical data in humans indicates that doses expressed in these units are not toxicologically equivalent in animals of different sizes, and that a dose producing no effect in mice might indeed produce an effect in humans.

Studies of effect levels of chemicals indicate that a better correlation among different species exists between toxicity and dose when doses are expressed in units of mg chemical per unit surface area (mg/m^2). The theoretical basis for this correlation is not obvious, but a large number of physiological parameters, including surface area, are approximately proportional to the two-thirds power of body weight. These parameters include metabolic rates, oxygen consumption, blood volume, kidney function, thyroid function, brain weight, liver weight, cardiac output, blood pressure and extracellular water volume. Since many of these parameters are related to the absorption, distribution, excretion, metabolism and mechanisms of toxicity of chemicals, surface area is used as a convenient physical parameter which is proportional to the physiological rates and functions that are directly affected.

The use of surface area equivalence in dose conversions yields calculated doses that are lower than if weight equivalence is used. Comparative data are presented in Table 6-3, which shows the doses in several species calculated to be equivalent to a dose of 2 mg in a 20 g mouse (100 mg/kg). Using weight equivalence, the same dose in a 70-kg human is 7,000 mg (100 mg/kg), but if surface area equivalence is used, the dose is only 776 mg (11 mg/kg).

Conversion of doses (expressed in units of mg/kg) in one species to surface-area equivalent doses (also expressed as mg/kg) in another species may be accomplished by simply multiplying by a conversion factor, as follows:

TABLE 6-1 STATISTICAL TERMS AND THEIR USE

Sample Size (N) - The sample size must be sufficient to establish statistical significance of the results. Statistical significance increases as function of the number of animals used in the experiment. The acceptable number needed for an experiment depends on the type of effect studied (acute, chronic, etc.), the length of the experiment, and the degree of control that can be placed over the experiment.

Mean - In samples, as well as in populations, there are generally a preponderance of values somewhere around the middle of the range of observed values. The mean is the most widely used measure of this central tendency. The mean is calculated as the sum of all the sample values divided by the sample size.

$$\bar{X} = \frac{\Sigma X_i}{N}$$

Measures of Dispersion and Variability - Whereas the mean provides a measure of central tendency, other measures provide an indication of how tightly the data are distributed around a mean or, conversely, how variable the measurements are. Many such distributions are found to conform to a normal "bell shaped" distribution. Generally, statistical significance increases as variability decreases. The most common measure of variability is variance, the sum of the squares of the deviations from the mean divided by N-1.

$$\text{Variance} = \frac{\Sigma(X_i - \bar{X})^2}{N-1}$$

The positive square root of the variance is called the standard deviation (SD). If random samples of size N are drawn from a normal population, the standard error of the means (SEM) is calculated by dividing the SD by the square root of the sample size.

$$\text{SEM} = \frac{SD}{N^{0.5}}$$

Statistical Significance - Many investigations address the hypothesis that a given chemical produces a significant effect in a treated group as compared to an untreated control group (i.e., the sample data are derived from two statistically different populations). The P-value is the probability that the hypothesis is false based on the data collected. The smaller the P-value the greater the confidence in the truth of the hypothesis. Generally, the greater the observed differences between the treated and untreated groups the smaller the P-value. The significance level α is the criteria by which the hypothesis is rejected or accepted. Many studies use a 5% significance level, i.e., if $P>.05$, the hypothesis is rejected. However, if a test result fails to meet the criteria of significance for proving an effect has occurred, that does not prove the effect did not occur. The strength of a negative conclusion is evaluated by a power test. A power test provides an estimate of the probability that an experiment would detect an effect if it were present. For small effects, tests with high power (large numbers of animals, precise measuring techniques) are required.

TABLE 6-2 STATISTICAL ANALYSIS OF HYPOTHETICAL DATA

	Blood Pressure, mm Hg	
	Control (Not Exposed)	Exposed to Chemical
	100	107
	110	92
	93	115
	107	116
	85	112
	108	96
	94	101
	86	115
	105	111
	78	99
$\bar{X}$	96.9	106.4
SD	11.1	8.8

$P<0.05$

Assume 20 animals are divided into two groups of ten. The first group (control) is not exposed to the chemical, while the second group is exposed to a dose of some chemical suspected of causing increased blood pressure. After exposure, the mean blood pressure of each animal is measured, with the results shown above. Exposure to the chemical did produce an increase in mean blood pressure ($\bar{X}$), but is this really caused by the chemical or is this change random? To answer this, first the standard deviations are derived, and then a P value is calculated. Since P is less than 0.05, there is a 95% probability that the change observed did not occur by random, but is a real effect caused by the chemical.

TABLE 6-3 COMPARISON OF DOSE CONVERSIONS USING SURFACE AREA AND WEIGHT EQUIVALENCE

Species	Weight, g	Surface Area, cm^2	Calculated Dose, mg[(a)]	
			Weight Equivalence	Surface Area Equivalence
Mouse	20	46	2	2
Rat	200	325	20	14
Guinea Pig	400	564	40	24
Rabbit	1,500	1,272	150	55
Cat	2,000	1,381	200	59
Monkey	4,000	2,975	400	128
Dog	12,000	5,766	1,200	248
Human	70,000	18,000	7,000	776

(a) Based on a dose of 2 mg in a 20 g mouse (100 mg/kg).

Adapted from Klaassen and Doull (1980).

$$D_H = (D_A)(W_A/W_H)^{1/3}$$

where:

D_H = the human equivalent dose (mg/kg)
D_A = the animal dose (mg/kg)
W_H = human body weight (kg)
W_A = animal body weight (kg)

Table 6-4 lists the factors for converting doses in animals to doses in humans employing the weights given in Table 6-3. The term $(W_A/W_H)^{1/3}$ to estimate body surface area ratios is employed because weight can be measured easily but surface area cannot. If actual surface areas were employed the result would differ slightly. A sample calculation using this equation is presented below.

Sample Calculation

To convert a dose of 100 mg/kg in the mouse to an equivalent dose in humans, simply multiply by the conversion factor for the mouse:

$$(100 \text{ mg/kg})(0.066) = 6.6 \text{ mg/kg}$$

where:

100 mg/kg = dose in mouse
0.066 = dose conversion factor (assuming surface area equivalence)
6.6 mg/kg = calculated equivalent dose in humans

6.3 Correction for Toxicokinetic Differences

The principal reason that data from animals are not directly applicable to humans is that there are toxicokinetic differences (differences in ingestion, inhalation, absorption or metabolism) among species. Whenever there are good quantitative data that characterize these differences, it is possible to correct for them. For example, if an oral dose of 100 mg/kg is 30% absorbed in rats, this corresponds to an absorbed dose of 30 mg/kg. If an oral dose of 100 mg/kg of the same chemical is 60% absorbed in humans, this corresponds to an absorbed dose of 60 mg/kg. Clearly, the same oral dose (100 mg/kg) would be more effective in humans than in rats. This is simply corrected for, as follows:

$$\frac{(\text{Dose in animal})\ (\text{Absorption in animal})}{(\text{Absorption in humans})} = \text{Equivalent dose in humans}$$

For the above example the calculation would be as follows:

$$(100 \text{ mg/kg})(30\%/60\%) = 50 \text{ mg/kg}$$

where:

100 mg/kg = dose in rat
30% = absorption in rat
60% = absorption in humans
50 mg/kg = equivalent dose in humans

TABLE 6-4 DOSE CONVERSION FACTORS

Species	Weight, kg	$(W_A/W_H)^{1/3}$
Mouse	0.02	0.066
Rat	0.2	0.142
Guinea Pig	0.4	0.179
Rabbit	1.5	0.278
Cat	2.0	0.306
Monkey	4.0	0.385
Dog	12.0	0.555
Human	70.0	1.000

Similar corrections may be employed to adjust for differences in absorption across skin or lung and differences in rates of metabolism. Unfortunately, reliable quantitative toxicokinetic data in animals and/or humans are frequently lacking and objective corrections for differences in absorption or metabolism are not possible in all cases.

6.4 High-Dose to Low-Dose Extrapolation

The design of experimental dose-response studies is limited by two important practical considerations. First, the researcher is restricted to the study of animal populations of manageable size, usually 100 to 1,000 animals. Second, the researcher is limited to the use of exposures that will produce a measurable response in a test population of the size studied. The task of the toxicologist is then to extrapolate results obtained at high doses where effects can be detected to expected results at low levels (more characteristic of human exposure from the environment) where effects cannot be measured directly.

Despite wide gaps in our knowledge of the metabolism and ultimate fate of chemicals in man, properly conducted animal experiments have yielded results that are predictive of the effects in humans. Using appropriate statistical treatment of the results of experiments on animals, a plausible estimate of risk can be calculated that approximates the true risk in populations exposed to known concentrations of a toxic chemical. Such estimates are designed to be highly conservative (i.e., the true risk is almost certainly lower than the estimate).

Animal experiments, using relatively small numbers of subjects, must be performed at doses high enough to provide measurable toxic effects in a relatively short time period. Since adverse effects at very low exposures are often not apparent, estimates of risk from low exposures are based on the downward extrapolation of the dose-response curve from relatively high dose levels. Therefore, to estimate the probability of effects (response) at dose levels outside the experimental range, it is necessary to make an assumption concerning the shape of the dose-response curve at the low dose range.

Many mathematical models have been developed which make this downward extrapolation. The dose-response curve, depending on the mathematical model used, may be convex, linear, or concave at low doses (see Figure 6-1). A curve that is concave at low doses will lie above one that is linear and a curve that is convex will lie below one that is linear. Consequently, if the curve is approximated at low doses by a straight line, the approximation will overestimate risk if the true response curve is convex, and underestimate risk if the true response curve is concave.

Six models for extrapolating to low dose are routinely used: the probit model, logit model, Weilbull model, linearized multistage model, one-hit model, and the gamma multi-hit model. (These models are discussed in detail in Section 6.4.1) The models are used to estimate a virtually safe dose (VSD), which

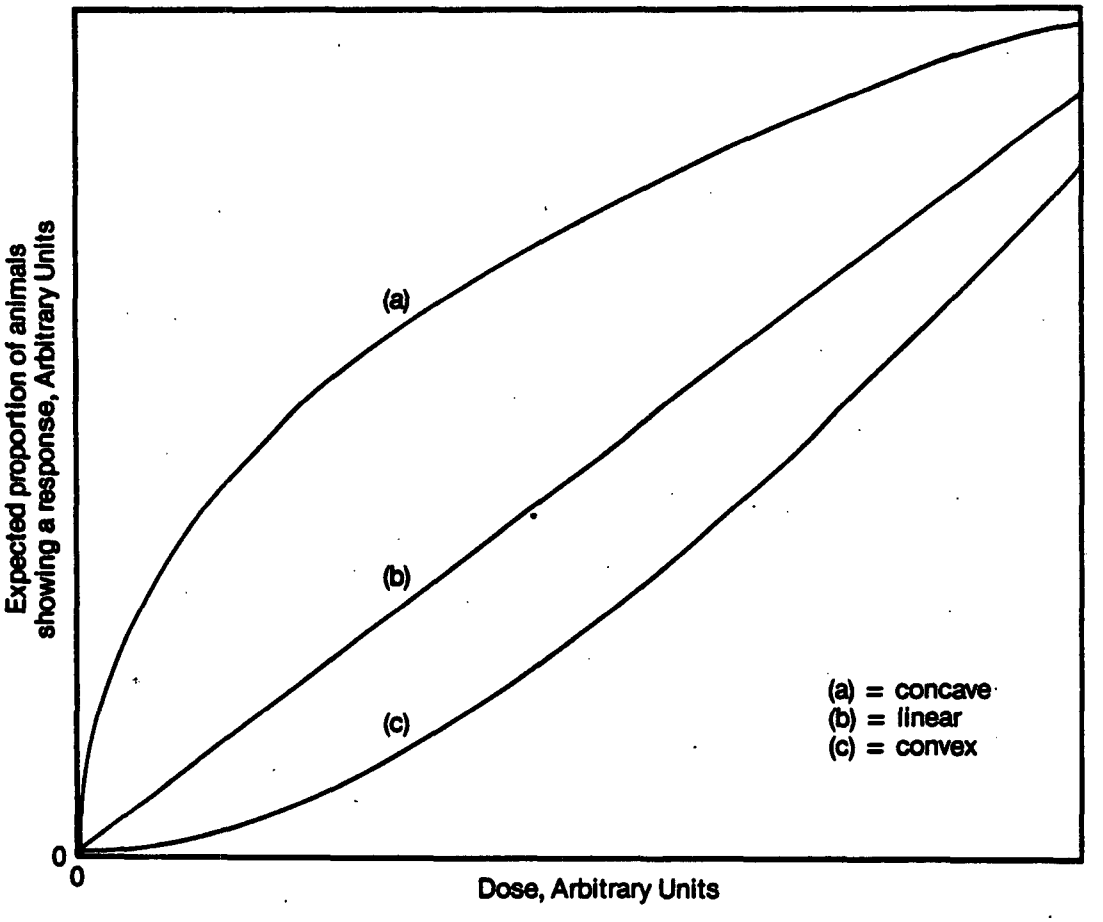

FIGURE 6-1 ILLUSTRATION OF DIFFERENT POSSIBLE SHAPES OF THE DOSE-RESPONSE CURVE IN THE LOW DOSE RANGE

is the dose level corresponding to a desired low level of response. Although each of the six models are frequently used in the assessment of risk, there is substantial scientific disagreement about their relative merits. The Proposed Guidelines for Carcinogen Risk Assessment (USEPA 1984) recommends that the linearized multistage model be utilized for high-to-low dose extrapolation unless there is mechanistic or other biological evidence that indicates the greater suitability of an alternative extrapolation model, or there is statistical or biological evidence that excludes the use of the linearized multistage model.

Figure 6-2 is an example of how the six models are applied to extrapolating low dose-response from incidence of liver tumors in mice exposed to high levels of DDT. While each of the models was found to fit the experimental data nearly equally well, they lead to very large differences when extrapolated to low doses, differing in this case by three to five orders of magnitude (1,000- to 100,000-fold different). The one hit and the linearized multi-stage models are most conservative (i.e., give the highest risk estimate).

6.4.1 Dose-Response Models

The following section describes the six models currently being used for high-to-low dose extrapolation, their assumptions, similarities and limitations.

There are two basic classes of dose-response models:

- Tolerance Distribution Models
- Stochastic or Mechanistic Models

These categories are not always distinct and some models may belong to both categories.

Tolerance distribution models are based on the concept that each individual in the population has its own tolerance to the test chemical. If a dose does not exceed the tolerance of an individual, then there will be no response by that individual. If the dose exceeds the tolerance, then a response will be observed. It is assumed that the distribution of tolerances within the population follows a normal (bell-shaped) distribution. The probit and logit models fall into this class.

Stochastic or mechanistic models including the one-hit, gamma multi-hit, linearized multistage and Weibull models, are derived from assumptions regarding the mechanism of action of the toxic chemical upon its target site. The "hit" theory for interaction between radiation particles and susceptible biological targets has generated this general class of models. This "hit" theory is also applicable to the action of toxic chemicals upon their target sites. The assumptions forming the basis of this theory include:

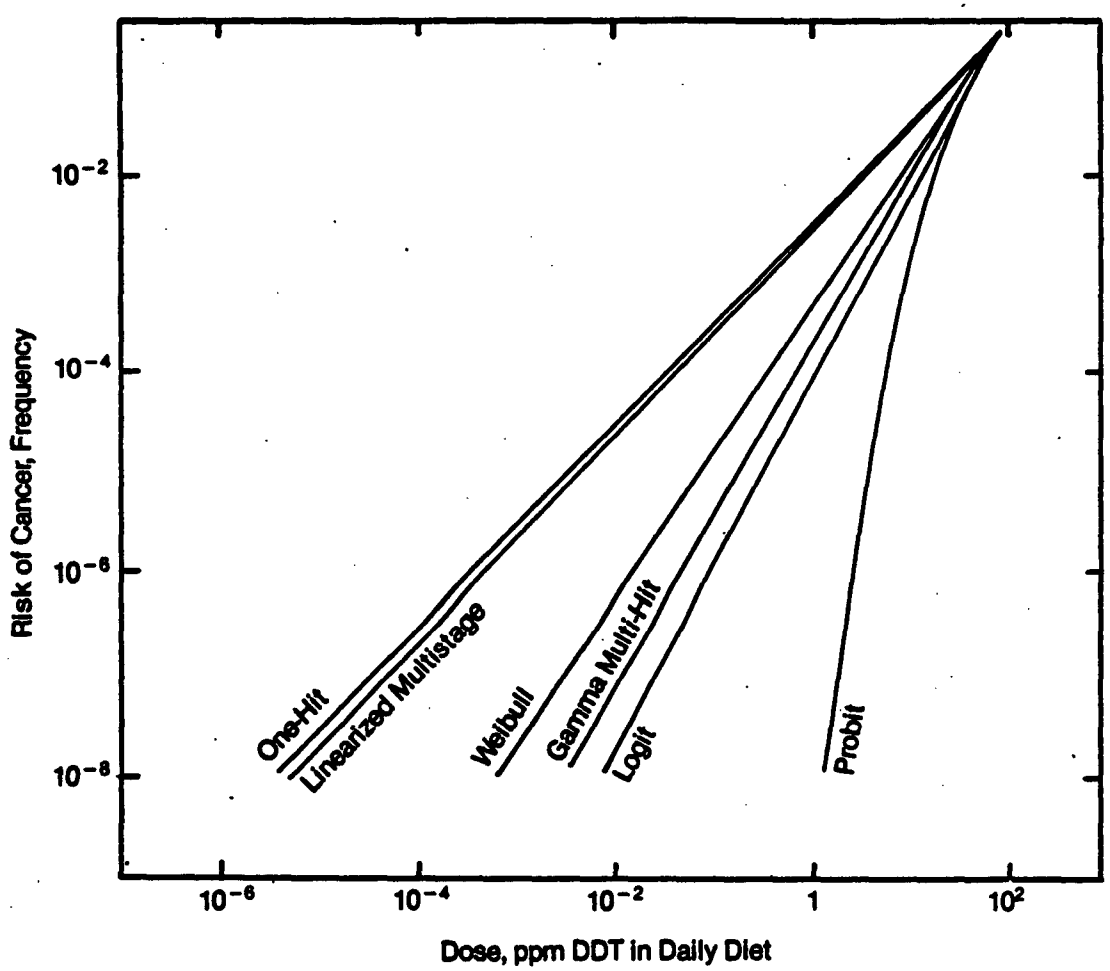

FIGURE 6-2 DOSE-RESPONSE CURVE EXTRAPOLATIONS BY SIX MATHEMATICAL MODELS

This figure illustrates the large differences between six mathematical models for extrapolation to the low-dose range from a set of cancer frequency data at high doses of DDT.

Adapted from Brown (1974).

1. The organism has some number of critical targets (susceptible cells or molecules);

2. The organism responds if one or more of these critical targets are injured;

3. A critical target is injured if it is "hit" by one or more toxic chemicals; and

4. The probability of a "hit" in the low-dose region is proportional to the dose level of the toxic chemical.

It should be noted that stochastic/mechanistic models of carcinogenesis generally become linear at low dose levels. This is because they assume that all population members have identical susceptibility to the carcinogen. If this was not the case, the population dose-response relationship at very low dose rates would be concave even though no absolute threshold for the carcinogen exists. Many researchers believe that the shape of the true dose-response curve at low exposure levels is convex, i.e., may have some degree of upward curvature (see Figure 6-1). Therefore, linearity provides conservative or overestimated extrapolated risk estimates at low doses.

6.4.2 Threshold Versus Nonthreshold Models

All the models described previously are nonthreshold models; i.e., they assume some positive probability of observing a response no matter how low the dose. There has been much discussion over the existence of thresholds. Some scientists argue that responses to carcinogens are less likely to show a threshold effect than responses to other toxic substances because cancer may be produced by an event in a single cell. Even if a threshold level does exist, this level would probably vary among members of the population at risk and the resulting dose-response curves would be indistinguishable from those described by nonthreshold models. For this reason, it appears that nonthreshold models are appropriate to use in extrapolating the risk from exposure to toxic chemicals.

6.4.3 Toxicokinetic Considerations in High-To-Low Dose Extrapolation

Toxicokinetics is the study of the time course of an administered chemical and its metabolites in the body. The six models currently available for high-to-low dose extrapolation described above assume that the biological fate of the administered chemical is directly proportional to the administered dose. However, this circumstance is not always the case. It is now known that many chemicals are only carcinogenic after they have been metabolized and that the metabolic processes involved may not be proportional to the administered dose. The high doses typically used in carcinogenesis bioassays often saturate normal metabolic (detoxification) processes, resulting in nonproportional relationships between nominal and effective doses. If detoxification pathways are saturated, then the effective dose will increase more rapidly than a proportional relationship would predict. The overall consequence of a nonproportional relationship is that the carcinogenic response may increase in

a nonlinear manner with increasing dose. Therefore the mathematical models used for extrapolation may overestimate the risks associated with low dose levels of toxic chemicals.

6.5 Sources of Uncertainty

In the assessment of risks associated with toxic chemical exposure, there are a number of sources of significant uncertainty. Many of the sources of uncertainty have been described in preceding sections, and are only summarized here.

6.5.1 Sources of Uncertainty in Extrapolation from Animal Studies

6.5.1.1 High-to-Low Dose Extrapolations

Toxicological studies are often conducted at doses of chemicals much higher than those to which human populations are exposed in the environment. For responses thought to have no threshold (e.g., cancer), prediction of the toxicological response at low doses of a chemical must be done mathematically. Frequently, there is considerable uncertainty in this process, most often because the data are too limited to define the dose-response curve precisely. In addition, the best mathematical equation to describe the dose-response curve may not be know.

6.5.1.2 Scaling Factors

Rodents are smaller than humans, they live approximately 1/35th as long, and their rates of metabolism and cell division are much faster. These differences influence chemical toxicity. There is some uncertainty whether the proper way to scale or convert doses from animals to humans is on the basis of relative body weights, relative surface areas, relative life spans, or on some other basis.

6.5.1.3 Differences in Species' Sensitivity

There is a large data base of evidence that different species, even different strains within species, have markedly different sensitivities to toxic chemicals. Animal species can exhibit differences in sensitivity to a chemical of as much as 100-fold. These differences are not accounted for in scaling corrections and assumptions.

6.5.1.4 Individual Variation in Human Sensitivity

Even beyond differences among species, there is greater individual variation in sensitivity among humans than among test animals. This variation is a result of the fact that humans are genetically heterogeneous, while test animals are bred for genetic homogeneity. The range of human sensitivities creates an important source of uncertainty of unquantified magnitude in assessing human risks.

6.5.1.5 Interactions and Synergisms

Animals are usually exposed to only one test chemical in carefully controlled settings. Humans, however, are exposed to a wide variety of other chemicals and environmental conditions. The risk from multiple exposures may be greater than the sum of the risks from exposure to individual toxic chemicals. Yet, most synergisms have not been identified, let alone quantified.

6.5.2 Sources of Uncertainty in Epidemiological Studies

6.5.2.1 Confounding Factors

Epidemiological studies are performed in an uncontrolled setting, introducing many unknown factors that can obscure true relationships between cause and disease. While it is usually possible to identify some confounding factors and take them into account, it is very difficult to eliminate this problem entirely. Even the best epidemiological studies cannot usually detect effects occurring at less than 50% above the normal rate.

6.5.2.2 Effects of Latency

In the case of carcinogens, latency periods (the time from the first exposure to diagnosis of disease) of 20 years or more are common. Studies which do not follow exposed subjects for their full lifetime can fail to detect a carcinogenic effect entirely or can miss the majority of later occurring cases.

6.5.2.3 Failure to Follow All Members of the Exposed Group

A common failure of occupational studies is the failure to follow employees who change jobs. Since ill employees are the most likely to leave employment, ignoring this factor can be significant. Their state of health, or even whether they are still alive, is often not determined. This tends to understate the true risk. Follow-up problems are even greater for many nonoccupational studies.

6.5.2.4 Ignorance of True Exposure Levels and Duration

Dose information is often highly speculative, especially for exposure incidences taking place two or more decades in the past. This problem is especially severe in epidemiological studies concerning toxic chemicals released in the environment.

6.5.2.5 Errors in Describing the Study and Control or Failures to Adjust for Age, Socioeconomic Status, and Other Variables

Errors in describing the group exposed and the comparison control group can introduce serious errors into quantitative estimates. Similar errors can occur from failure to adjust for age, income, race and other variables.

6.6 Approaches to Dealing with Uncertainty

When there are insufficient data to permit clear decisions in the toxicity assessment process, two strategies are available. The first is to assume worst-case values; this strategy almost certainly ensures that calculated values will not be too high, but may yield values that are lower than really necessary. The EPA's calculation of cancer risk estimates is a prominent example of the application of this strategy.

A second approach to dealing with uncertainty is to employ uncertainty factors. These factors are intended to provide a sufficient "margin of error" to account for uncertainties arising from all of the possible sources described in sections 6.5.1 and 6.5.2. It is a standard practice to employ uncertainty factors in the derivation of noncarcinogenic guidelines and standards.

Table 6-5 provides EPA guidelines for selection of uncertainty factors for evaluating acceptable daily intake (ADI) of noncarcinogenic chemicals based on a NOAEL. A minimum uncertainty factor of 10 is generally employed to account for variations between individuals and to provide a basic margin of safety. Additional uncertainty is added (usually by factors of 10) to account for use of data from animals, or for use of limited or poor quality data, or data from short-term studies.

The following is a hypothetical example of the use of this approach in estimating an ADI for humans.

Assume a hypothetical compound is widely used in the United States, and that it is toxic to humans and animals, causing injury primarily to lung tissue. A two-year feeding study in rats indicates that doses of up to 170 ppm in the diet (8.5 mg/kg/day) do not cause significant injury to lung or other tissues. This is identified as the NOAEL. Since no useful long-term or acute human data exist, an uncertainty factor of 100 is appropriate, and the ADI is calculated as follows:

$$\text{ADI} = \frac{8.5 \text{ mg/kg/day}}{100} = 0.085 \text{ mg/kg/day}$$

Assuming 70 kg as the average weight of an adult human, and assuming consumption of 2 L/day of water, if exposure were entirely through water, the maximum acceptable water concentration would be:

$$\frac{(0.085 \text{ mg/kg/day})(70 \text{ kg})}{(2 \text{ L/day})} = 3.0 \text{ mg/L}$$

If exposure were also occurring by other routes, the permissible level in water would be reduced accordingly. Assuming that 20% of the ADI could come from water, the maximum permissable level from water would be:

$$(0.20)(3.0 \text{ mg/L}) = 0.6 \text{ mg/L}$$

TABLE 6-5 GUIDELINES FOR SELECTION OF UNCERTAINTY FACTORS

	Uncertainty Factor
1.	Use a 10-fold factor when extrapolating from valid experimental results of studies on prolonged ingestion by humans. This 10-fold factor protects the sensitive members of the human population estimated from data gathered on average healthy individuals.
2.	Use a 100-fold factor when extrapolating from valid results of long-term feeding studies on experimental animals when results of studies of human ingestion are not available or scanty (e.g., acute exposure only). This represents an additional 10-fold uncertainty factor in extrapolating data from the average animal to the average human.
3.	Use a 1,000-fold factor when extrapolating from short-term study results from experimental animals when no useful long-term or acute human data are available. This represents an additional 10-fold uncertainty factor in extrapolating from short-term to chronic exposures.
4.	Use an additional uncertainty factor of between 1 and 10, depending on the sensitivity of the adverse effect, when deriving an ADI from a LOAEL. This uncertainty factor drops the LOAEL into the range of a NOAEL.

Adapted from Dourson and Stara (1983).

Application of appropriate uncertainty factors in calculations of this sort ensures that the resulting values will be sufficiently conservative that no adverse effects will occur in any exposed human population.

It is important to realize that methods for calculating ADI's are constantly being reviewed by EPA for consistency with the latest toxicological knowledge. The method illustrated in this example, therefore, may be revised.

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7.0 EXPOSURE ASSESSMENTS

Exposure assessment is the process of collecting all the necessary information to answer the following questions for a specific hazardous waste site:

- What chemicals are present?
- In what media (air, soil, water) do these chemicals occur?
- What amount or concentration of each chemical is present?
- What living organisms (humans, wildlife, plants) are exposed?
- By what routes are these organisms exposed?
- What pattern and degree of exposure is expected in the future?

When this information is combined with toxicological data derived from the toxicity assessment process (chapters 3.0 through 6.0), it is possible to derive reasonable estimates of the risks posed to the exposed organisms.

The purpose of this chapter is to explain various aspects of exposure assessments as related to actions at hazardous waste sites. This chapter does not describe the technical means of collecting and analyzing exposure data or the methodology of conducting exposure assessments. Rather, it explains the types of data which are required to permit the toxicologist to perform a risk assessment. To accomplish this purpose, this chapter describes the steps in assessing present, past, and future exposures.

7.1 Collection of Occurrence Data

Adverse effects in living organisms are not produced unless a toxic chemical contacts the organism at a sufficient concentration and for a sufficient duration to initiate a toxic effect. Therefore, whether or not a toxic effect occurs depends not only on the properties of the chemical and the characteristics of the organism in question, but also on a number of exposure-related factors. The major exposure-related factors that influence toxicity are route, duration and frequency of exposure. Therefore, one type of information required in conducting an exposure assessment is knowledge of the identity and concentrations of toxic chemicals in each possible exposure medium (i.e., air, groundwater, surface water, soil and biota). Two important aspects of collecting this type of information include an evaluation of the history of the hazardous waste site and the collection and analysis of sampling data. An important component of the collection/analysis of data involves the consideration of certain legal requirements (e.g., chain-of-custody requirements, quality assurance/quality control (QA/QC) procedures) to ensure the integrity of the data.

7.1.1 Site History

In the assessment of exposure resulting from the presence of toxic chemicals at a hazardous waste site, an important initial step is an evaluation of the history of the site. The types of information typically required in order to adequately characterize the history of a hazardous waste site are presented in Table 7-1. Especially important to the performance of an exposure assessment is information on the identity of chemicals present at the site, their concentrations, and the manner in which they were originally disposed or stored.

TABLE 7-1 CHECKLIST OF IMPORTANT SITE HISTORY INFORMATION

1. ☐ Facility Ownership _____
2. ☐ Facility Type _____
3. ☐ Facility Location, Size Configuration, Physical Description _____
4. ☐ Time-Frame of Waste-Related Activities _____
5. ☐ Types of Activities/Operations at the Site _____
6. ☐ Waste Disposal/Storage Methods _____
7. ☐ Identity/Quantity of Disposed Waste _____
8. ☐ Chemical Composition of Waste _____
9. ☐ Site Incidents (e.g., fires, explosions) _____
10. ☐ Records of Previous Site Investigations _____
11. ☐ Records of Previous Sampling Activities _____
12. ☐ Records of Previous Response Actions _____

N/A = Not applicable.
D/A = Documented and Attached.

Once this information is obtained, predictions can be made regarding which chemicals warrant concern, what media are likely to be contaminated and which exposure routes will be of concern. Based on this information, a sampling plan can be devised. Therefore, the results of the site history analysis serve to focus and direct subsequent exposure assessment activities.

7.1.2 Sampling and Analysis of Environmental Data

A second important aspect of the collection of occurrence data at hazardous waste sites involves the sampling and analysis of environmental media for chemicals of concern. To a large extent, this activity receives its direction from the results of the site history analysis. Since site history analyses typically provide information on those chemicals expected to be of concern and in what environmental media those chemicals may occur, sampling efforts can be directed accordingly. However, while site history analysis is an important initial step in performing an exposure assessment, the available site history information is often incomplete and/or unreliable. In such cases, it may be necessary to collect sampling data on a variety of chemicals that may be present at the site. Table 7-2 presents a list of chemicals for which sampling may be required when site history data are insufficient.

The results of sampling efforts should yield data on the amount of toxic chemicals released from each on-site source into each environmental medium. Estimates of chemical release to each environmental medium may be qualitative or quantitative. Qualitative information is useful for discriminating between major and minor sources of releases and for estimating the nature and relative magnitude of releases. However, quantitative release data are required since it is these data that will be used in subsequent steps of the exposure assessment process to calculate doses of toxic chemicals incurred by exposed populations (receptors). Quantitative data may be obtained through either modeling or monitoring (sampling) activities or a combination of modeling and monitoring. The media which must be analyzed for toxic chemicals include the atmosphere, surface water, groundwater, soil, and the tissues of organisms (biota) which are consumed by others (especially species consumed by humans).

7.1.2.1 Atmospheric Contamination

Emissions of contaminated fugitive dusts (airborne wastes and contaminated soil particles) and volatilization of toxic chemicals are the most likely sources of atmospheric contamination at hazardous waste sites. Fugitive dust emissions can result from wind erosion of waste and contaminated soil, vehicular traffic over contaminated roads, heavy equipment activity at the site or incineration of wastes during remediation. Volatilization of contaminants at hazardous waste sites can occur from lagoons, inadequately covered landfills or from spills or leaks.

Mathematical equations for making quantitative estimates of atmospheric contamination patterns are termed atmospheric dispersion models. These models are employed to determine airborne chemical concentrations as a function of space and time and they typically require information on characteristics of the source, physical and chemical properties, and data on local meteorology.

TABLE 7-2 CHEMICALS FREQUENTLY OCCURRING AT HAZARDOUS WASTE SITES FOR WHICH SAMPLING DATA MAY BE REQUIRED

Number	Volatiles	Chemical Abstract Service (CAS) Number
1.	Chloromethane	74-87-3
2.	Bromomethane	74-83-9
3.	Vinyl chloride	75-01-4
4.	Chloroethane	75-00-3
5.	Methylene chloride	75-09-2
6.	Acetone	67-64-1
7.	Carbon disulfide	75-15-0
8.	1,1-Dichloroethene	75-35-4
9.	1,1-Dichloroethane	75-34-3
10.	trans-1,2-Dichloroethene	156-60-5
11.	Chloroform	67-66-3
12.	1,2-Dichloroethane	107-06-2
13.	2-Butanone	78-93-3
14.	1,1,1-Trichloroethane	71-55-6
15.	Carbon tetrachloride	56-23-5
16.	Vinyl acetate	108-05-4
17.	Bromodichloromethane	75-27-4
18.	1,1,2,2-Tetrachloroethane	79-34-5
19.	1,2-Dichloropropane	78-87-5
20.	trans-1,3-Dichloropropene	10061-02-6
21.	Trichloroethene	79-01-6
22.	Dibromochloromethane	124-48-1
23.	1,1,2-Trichloroethane	79-00-5
24.	Benzene	71-43-2
25.	cis-1,3-Dichloropropene	10061-01-5
26.	2-Chloroethyl vinyl ether	110-75-8
27.	Bromoform	75-25-2
28.	2-Hexanone	591-78-6
29.	4-Methyl-2-pentanone	108-10-1
30.	Tetrachloroethene	127-18-4
31.	Toluene	108-88-3
32.	Chlorobenzene	108-90-7
33.	Ethylbenzene	100-41-4
34.	Styrene	100-42-5
35.	Xylene	1330-20-7

continued-

Table 7-2 - continued

Number	Semi-Volatiles	Chemical Abstract Service (CAS) Number
36.	N-Nitrosodimethylamine	62-75-9
37.	Phenol	108-95-2
38.	Aniline	62-53-3
39.	bis(2-Chloroethyl) ether	111-44-4
40.	2-Chlorophenol	95-57-8
41.	1,3-Dichlorobenzene	541-73-1
42.	1,4-Dichlorobenzene	106-46-7
43.	Benzyl alcohol	100-51-6
44.	1,2-Dichlorobenzene	95-50-1
45.	2-Methylphenol	95-48-7
46.	bis(2-Chloroisopropyl) ether	39638-32-9
47.	4-Methylphenol	106-44-5
48.	N-Nitroso-N-dipropylamine	621-64-7
49.	Hexachloroethane	67-72-1
50.	Nitrobenzene	98-95-3
51.	Isophorone	78-59-1
52.	2-Nitrophenol	88-75-5
53.	2,4-Dimethylphenol	105-67-9
54.	Benzoic acid	65-85-0
55.	bis(2-Chloroethyoxy) methane	111-91-1
56.	2,4-Dichlorophenol	120-83-2
57.	1,2,4-Trichlorobenzene	120-82-1
58.	Naphthalene	91-20-3
59.	4-Chloroaniline	106-47-8
60.	Hexachlorobutadiene	87-68-3
61.	4-Chloro-3-methylphenol (p-Chloro-m-cresol)	59-50-7
62.	2-Methylnaphthalene	91-57-6
63.	Hexachlorocyclopentadiene	77-47-4
64.	2,4,6-Trichlorophenol	88-06-2
65.	2,4,5-Trichlorophenol	95-95-4
66.	2-Chloronaphthalene	91-58-7
67.	2-Nitroaniline	88-74-4
68.	Dimethyl phthalate	131-11-3
69.	Acenaphthylene	208-96-8
70.	3-Nitroaniline	99-09-2

continued-

Table 7-2 - continued

Number	Semi-Volatiles	Chemical Abstract Service (CAS) Number
71.	Acenaphthene	83-32-9
72.	2,4-Dinitrophenol	51-28-5
73.	4-Nitrophenol	100-02-7
74.	Dibenzofuran	132-64-9
75.	2,4-Dinitrotoluene	121-14-2
76.	2,6-Dinitrotoluene	606-20-2
77.	Diethyl phthalate	84-66-2
78.	4-Chlorophenyl phenyl ether	7005-72-3
79.	Fluorene	86-73-7
80.	4-Nitroaniline	100-01-6
81.	4,6-Dinitro-2-methylphenol	534-52-1
82.	N-Nitrosodiphenylamine	86-30-6
83.	4-Bromophenyl phenyl ether	101-55-3
84.	Hexachlorobenzene	118-74-1
85.	Pentachlorophenol	87-86-5
86.	Phenanthrene	85-01-8
87.	Anthracene	120-12-7
88.	Di-n-butyl phthalate	84-74-2
89.	Fluoranthene	206-44-0
90.	Benzidine	92-87-5
91.	Pyrene	129-00-0
92.	Butyl benzyl phthalate	85-68-7
93.	3,3'-Dichlorobenzidine	91-94-1
94.	Benzo(a)anthracene	56-55-3
95.	bis(2-Ethylhexyl) phthalate	117-81-7
96.	Chrysene	218-01-9
97.	Di-n-octyl phthalate	117-84-0
98.	Benzo(b)fluoranthene	205-99-2
99.	Benzo(k)fluoranthene	207-08-9
100.	Benzo(a)pyrene	50-32-8
101.	Indeno(1,2,3-cd)pyrene	193-39-5
102.	Dibenz(a,h)anthracene	53-70-3
103.	Benzo(ghi)perylene	191-24-2

continued-

Table 7-2 - continued

Number	Pesticides	Chemical Abstract Service (CAS) Number
104.	alpha-BHC	319-84-6
105.	beta-BHC	319-85-7
106.	delta-BHC	319-86-8
107.	gamma-BHC (Lindane)	58-89-9
108.	Heptachlor	76-44-8
109.	Aldrin	309-00-2
110.	Heptachlor epoxide	1024-57-3
111.	Endosulfan I	959-98-8
112.	Dieldrin	60-57-1
113.	4,4'-DDE	72-55-9
114.	Endrin	72-20-8
115.	Endosulfan II	33213-65-9
116.	4,4'-DDD	72-54-8
117.	Endrin aldehyde	7421-93-4
118.	Endosulfan sulfate	1031-07-8
119.	4,4'-DDT	50-29-3
120.	Endrin ketone	53494-70-5
121.	Methoxychlor	72-43-5
122.	Chlordane	57-74-9
123.	Toxaphene	8001-35-2
124.	Aroclor-1016	12674-11-2
125.	Aroclor-1221	11104-28-2
126.	Aroclor-1232	11141-16-5
127.	Aroclor-1242	53469-21-9
128.	Aroclor-1248	12672-29-6
129.	Aroclor-1254	11097-69-1
130.	Aroclor-1260	11096-82-5

When sampling is employed to obtain quantitative data on atmospheric contamination it must be recognized that the distribution of a wind-borne chemical varies over time with down-wind distance from the source. Therefore, sampling may have to be conducted over extended time periods and distances.

Air quality data should provide adequate temporal as well as spatial resolution of air pollutant concentrations. An air sampling survey designed to provide good temporal resolution should include samples having a short enough averaging time to measure the effects resulting from a variety of possible combinations of source strength and meteorological phenomena. Obtaining samples averaged over a short interval requires either continuous sampling or frequent grab samples and results in a large quantity of data. Monitoring long enough to sample all variations of source strength and meteorology may take as long as one year. Continuous monitoring for one year usually assures that samples are taken during each season, on all days, and during all hours. Such sampling provides the best data describing the temporal distribution of toxic chemical concentrations. The cost, however, may be unreasonably high for maintaining and operating monitoring stations. There is also a long lag time between initiation of the monitoring program and receipt of the final results. A compromise is often made between the two situations, using the best available methodology and ensuring that the sampling schedule includes the time period expected to yield the highest toxic chemical concentrations.

7.1.2.2 Surface Water/Groundwater Contamination

Runoff and overland flow of toxic chemicals (from leaks, spills, etc.) are the most likely sources of surface water contamination at hazardous waste sites. Leaching of toxic chemicals from contaminated soils and the vertical migration of toxic chemicals from lagoons are the most likely sources of groundwater contamination at hazardous waste sites.

Quantitative estimations of the degree of contamination of surface waters are often derived by the application of mathematical models designed to provide information on runoff releases and overland flow of toxic chemicals. These models require data on the sorption partition coefficients for contaminants of concern (sorption partion coefficients may be derived from octanol-water partition coefficients). A model also exists for the rapid estimation of the extent of groundwater contamination. This model requires data on various site-specific and chemical-specific characteristics and provides order-of-magnitude estimates of groundwater contamination.

Surface and groundwater contamination data may also be obtained via sampling. As with air quality data, water sampling data should provide adequate temporal and spatial description of water-borne chemical concentrations. The variation of chemical concentrations in groundwater samples will generally be less than chemical concentrations in surface water. Of particular concern when dealing with water samples is the stability (integrity) of the sample. Complete preservation of a sample is nearly impossible since biological, chemical or physical changes in the sample will usually occur. However, to the extent possible, adequate integrity of samples must be maintained.

7.1.2.3 Soil Contamination

Sources of surface soil contamination at hazardous waste sites include spills, lagoon failure, contaminated runoff or intentional placement of waste on or in the ground. Toxic chemicals can also be leached from surface soils to subsurface layers. Generally, the substances of concern at uncontrolled hazardous waste sites are non-polar and will bind (adsorb) strongly to organic soil particles as a result of their hydrophobic properties.

No modeling methods are currently available to estimate toxic chemical concentrations in surface soils and direct sampling must be conducted to obtain data on surface soil contamination. Analyses of toxic chemical concentrations in subsurface soils may be performed by means of modeling, sampling, or a combination of both.

7.1.2.4 Contamination of Biota

In addition to assessing concentrations of toxic chemicals in air, water and soils, performance of an exposure assessment requires the collection of data on the occurrence of toxic chemicals in the tissues of plants and animals that may be consumed as food by other organisms. Of special concern are organisms (such as fish or shellfish) which are consumed by humans. The quality of results in assessing toxic chemical concentrations in food depends entirely on the method of sampling and sample preparation. Since even processed foods are not homogeneous in quality, the design, sampling and interpretation of the results requires extra care due to possible interactions between chemical, food and nutrients. Important factors that must be considered in the sampling and analysis of biota include both the characteristics of the chemical of concern and the food of concern. Significant characteristics of chemicals include the physical/chemical properties and sources of release. The food characteristics that are important include the potential for contamination, consumption data (regional patterns) and dynamics of food consumption.

7.1.2.5 Data Validation and Interpretation

In the collection of occurrence data for use in an exposure assessment, the first task is to obtain relevant data that are complete, accurate and representative of the actual situation and conditions. The measurements should be comparable and maintained in consistent units. After collection of data, the next task is to verify and validate the data values. This involves removing erroneous and irrelevant measurements, testing for outliers (extremely high or low values) and testing data for accuracy, precision and representativeness. A detailed explanation should be given to justify the removal or inclusion of questionable data values in the final data set. A decision must be made on how to handle values below the detection limit or data reported with negative values. The final task is to perform statistical tests which will allow for interpretation of the data. Since the statistical methods employed are usually based on the assumption of the normality of the data values, appropriate tests should be performed and transformations (such as logarithmic) should be used if needed. The accuracy and precision of the estimates should be reported in the form of standard deviation, coefficient of variation and confidence limits.

7.1.2.6 Collection of Occurrence Data--A Hypothetical Example

In order to illustrate the nature of the data required in evaluating the occurrence of a toxic chemical in the environment, the following hypothetical example is presented.

Prior to initiating an enforcement action at a hazardous waste site pursuant to Section 106 of CERCLA, an endangerment assessment is required. One of the first steps performed in preparing the endangerment assessment is an evaluation of the history of the site. In evaluating the history of a particular site it is determined that the waste present in the largest quantity is 1,1,2,2-tetrachloroethylene (perchloroethylene).

Results of the site history analysis indicate that the perchloroethylene was disposed of in steel drums buried in deep, unlined pits. The site is located several thousand feet from a reservoir which is a popular fishing location and which also serves as the drinking water supply for several nearby towns. Available toxicological information on perchloroethylene indicates that this compound is mutagenic, carcinogenic and hepatotoxic. There is, therefore, a high level of concern regarding the presence of this chemical at the site. A review of data on the physical and chemical characteristics of perchloroethylene reveals that the chemical is expected to be mobile in groundwater and, therefore, could be expected to migrate to the reservoir if released from the buried drums. A sampling plan, is subsequently devised to obtain data on concentrations of perchloroethylene in the groundwater between the site and the reservoir, and in the reservoir itself. Sampling is also scheduled for game fish species present in the reservoir because of the potential for human exposure through ingestion of contaminated fish. Air sampling is not included in the initial sampling plan since the depth of burial of the waste-filled drums reduces concern for volatilized emissions. Similarly, soil sampling is not scheduled because of the depth of waste location and because no human exposure through contact with potentially contaminated soils is expected due to the nature of the site (on relatively inaccessible private property and fenced in).

When sampling is concluded and valid occurrence data for perchloroethylene are available, the data can be used to calculate predicted human exposure levels for the two expected exposure routes (drinking water and ingestion of contaminated biota).

7.1.3 Legal Requirements

It is not always possible to know in advance whether or not monitoring data in general, and exposure monitoring data in particular, may be used in enforcement actions. Therefore, it is good practice to make the assumption, when collecting monitoring data, that the data may be used in enforcement actions. Accordingly, there are certain policies and procedures which should be followed in order to ensure that the collected monitoring data will be acceptable as evidence in a court of law. The purpose of this section is to present a brief summary of some of the more important factors which must be taken into consideration so that monitoring data may be acceptable for use in enforcement cases. For more detailed information on this topic, the reader is referred to the list of publications at the end of this chapter.

Prior to the collection and analysis of samples, a draft sampling/analysis plan should be prepared and circulated to appropriate technical and legal personnel for review and comment. The final sampling/analysis plan should then formally incorporate or rebut all comments received. The final plan should also detail the sampling project's objectives, survey methods, personnel and equipment requirements, safety program and equipment, custody procedures, quality assurance procedures and report schedules.

Once samples have been collected they should be appropriately marked with tags and maintained under Chain-of-Custody procedures. Possession of all samples must be traceable from the time they are collected until they are introduced as evidence in legal proceedings. Samples are accompanied by a Chain-of-Custody Record, and all transfers between individuals must be documented by signatures and records of the dates and times of transfer. Samples are shipped (if by mail, registered with return receipt requested) to an appropriate laboratory for analysis, with a separate custody record identifying the sample contents accompanying each shipment.

At the receiving laboratory, a designated sample custodian accepts custody of the samples and verifies that the information on the sample tags matches that on the Chain-of-Custody Record. After assigning a unique laboratory identification number to each sample tag, the sample custodian enters the sample tag data into a bound logbook arranged by project code and station number. The sample custodian then distributes samples to appropriate analysts who also maintain a record to show the Chain-of-Custody for each sample or sample aliquot. All laboratory observations and calculations relevant to a sample are recorded by the analysts in serialized logbooks. The logbooks must contain information sufficient to allow the analysts to recall and describe succinctly each step of the analyses performed. Moreover, sufficient detail is necessary to enable others to reconstruct the procedures followed should the original analyst be unavailable for testimony. Any irregularities observed during the analytical process need to be noted.

After an organization has completed its work for a particular project, all documents generated should be assembled in the organization's files. The file then becomes accountable. Any records taken from the file must be signed out.

Using proper Chain-of-Custody procedures, data obtained from the analyses of samples are returned to the Sampling/Analysis Project Leader for use in the preparation of a draft project report. In preparing reports, the ability to substantiate and defend the contents is of foremost importance. All draft reports should be dated and numbered and be accountable. All draft copies of the report must be returned to the Sampling/Analysis Project Leader after review. Once comments have been incorporated and the final report is available, all draft copies should be disposed of properly.

The following is a list of documents which provide more detailed information on the procedures which should be followed to ensure that sampling data will be acceptable as evidence in a court of law.

1. The EPA's "Handbook for Analytical Quality Control in Water and Wastewater Laboratories" (Booth 1979) addresses in detail the following areas: laboratory facilities, instruments, glassware requirements, and reagents. It further deals with analytical performance, data handling, and data reporting. Separate chapters are devoted to the special requirements for trace organic analysis, water and wastewater sampling, microbiology, aquatic biology, and safety.

2. The "Quality Assurance Guidelines for Biological Testing" (Stanley 1978) discusses the following elements of quality assurance: quality assurance policy and objectives, design and analysis of experiments, sampling, precision and assurance of tests, physical environment of research, chemicals and reagents, control of performance, and data handling and reporting.

3. The "Handbook for Sampling and Preservation of Water and Wastewater" (Berg 1982) is not an official EPA manual, but is a reference to be used as an input to EPA manuals and guidelines. The report presents general considerations for sampling; sample preservation and handling methods, sampling methods for wastewaters, surface waters, and bottom sediments; methods for collection of microbial samples, and statistical analysis methods.

4. The "Quality Assurance Guidelines for IERL-CI Project Officers" (Stratton and Bonds 1979) is designed for use, as the title implies, by EPA project officers. The report provides quality assurance guidelines for procurement of projects requiring sampling and analysis, guidelines for monitoring such projects and auditing procedures. The document should assist in providing an understanding of what EPA project officers expect in terms of quality assurance.

5. Another source of EPA quality assurance procedures is the "Manual of Analytical Quality Control for Pesticides and Related Compounds in Human and Environmental Samples" (Sherma 1977). A general description of pesticide residue analytical procedures is provided following a discussion on inter- and intra-laboratory quality control. Also covered are procedures for analysis of samples including extraction, isolation, and gas chromatography. Although the manual deals primarily with pesticides, many of the procedures and recommendations apply to the analysis of any organic chemical.

7.2 Identification and Analysis of Exposed Populations

The identification and analysis of exposed human populations generally involves four distinct steps, each of which requires unique information. In the first step, population data are compared with occurrence data in order to quantify the population that will actually or potentially come into contact with contaminated air, water, soil or food. The second step, population characterization, involves determining whether any groups within the exposed population may experience a greater risk than the average population as a

result of exposure. High risk groups might include women of childbearing age, the chronically ill, infants, children or the elderly. The third step, activity analysis, involves an examination of the activities in which a potentially or actually exposed population engages. The final step, development of exposure coefficients, yields information critical to the calculation of exposure levels. Each of these steps is discussed in further detail below. The analysis of nonhuman populations is also discussed.

7.2.1 Population Identification and Enumeration

The first step in identifying and analyzing exposed populations is to determine the number of individuals exposed and the routes of exposure. For humans, the Census of Population (most recently conducted in 1980) can be accessed to determine the size, distribution and demographic characteristics of a geographically defined population. Census data are especially useful in quantifying populations exposed as a result of their presence in a specific locale (e.g., those exposed to toxic chemicals in ambient air or soil). For example, identification/enumeration of populations exposed to airborne toxic chemicals may be accomplished by overlaying an isopleth map of chemical concentrations around a source on census maps.

In the analysis of populations that may be exposed to chemicals present in surface or groundwater, all persons in the service area of a water supply system that draw water from a contaminated source must be considered as potentially exposed through ingestion and dermal exposure while bathing. Information concerning local drinking water sources and populations served can usually be obtained from the local Department of Public Works, Planning Department or Health Department. Information on public departments or private drinking water treatment companies that use groundwater as their raw water supply, as well as the number of households drawing water from private wells, will also generally be available from these sources. Swimmers in contaminated waters may also comprise a portion of the exposed population.

Dermal exposure to contaminated soils could constitute an exposure route for individuals who work outdoors or for children playing outdoors. In addition, children sometimes ingest toxic chemicals contained in soil through pica behavior (eating soil). Direct soil exposure is, in most cases, minor in magnitude when compared to other routes, and it is often difficult to quantify the actual level of transmission of soil-absorbed toxic chemicals across skin. Exposure to airborne contaminated soil particulates and substances volatilizing from soil may, however, be quite significant, and must be considered as an integral component of the overall site air contamination/ exposure analysis.

Human exposure to contaminated food will usually be associated with fruit and vegetables grown in home gardens, or with fish or game residing in contaminated areas.

7.2.2 Population Characterization

After exposed populations have been identified and enumerated, they should be characterized by age and sex factors since the physiological parameters that determine dose (e.g., breathing rate, skin surface area, food and water

ingestion) are often age- or sex-specific. Also, from a toxicity standpoint, subpopulations defined by age or sex may be especially susceptible to toxic chemicals. Thus, characterization of exposed populations permits the determination of exposure distributions within the population at large, and the delineation of specific high-risk subpopulations.

7.2.3 Activity Analysis

Activities engaged in by members of a given population or subpopulation can dramatically affect the level of exposure to environmental chemicals. For example, persons whose lifestyle or employment involves frequent strenuous activity will ventilate larger volumes of air per unit time than will those living a less strenuous life and will, therefore, experience a greater level of exposure to airborne chemicals. Another example would be if activities take persons away from the source of the exposure such as weekends in the country. Also seasonal factors can affect exposure levels, e.g., dermal contact with contaminated soil is considerably reduced during freezing weather.

Key activity-related exposure determinants to be quantified in the activity analysis phase for each exposure mechanism are:

- Ingestion: amount of contaminated food or water ingested (per unit time).
- Inhalation: length of time (frequency and duration) spent in each related activity; nature of the activity in terms of light, medium, heavy, or maximum exertion (per unit time).
- Dermal exposure: length of time (frequency and duration) spent in each related activity (per unit time).

7.2.4 Development of Exposure Coefficients

The final component of the identification and analysis of exposed human populations is the development of exposure coefficients. An exposure coefficient is a term which combines information on the frequency, mode and magnitude of contact with each contaminated medium to yield a quantitative value of the amount of contaminated medium contacted per day. Exposure coefficients are developed for each exposure route and are used in calculating exposure levels. Developing an exposure coefficient requires that certain assumptions be made regarding intake of air, food and water. Some standard intake assumptions are provided in Table 7-3.

7.2.5 Nonhuman Population Analyses

Nonhuman populations are generally more difficult to quantify and characterize than are human populations because less information is available and the data that are available are often measured in units other than numbers of organisms. Populations may, for example, be expressed as herds or biomass. The adequacy and sources of nonhuman population data vary considerably, depending on the organism. As might be expected, most research effort has been invested in plant and animals species that have economic significance.

TABLE 7-3 STANDARD INTAKE ASSUMPTIONS FOR HUMANS

Respiratory Rate[a]	Adult Male	Adult Female	Child (10 yrs)
Resting (L/min)	7.5	7.0	4.8
Light Activity (L/min)	20.0	19.0	13.0
Fluid Intake[b]			
Milk (L/day)	0.30	0.20	0.45
Tap Water (L/day)	0.15	0.10	0.20
Other (L/day)	1.50	1.10	0.75
Total (L/day)[c]	1.95	1.40	1.40
Food Consumption[a]	1,500 g/day	--	--

(a) Adapted from Callahan et al. (1983)
(b) Adapted from International Commission on Radiological Protection (IRCP) (1975).
(c) EPA usually uses 2 L/day as total fluid consumption for adults and 1 L/day for children.

In most cases, it is necessary to gather the data from diverse sources to enumerate nonhuman populations. The major sources of data include journal articles and other publications, Federal and state agencies, and selected private agencies. When data from these sources are not available, field surveys may be required.

7.3 Calculation of Exposure

Calculation of doses of contaminants incurred by exposed populations is accomplished by integrating the results of the collection of chemical occurrence data (described in section 7.1) with the results of exposed population analyses (described in section 7.2).

To calculate the dose incurred, the concentration of the chemical in an environmental medium is first multiplied by the appropriate exposure coefficient. This calculation provides an estimate of the total amount of each chemical to which the population is exposed on a daily basis. However, this value must be adjusted to account for the extent to which each chemical is transferred across the membranes of the exposed organism (i.e., the extent of absorption). This adjustment is accomplished by multiplying total daily exposure values by an absorption factor. Usually, absorption factors cited in the toxicological literature are employed in this calculation. When empirically derived absorption factors are not available, an absorption factor of unity is applied, thereby generating a conservative (worst-case) estimate of the dose incurred. Finally, this whole-body dose estimate (mg/day) is converted to terms of mg of contaminant/kg of body mass/day by dividing it by the body mass representative of the receptor population.

Sample Calculation

The following hypothetical example illustrates the procedure for calculation of dose incurred:

A factory is located near a toxic dump site which has improperly disposed of large quantities of carbon tetrachloride (CCl_4). The factory draws water for drinking and industrial uses from a well which is found to contain 1.6 mg/L of CCl_4, and average air concentrations are 0.1 ppm (0.63 mg/m^3). The factory employs adult males who work 8-hour shifts doing light physical labor.

Calculation of Exposure Coefficient:

Since the adult males spend about one-half of their waking hours in the factory, the amount of water they consume may be estimated to be one-half of average total water consumption for adults (2 L/day/2 = 1 L/day). The inhalation exposure coefficient is calculated as follows:

$$(20\ \text{L/min})\ (60\ \text{min/hr})\ (8\ \text{hr/day}) = 9{,}600\ \text{L/day}$$

Converting to more convenient units, this becomes:

$$(9{,}600\ \text{L/day})\ (10^{-3}\ \text{m}^3/\text{L}) = 9.6\ \text{m}^3/\text{day}$$

Absorption Coefficients:

Inspection of published toxicokinetic information on CCl_4 reveals that gastrointestinal absorption is about 90% and absorption in the lung is about 30%, so the absorption coefficients are 0.90 and 0.30, respectively.

Dose Calculation:

A. Daily Dose via Water = (Exposure Coefficient)(Water Concentration)
= (1 L/day)(1.6 mg/L)
= 1.6 mg/day

Daily Dose via Air = (Exposure Coefficient)(Air Concentration)
= ($9.6\ m^3$/day)($0.63\ mg/m^3$)
= 6.0 mg/day

B. Absorbed Dose = (Daily Dose)(Absorption Coefficient)
For Water: (1.6 mg/day)(0.90) = 1.4 mg/day
For Air: (6.0 mg/day)(0.30) = 1.8 mg/day
Total Absorbed Dose: 1.4 mg/day + 1.8 mg/day = 3.2 mg/day

C. Conversion to mg/kg basis for adult (70 kg):

$$\frac{(3.2\ mg/day)}{(70\ kg)} = 0.05\ mg/kg/day$$

It is important in all caculations of exposure levels that assumptions regarding body weight, water and air intake, food consumption, etc., be clearly identified as such, to distinguish assumed average values from actual measured values.

7.4 Estimation of Past Exposure

In some instances, it may be desirable to obtain estimates of past exposure levels to certain chemicals. This can sometimes be accomplished by measuring the concentration of the chemical in body fluids or tissues of exposed organisms.

Following the absorption of a chemical by a particular organism, the chemical distributes throughout the organism and may accumulate in various parts of the organism. The site of accumulation may or may not be the major site of toxic action. When a chemical accumulates at a site other than the site at which it produces toxic action, the site of accumulation may be referred to as a "storage site." The accumulation in this storage site may serve as a protective mechanism, whereby the concentration of the chemical in the storage site keeps the concentration of the chemical in a vulnerable organ at a low level. However, the concentration of the chemical in the storage site is in equilibrium with the chemical in blood, and therefore it is slowly released into the circulation at a rate equal to the rate at which the chemical is excreted or removed from the blood. This tends to maintain the chemical in the blood for long periods of time. Some of the major storage areas for chemicals include plasma proteins, liver, kidney, fat and bone.

Therefore, measurements of toxic chemical concentrations in these storage areas are the most useful in terms of estimating past exposure levels.

It should be noted that this means of estimating past exposure levels has a major limitation. Not all chemicals accumulate to the same extent. Therefore, estimation of past exposure levels by measuring accumulated chemical concentrations is useful only for those chemicals that tend to accumulate. This method provides no useful information on past exposure to chemicals that are efficiently cleared from the body.

7.5 Prediction of Future Exposure

In order to predict future exposure scenarios, it is necessary to generate estimates of the direction of travel and future ambient concentrations of toxic chemicals within all environmental media. Some important physical/chemical properties of chemicals that may influence their environmental fate are presented in Table 7-4. Some important site characteristics that may influence environmental fate are presented in Table 7-5. Whenever mathematical models are employed to calculate expected future exposures, it is important to identify calculated values as such (to avoid confusion with measured values) and to cite the model employed. Population predictions, such as future population characteristics and distribution patterns, are also necessary so that they can be compared with future chemical distribution/concentration data in order to predict future exposure scenarios.

7.5.1 Environmental Stability

In analyses of the environmental fate of chemicals, all processes that are likely to lead to transformation of chemicals in various environmental media must be identified. In conducting environmental fate analyses, each transformation process is assessed and, when possible, rates of all processes are quantified. Usually, information regarding the significance and rates of transformation processes in various media is available in the technical literature. Some transformation processes likely to be important in assessing the fate of chemicals are described below.

Photolysis is the degradation of a chemical caused by exposure to light. This process may be important to chemicals in the atmosphere, in surface water of sufficient clarity for penetration of light, and on the surface of the soil. Photolysis typically results in the degradation of chemicals through rupture of covalent bonds. Factors determining the rate of photolysis include: flux of photons to the substrate media, chemical-specific light adsorption coefficients, and reactor yield constants (i.e., the efficiency of the degradation process with available photon energy).

Examples of photolysis include the splitting of hydrogen iodide by the reaction:

$$2HI \xrightarrow{\text{light}} H_2 + I_2$$

and the splitting of ketene (CH_2CO) into carbon monoxide (CO) and carbene (methylene) (CH_2).

TABLE 7-4 PHYSICAL AND CHEMICAL PROPERTIES THAT MAY INFLUENCE ENVIRONMENTAL FATE

Adsorption Coefficient

Biodegradation Rate

Boiling Point

Henry's Law Constant

Hydrolysis Rate

Melting Point

Molecular Weight

Octanol/Water Partition Coefficient

Photolysis Rate

Vapor Pressure

Volatilization Rate

Water Solubility

TABLE 7-5 SITE CHARACTERISTICS THAT MAY INFLUENCE ENVIRONMENTAL FATE

Ambient Moisture

Ambient Humidity Levels

Ambient Temperatures

Ambient Wind Velocity and Direction

Geologic Characteristics

Hydrologic Characteristics

Soil Characteristics

Topographic Features of Site and Surrounding Area

Vegetative Cover of Site and Surrounding Area

Watershed/Land Use Characteristics

Indirect photolysis is an important transformation process for many chemicals. This process involves intermediate compounds present in the environmental medium which undergo direct photolytic transformation and thereby become reactive with a toxic chemical (usually as a strong oxidizer). These intermediates subsequently react with a chemical, effecting its transformation. Indirect photolysis rates are governed by the concentration and light adsorption coefficient of the intermediate compound and the rate of its subsequent reaction with the toxic chemical.

Oxidation is the process of removing electrons from a chemical. These are accepted by another chemical (an oxidizing agent). Chlorine in drinking water and ozone in the atmosphere are two important oxidizing agents. Oxidation by oxygen is also important, and occurs in air, surface water, groundwater and soil. The rate of oxidation is determined by the concentration of the oxidizing agent, and its reaction rate constant with respect to the chemical.

For example, cupric ion is the oxidizing agent in the following reaction:

$$Fe + Cu^{2+} \rightarrow Fe^{2+} + Cu$$

Here, two electrons are transferred from the Fe atom to the Cu atom. Thus, the Fe atom is oxidized (becomes positively charged by the loss of two electrons) while the Cu atom receives the two electrons and becomes neutral (is reduced).

Hydrolysis is the splitting of a chemical bond by insertion of water. Hydrolysis may occur in the air, surface water, groundwater or soil. Hydrolysis is most important for organic chemicals which contain as part of their structure one or more easily polarized, electronegative groups covalently bonded to carbon atoms. Covalent inorganic chemical compounds also undergo hydrolytic transformation, usually at rapid rates. Hydrolysis is highly pH-dependent, and can be acid- or base-catalyzed.

The hydrolysis of an organic cyanide (nitrile) is shown below as an example of this type of reaction:

$$RC{\equiv}N \xrightarrow[H^+]{2H_2O} RCO_2H + NH_4^+$$

Chemical Speciation. Metallic chemical compounds undergo a wide range of transformation processes, forming and breaking down complexes with inorganic or organic ligands present in the substrate medium. These processes, referred to collectively as speciation, can occur in all environmental media. The final speciation of a metal in a given environment (i.e., its degree of association with various complexing agents) affects its bioavailability, solubility, volatility and sorption properties. For example, lead chloride ($PbCl_2$) is a fairly soluble form of lead (Pb^{2+}), and could easily dissolve in groundwater and escape from a toxic dump site. However, if the soil were rich in carbonates (CO_3^{-2}), the lead would form lead carbonate ($PbCO_3$), which is much less soluble!

$$PbCl_2 + CO_3^{2-} \rightarrow PbCO_3 + 2Cl^-$$

Bioconcentration and biotransformation are processes mediated by organisms. Bioconcentration is the uptake and accumulation of a chemical within the tissue of exposed organisms (fish, plants, birds, etc.), often within specific tissues or organs. Bioconcentration rates are a function of the uptake and excretion rates of the exposed organisms and the lipophilic properties of the chemical. Biotransformation is a collective term for the various reactions that occur as a result of metabolism by organisms such as soil bacteria. Biotransformation commonly causes the degradation and detoxification of toxic organic chemicals. Both bioconcentration and biotransformation are dependent on the metabolic rates of the organisms which, in turn, are functions of various environmental parameters, including the availability of nutrients, sunlight, moisture, ambient temperature, and pH. These transformation processes occur in the water, soil, groundwater and, to a limited extent, in the air.

An example of a biodegradation pathway is illustrated by the microbial degradation of the insecticide parathion. In this pathway, Pseudomonas stutzeri initially converts parathion to p-nitrophenol which is subsequently utilized by Pseudomonas acruginosa.

$$\text{Parathion} \xrightarrow{\text{P. stutzeri}} \text{p-Nitrophenol} \xrightarrow{\text{P. aeruginosa}} \text{Further Degradation Products}$$

Table 7-6 presents some information on the environmental stability (persistence) of selected chemicals from Table 7-2.

7.5.2 Environmental Mobility

The movement of chemicals in the environment is determined by the physical and chemical properties of the chemicals themselves and by the characteristics of the environment. Chemicals may move both within media (intramedia transport) and from one environmental medium to another (intermedia transport).

7.5.2.1 Intramedia Transport

Atmospheric transport of chemicals is governed by diffusion and by meteorological variables such as air currents (wind speed and direction), humidity, particulate concentrations and air temperature differentials. The size of the particle to which a toxic chemical is adsorbed is an important determinant of the extent to which it will be transported through the atmosphere.

In water, diffusion through the water and currents within the water are the principal routes of transport. Aquatic transport processes are, therefore, dependent on whether the water body is a lake, slow river, swift stream, estuary or ocean. For example, tidal movement is the overriding transport mechanism within an estuary, while thermal stratification may control movement within a deep lake or reservoir.

Chemicals are transported within soil principally by water flow. Aside from the rate of water flow, the key parameter is the interaction (sorption and

TABLE 7-6 ENVIRONMENTAL PERSISTENCE OF SOME COMPOUNDS THAT MAY OCCUR AT HAZARDOUS WASTE SITES

Chemical	Environmental Persistence
Volatiles:	
Vinyl chloride	Half life: 25 minutes
Chloroform	Half life in bluegill: 1 day Half life in water: 15 months
1,1,1-Trichloroethane	Hydrolysis half life: freshwater: 5-9 months marine : 39 months
Carbon tetrachloride	Half life in bluegill: 1 day
Dibromochloromethane	Half life projection: 274 years
Bromoform	Hydrolysis half life: 686 years
Chlorobenzene	Half life in water: 5.8 hours
Semi-Volatiles:	
bis(2-Chloroethyl) ether	Half life in bluegill: 4-7 days
2-Chlorophenol	Half life in bluegill: 1 day
2,4-Dimethylphenol	Complete biodegradation: 2 months Half life: less than 1 day
2,4-Dichlorophenol	Half life in aerated water: 8-9 days
Hexachlorocylcopentadiene	Half life: 11 days
2,4,6-Trichlorophenol	Half life in rat blood: 20 hours
2-Chloronaphthalene	Biomagnified in water
2,4-Dinitrophenol	No significant microbial degradation in 64 days
2,6-Dinitrotoluene	Greater than 64 days
Benzidine	Half life in dogs and rats: 68-88 hours

continued-

Table 7-6 - continued

Chemical	Environmental Persistence
Semi-Volatiles (continued):	
3,3'-Dichlorobenzidine	Long life in soil
Pesticides:	
Dieldrin	Half life in water: 723 days
Chlordane	Very persistent

Adapted from McNelis et al. (1984)

desorption) of the chemical with soil particles. Sorption is controlled by the characteristics of the chemical and by the type (i.e., organic content) of the soil. Site-specific information on soil composition should be used whenever possible to characterize soil transport when disposal sites or other areas of soil contamination are identified by location.

7.5.2.2 Intermedia Transport

The physical and chemical properties of a particular chemical typically have the greatest impact on intermedia transport. Transport from air to water is dominated by two process: (1) scavenging via liquid deposition (rain, snow), and (2) gravitational settling (dry deposition). Transport from water to air is mediated by air-stripping, aerosol formation and volatilization. Air-stripping and aerosol formation are most important under agitated conditions. The importance of volatilization for a chemical can be evaluated by assessing the chemical's solubility in water, vapor pressure, Henry's Law Constant, molecular weight and activity coefficient.

Transport from ground to air may also occur through volatilization from soil. Volatilization of organic chemicals from soil is strongly influenced by sorption phenomena and local frequency of precipitation. Volatilization from soil may also be influenced by seasonal differences in air and ground temperatures; volatility from soil is enhanced when the land is warmer than surrounding air. The other major ground-to-air transport process, resuspension of dusts, also is affected by precipitation as well as by wind speed. Chemicals are transported from air to ground by dry and wet deposition.

Transport between water and land is, to a large extent, controlled by the solubility and leachability of a chemical and the affinity of the chemical for the organic content of soil and sediment. Soil particles upon which chemicals are sorbed are washed into water bodies by surface runoff; once the chemical is in the water, equilibrium partitioning between water and suspended solids is established.

7.5.3 Environmental Partitioning

Partitioning refers to the relative distribution of a chemical among environmental compartments. Partitioning analysis is essentially a qualitative effort that is undertaken when the time or financial resources do not allow for a detailed analysis of the environmental transport and transformation of a chemical. A partitioning analysis may be based on examination of a chemical's physical and chemical properties, analogy with other chemicals whose fate is fairly well documented or mathematical modeling.

Examination of a chemical's physical and chemical properties can often permit an estimate of its environmental partitioning. The following are the most useful parameters for estimating the relative partitioning of a chemical:

- Vapor pressure is indicative of a chemical's ability to volatilize to the atmosphere.

- Water solubility of a chemical is indicative of its ability to be distributed by the hydrologic cycle. Water solubility also affects other degradation pathways (e.g., photolysis, hydrolysis, oxidation) and specialized transport pathways (e.g., volatilization from solution and washout from the atmosphere by rain).

- Octanol/water partition coefficient (K_{ow}) is indicative of a chemical's tendency to partition itself between an organic phase (e.g., fish, soil) and an aqueous phase. It is particularly important for estimating bioconcentration factors for aquatic life.

- Boiling point, in addition to serving as an indicator of the physical state of a chemical, provides an indication of its volatility.

- Henry's Law Constant is indicative of an organic chemical's tendency to volatilize from water to air.

- Adsorption coefficient (K_{oc}) is indicative of the extent to which a chemical partitions between solid and solution phases (e.g., water-saturated or unsaturated soil, runoff water, and sediment). Adsorption potential to soil/sediment is indicative of a chemical's environmental mobility.

Qualitative analysis of the fate of a chemical can also be made by analogy with other chemicals whose fate is well documented. If the chemical under study is structurally similar to a previously studied chemical, some parallel can be drawn to the environmental fate of the analogue.

A more detailed mathematical modeling analysis of partitioning may also be undertaken when resources permit and where environmental loadings (i.e., emission) data are available.

7.5.4. Population Predictions

As noted in section 7.2, performing an exposure assessment requires a characterization of exposed populations (number, age, sex, race, activities, etc.). Generally, predictions regarding future exposure scenarios require the same types of information on exposed populations as assessments of present exposures. That is, potentially exposed populations must be identified, enumerated and characterized, an activities analysis must be performed and exposure coefficients must be estimated. (Section 7.2 of this handbook provides descriptions of these aspects of exposed population analyses.) Based on this information, predictions regarding future exposure levels can be made.

7.6 Key Guidance and Implementation Documents

In 1984, the EPA proposed guidelines for use in conducting exposure assessments (49 FR 46304). These guidelines, which will be revised by EPA as necessary to reflect the benefit of experience, provide a general approach and framework for carrying out exposure assessments for specified pollutants. In some cases these guidelines may be useful only as a rough template; in other cases they may serve as a model which can be closely followed.

According to the guidelines, the five major topics to be addressed in most exposure assessments include:

- sources
- exposure pathways
- monitored or estimated concentration levels and duration
- exposed population(s)
- integrated exposure analysis

These five topics are appropriate for exposure assessments, whether the assessments are of a global, national, regional, local, site-specific or workplace-related nature. The topics are also appropriate for exposure assessments on new or existing chemicals and radionuclides. They are also applicable to both single media and multimedia assessments.

In general, the guidelines should be followed, to the extent possible, when an exposure assessment is conducted as a required element in a regulatory process or when an exposure assessment is conducted on a discretionary basis to support regulatory or programmatic decisions. Application of these guidelines to the conduct of exposure assessments results in a number of advantages including the following:

1. While there will usually be gaps in the data required to perform an exposure assessment, the use of the guidelines should help avoid inadvertent errors of omission.

2. Use of the guidelines should promote consistency among various exposure assessment activities. For example, use of the guidelines should result in consistency with respect to common physical, chemical and biological parameters and with respect to assumptions about typical exposure situations. Consistent presentation of the possible range estimates will enhance the comparability of results and allow for improvement of exposure assessment procedures through the sharing of common data and experiences.

3. The primary objective of most exposure assessments is to provide reliable data and/or estimates for subsequent use in conducting a risk assessment. The guidelines provide a common approach to format which simplifies the process of reading and evaluating exposure assessments, thereby facilitating the integration of the exposure and hazard assessments.

In addition to EPA's proposed exposure assessment guidelines, a number of other documents provide valuable information regarding the performance of exposure assessments. These documents are listed below.

1. Superfund Exposure Assessment Manual (Versar 1984)--This document presents an integrated methodology designed to guide the execution of the major components of qualitative and quantitative exposure assessments for hazardous waste sites.

2. Exposure Assessment Methodologies for Hazardous Waste Sites (McNelis et al. 1984)--This document presents a uniform approach to the estimation of exposures to important receptors from defined hazardous waste constituents via all important exposure pathways. The efficient use of available or historical information and the need for exploratory programs is emphasized. Applicable theoretical and empirical models which are accessible are described in sufficient detail to facilitate decisions regarding their use in designing and conducting an exposure assessment program.

3. Remedial Investigations Guidance Document (JRB 1984)--This document provides a detailed structure for field studies involving data collection for remediation decisions pertaining to hazardous waste sites.

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8.0 RISK ASSESSMENTS

Risk assessments are invaluable scientific tools for hazardous waste site investigations. Management decisions regarding the regulation of chemicals present at hazardous waste sites require a thorough assessment of the potential risk to human health and the environment resulting from exposure to the chemicals present. Hazardous waste site risk assessments may be utilized for many purposes including:

- Screening and determining response priorities.
- Ranking the relative toxicities of a large number of chemicals at a site.
- Estimating risk associated with various clean-up alternatives.
- Supporting the assertion that an enforcement action is warranted under CERCLA.

This chapter will discuss the structure of the overall risk assessment process and explain how information developed during the toxicity assessment and exposure assessment processes is combined to determine the magnitude of the problem (i.e. probability of risk) at a site. This chapter will also introduce some specific applications of risk assessment at hazardous waste sites, such as the Hazard Ranking System, Endangerment Assessments and Feasibility Studies.

8.1 Risk Assessment Process

In the simplest sense, population risks from toxic chemicals are a function of two measurable factors: toxicity and exposure. In order to cause a risk to humans, a chemical must be (1) toxic, (2) present in the environment at some significant level and (3) accessible for human exposure. Risk assessment is an evaluation and interpretation of the available scientific evidence on these points, providing a judgment and, if appropriate, an estimate of the probability that risk exists.

The risk assessment process consists of one or more of the following four steps:

- Toxicological Evaluation
- Dose-Response Evaluation
- Exposure Assessment
- Risk Characterization

The toxicological evaluation and dose-response evaluation collectively comprise the toxicity assessment process at a hazardous waste site. The risk assessment process is usually initiated by performing a toxicological evaluation and a preliminary exposure assessment on the chemical(s) of

concern. If the results of either are negative (i.e., the chemical is not toxic or is not present at some significant level), then it is not necessary to proceed with the risk assessment.

Figure 8-1 provides an overview of the risk assessment process.

8.1.1 Toxicological Evaluation

The toxicological evaluation should answer the question "Does the chemical have an adverse effect?" It is a qualitative evaluation of the scientific data to determine the nature and severity of actual or potential health hazards associated with exposure to a chemical substance. This step involves a critical evaluation and interpretation of toxicity data from epidemiological, clinical, animal and in vitro studies. Factors that should be considered during the toxicological evaluation for each contaminant include routes of exposure, types of effects, reliability of data, dose, mixture effects and the strength of evidence supporting the conclusions of the toxicological evaluation. The toxicological evaluation should also identify any known quantitative indices of toxicity (e.g., NOAEL, LOAEL, carcinogenic risk factors, etc.). The elements of the toxicological evaluation are discussed in detail in Chapters 3.0, 4.0 and 5.0 of this handbook.

8.1.2 Dose-Response Evaluation

Once the toxicological evaluation indicates that a chemical is likely to cause a particular adverse effect, the next step is to determine the potency of the chemical. The product of the dose-response evaluation is an estimate of the relationship between the dose of a chemical and the incidence of the adverse effect in the human population.

The dose-response evaluation for non-carcinogenic chemicals provides an estimation of the NOAEL or LOAEL and the margin of safety associated with the conditions of exposure of the human populations at potential risk.

The dose-response evaluation for carcinogenic chemicals provides an estimation of the probability or range of probabilities that a carcinogenic effect will occur under the conditions of exposure of the human populations at risk. These estimates of probability are derived using mathematical models of the dose-response relationship.

The dose-response relationship is introduced in chapter 3.0 of this handbook and discussed in detail in chapter 6.0.

8.1.3 Exposure Assessment

The exposure assessment describes the likely degree of human exposure to a chemical of concern at a hazardous waste site. The objectives of a site-specific exposure assessment are to identify actual or potential routes of exposure, characterize the population exposed and determine the extent of the exposure at a site. The product of the exposure assessment process, as detailed in chapter 7.0, is an estimation of exposure levels or doses incurred for chemicals of concern at the site.

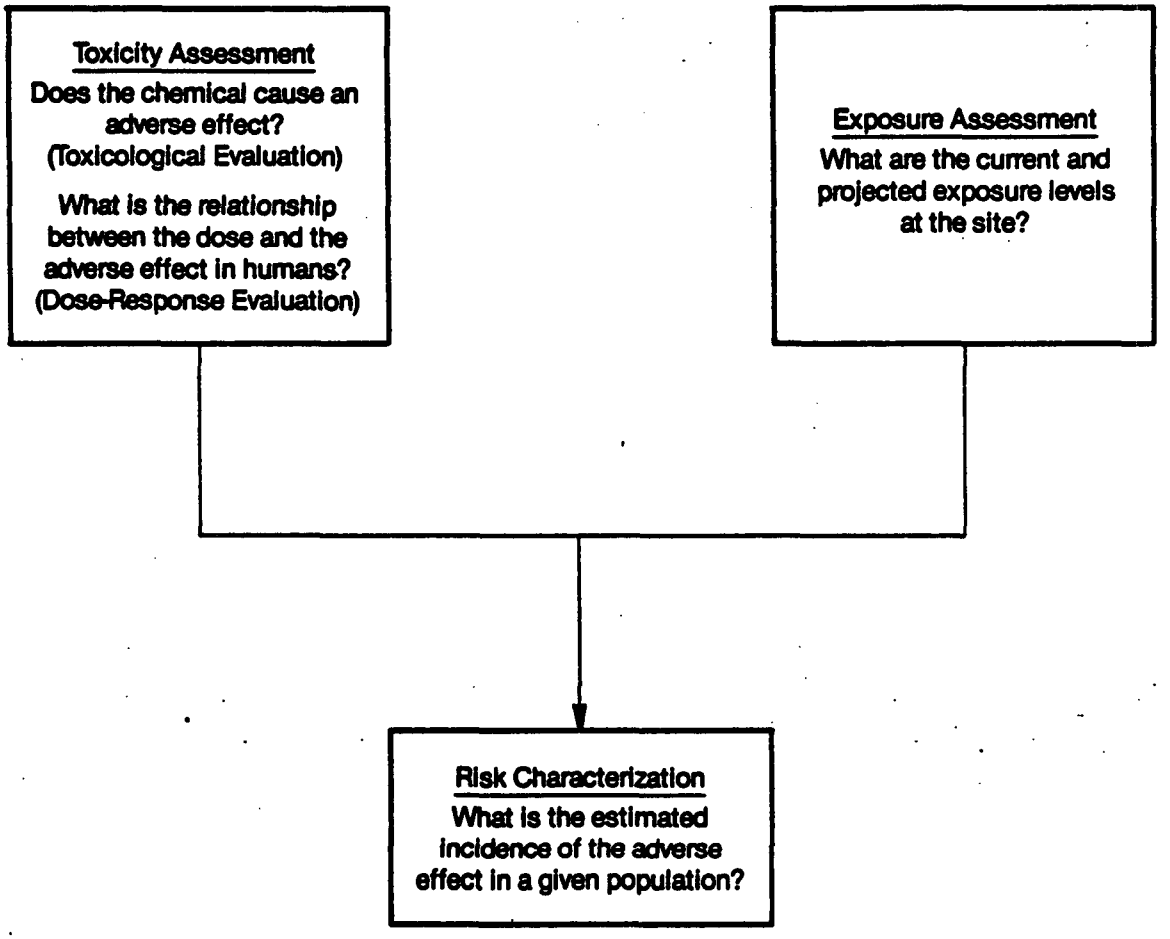

FIGURE 8-1 RISK ASSESSMENT PROCESS AT HAZARDOUS WASTES SITES

The risk assessment process at hazardous waste sites is an evaluation and interpretation of the available scientific evidence on the toxicity and exposure potential for a chemical of concern. The results of the Toxicity and Exposure Assessments are integrated to yield a complete characterization of risk at the site.

8.1.4 Risk Characterization

The final step in risk assessment, risk characterization, is the process of estimating the incidence of an adverse health effect under the conditions of exposure described in the exposure assessment. It is performed by integrating the information developed during the toxicity assessment (toxicological evaluation and dose-response evaluation) and the exposure assessment to yield a complete characterization of risk for a given hazardous waste site.

The final risk assessment should include a summary of the risks associated with a site and such factors as the weight of evidence associated with each step of the process, the estimated uncertainty of the component parts, the distribution of risk across various sectors of the population and the assumptions contained within the estimates.

8.2 Risk Characterization at Hazardous Waste Sites

This section discusses the characterization of risk at hazardous waste sites in greater detail. Two discrete steps are required to fully characterize potential risks from exposure to toxic chemicals at hazardous waste sites:

1. Characterize noncarcinogenic risks.
2. Characterize carcinogenic risks.

Typically, human populations are exposed to a mixture of chemicals at a hazardous waste site rather than a single chemical. This phenomenon occurs when a series of unrelated chemicals are placed in the same area for disposal or storage, eventually come in contact with each other, and are released as a mixture to the environment. The EPA has published "Proposed Guidelines for the Health Risk Assessment of Chemical Mixtures" (USEPA 1985) which provides guidelines for assessing the effects of multiple toxicant or multiple carcinogen exposures. The following discussion of risk characterization at hazardous waste sites is consistent with these proposed guidelines.

8.2.1 Characterization of Noncarcinogenic Risks

Risk assessment for single noncarcinogenic compounds at a site generally results in the derivation of an exposure level that is considered acceptable or is not expected to cause adverse effects. This exposure level may be expressed in a variety of ways such as the Acceptable Daily Intake (ADI), an Ambient Air Standard, or Water Quality Criteria. The term "acceptable level" (AL) will be used here to indicate any derived criteria, health standards or advisories.

Characterizing risks from noncarcinogenic compounds involves comparing the expected exposure level (E) to the AL. The resultant ratio (referred to as the hazard index, HI = E/AL) is a numerical indicator of the transition between acceptable and unacceptable exposure levels. When making this comparison it is important to ensure that the units for the exposure level and acceptable level are the same. It may be necessary to apply a scaling factor or exposure coefficient to the estimated exposure level, as appropriate, to

ERRATA (PAGES 8-4, 8-5)

Page 8-4, Section 8.2.1, Paragraph 2

Change the first sentence to read: "One method that toxicologists use to characterize noncarcinogenic risks involves comparing the expected exposure levels (E) to the "acceptable level" (AL) (USEPA 1985)."

Page 8-5, Paragraph 2

Add this paragraph immediately before Section 8.2.2: "This method of characterizing noncarcinogenic risks has not been adopted as official EPA policy. This is an oversimplification of the procedures used to estimate noncarcinogenic risks associated with exposure to multiple chemicals. There are many assumptions made in developing these calculation procedures which the user should be aware of. These assumptions are detailed in the proposed mixture guidelines (USEPA 1985)."

standardize the units in the ratio. When HI > 1 there is a potential health risk to the human populations associated with exposure to that chemical. Therefore, the HI of a mixture may be defined as:

$$HI = E_1/AL_1 + E_2/AL_2 + \ldots + E_i/AL_i$$

where:

E_i = exposure level to the i^{th} toxic chemical
AL_i = maximum acceptable level for the i^{th} toxic chemical

The EPA guidelines assume dose additivity for exposure to multiple noncarcinogens. This assumption of additivity is most properly applied to chemicals that induce the same effect by the same mechanism. Thus, it may be desirable to group the chemicals by type of adverse effect and derive a separate HI for each chemical group to avoid overestimating risk. As with single chemicals, when HI approaches unity, the potential for risks increases.

8.2.2 Characterization of Carcinogenic Risks

Carcinogenic risks are estimated as the probability or range of probabilities that a specific adverse effect will occur under the conditions of exposure of the human population at risk. One way of expressing this numerical estimate of risk is as a carcinogenic potency factor or "unit cancer risk" which is defined as the excess risk due to a continuous lifetime exposure to one unit of carcinogen concentration. This carcinogenic potency factor can be used to convert estimated intake directly to incremental risk. This relationship may be defined as:

$$P = dB$$

where:

P = incremental risk
d = exposure level (mg/kg/day)
B = carcinogenic potency factor ($mg/kg/day^{-1}$)

For multiple compounds, the above equation may be generalized to:

$$P_j = \Sigma d_j B_j$$

Total incremental risk at the site is then equal to $P_1 + P_2 + \ldots + P_j$

This equation is based on the following assumptions:

- Individual intakes are small.
- Actions by several carcinogens in mixture are independent (i.e., not synergistic or antagonistic).
- Route-specific cancer risks are additive.
- Total risk for the site is additive.

Therefore, characterizing the potential risks at a site from carcinogenic chemicals involves determining the incremental risk associated with exposure to each potential carcinogen at the site and summing the incremental risks to determine the total risk at the site.

For a detailed discussion of the uncertainties, assumptions and limitations associated with risk assessments of chemical mixtures refer to the proposed EPA guidelines (USEPA 1985).

8.3 Application of Risk Assessment at Hazardous Waste Sites

Risk assessments at hazardous waste sites may be performed for many reasons. Examples of some different applications of risk assessment are briefly described below. The Hazard Ranking System, which is described in Appendix A of the National Contingency Plan, is a risk assessment approach to screen hazardous waste sites for inclusion on the National Priorities List.

The Public Health Assessment component of the Remedial Investigation and Feasibility Studies at Superfund sites prescribes an initial risk assessment methodology for selecting indicator chemicals at a site. Chemicals are screened and assigned an "indicator score" based on concentrations present at the site and their toxicity. Chemicals with the top scores are designated as "indicator chemicals" for all analyses at the site. This allows subsequent analyses at the site to focus on five to ten "indicator chemicals" rather than potentially thousands of individual chemicals that may be present at a site.

The goal of the Feasibility Study at a Superfund site is to develop and evaluate alternative remedial actions. This evaluation involves an analysis of the effectiveness (i.e., risk assessment) of each proposed alternative at reducing potential public health risks at the site.

Finally, any time EPA initiates an enforcement action against a responsible party at a hazardous waste site, it must support and justify its actions with an endangerment assessment. The endangerment assessment, which is a type of risk assessment, evaluates the magnitude and probability of actual or potential harm to public health and welfare or the environment from the actual or threatened release of hazardous substances from a site. A more detailed discussion of the endangerment assessment process can be found in the Endangerment Assessment Handbook (ICAIR 1985).

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9.0 SUMMARY OF TOXICOLOGICAL INFORMATION ON DIOXINS AND FURANS

This chapter provides a summary of current toxicological information on dioxins and furans. This class of compounds was chosen for consideration here because of their high inherent toxicity and because they are often found as contaminants at hazardous waste sites. This chapter is similar to the sort of summary which might be prepared by a toxicologist for a hazard assessment, and therefore it is somewhat more technical and detailed than chapters 1 to 8 of this handbook. A nontoxicologist would not be expected to prepare a summary of this sort, but should, on the basis of the information presented in chapters 1 to 8 of the handbook, be able to understand and apply this information.

9.1 Chemical Properties and Environmental Stability

Dioxins are a class of compounds characterized by a structure comprising two benzene rings interconnected to each other through a pair of oxygen atoms (dibenzo-p-dioxin). Individual dioxins differ from each other with respect to substituents present on the benzene rings. Various chlorine-substituted dioxins have been found to be highly toxic, the degree of toxicity depending on the substitution pattern. Especially toxic is 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD).

Dioxins are stable and highly persistent in the environment. Under laboratory conditions, soil samples incubated with 1, 10 or 100 ppm of dioxin contained 75% to 85% of the original material up to 160 days after treatment (Kearney et al. 1972). In another study (Young 1974), soil samples from areas sprayed 10 years earlier contained dioxin levels of 10, 11, 30 and 710 ppt (parts per trillion).

Some studies indicate that dioxins are subject to photodecomposition or biodegradation. Crosby and Wong (1977) applied herbicide formulations containing chlorodioxins to glass petri dishes, rubber plant leaves or to loam soil. About 60% to 70% of the compound was lost in approximately six hours. Esposito et al. (1980) reviewed studies on the biodegradability of dioxins in aqueous and soil environments. However, due to problems of extracting dioxins from test soils, it was concluded that such transformation has not been conclusively established. Chlorodioxins (e.g., TCDD, 2,7-DCDD and DCDD) undergo photodecomposition in nonpolar solvents, but photodecomposition is very slow in aqueous solutions or in wet or dry soils (Crosby et al. 1971).

Dioxins are produced as unwanted contaminants in the industrial manufacture of chlorophenols and their derivatives. The primary sources of dioxins in the environment are the manufacturing and disposal sites of these industries. Important exposure routes for dioxins include dermal contact or ingestion of contaminated water or soil. Dioxins are lipophilic and tend to bioaccumulate in fatty tissue, so ingestion of fish (especially bottom-feeders) from contaminated waters is a significant human exposure route.

Furans are class of polyhalogenated aromatic compounds which are similar to dioxins. For example, 2,3,7,8-tetrachlorodibenzo-furan (TCDF) is similar in structure and toxicity to the chlorinated dioxin (TCDD) isomer. Very few

pertinent studies on polychlorinated dibenzofurans (PCDFs), including TCDF, have been located. Summaries of the available studies are included in the appropriate sections of this chapter, but several sections (i.e., Mutagenic and Carcinogenic Studies, Quantitative Indices of Toxicity) have no specific references to PCDFs.

9.2 Summary of Health Effects Data

Exposure to TCDD causes thymic atrophy, decreased body weight, liver damage, skin lesions (chloracne), renal function impairment, hematologic effects, adrenal atrophy, reproductive system damage, immunosuppression, fetotoxic, teratogenic and mutagenic effects and cancer. The chemical TCDD is an extremely toxic substance, with an LD_{50} value reported as low as 0.6 µg/kg following oral administration.

The effects of greatest concern associated with exposure to TCDD are liver damage, thymic atrophy, fetotoxic and teratogenic effects and carcinogenic potential.

Reported manifestations of TCDF toxicity are similar, including general edema (accumulation of fluid), liver effects, skin lesions (chloracne), thymic atrophy, immunosuppression and lethality.

9.2.1 Noncarcinogenic Studies

9.2.1.1 Acute Effects

Various chlorodioxins have produced lethality following exposures for short durations via oral administration in the rat, mouse, guinea pig, rabbit, monkey and dog (Schwetz et al. 1973, McConnell et al. 1978a, 1978b). Estimates of LD_{50} values for guinea pigs and mice exposed to various chlorodioxins range from 283.7 µg/kg to 5,000 µg/kg. These values are summarized in Table 9-1. The most toxic chlorodioxin is TCDD, which has been shown to induce lethality in a variety of animals (rat, guinea pig, mouse, monkey, rabbit) at dose levels between 0.6 µg/kg and 190 µg/kg following oral administration. The dermal LD_{50} value was 270 µg/kg. Estimates of LD_{50} values for this compound through various routes of exposure are summarized in Table 9-2.

A summary of studies providing data on the sub-lethal effects of acute exposure to TCDD is presented in Table 9-3. The effects were reported to occur following single exposures ranging from 0.1 to 300 µg/kg in four animal species (rat, guinea pig, chicken, mouse). Liver damage is the most consistently reported effect in most species. Rats receiving a single dose of 100 µg/kg of TCDD showed severe liver damage, thymic atrophy and jaundice (Gupta et al. 1973). In the same study, thymic and liver damage of lesser severity occurred at lower dose levels (25 and 50 µg/kg). In another study (Greig et al. 1973), rats exposed to TCDD (300 µg/kg) exhibited jaundice, multinucleated parenchymal cells of the liver and gastric hemorrhage. Histopathologic liver changes were observed five weeks after single oral doses

TABLE 9-1 ACUTE TOXICITIES OF VARIOUS CHLORODIOXINS BY ORAL EXPOSURE

Chlorine Positions	LD_{50}, µg/kg	
	Guinea Pig	Mouse
2,8	300,000	
2,3,7	29,400	3,000
2,3,7,8	0.6-2.0	284
1,2,3,7,8	3.1	337
1,2,4,7,8	1,125	5,000
1,2,3,4,7,8	72.5	825
1,2,3,6,7,8	70-100	1,250
1,2,3,7,8,9	60-100	1,440

Adapted from McConnell et al. (1978b).

TABLE 9-2 ACUTE LETHALITY OF TCDD

Species	Sex	Route of Exposure	LD_{50}, µg/kg	Reference
Rat	M	Oral	22	Schwetz et al. (1973)
Rat	M,F	Oral	100	Harris et al. (1973)
Rat	F	Oral	190	Greig et al. (1973)
Guinea Pig	M	Oral	0.6	Schwetz et al. (1973)
Rabbit	M,F	Oral	115	Schwetz et al. (1973)
	M,F	Dermal	272	
	M,F	Intra-peritoneal	252	
Mouse	M	Oral	114	Vos et al. (1974)
Mouse	M	Oral	283.7	McConnell et al. (1978b)
Monkey	F	Oral	<70	McConnell et al. (1978a)

Adapted from Esposito et al. (1980) and NTP (1982a,b).

TABLE 9-3 EFFECTS OF TCDD IN ANIMALS FOLLOWING ACUTE EXPOSURE

Species	Dose (µg/kg)	Route	Effects	Reference
Rat	25, 50 or 100		Liver damage, thymic atrophy	Gupta et al. (1973)
	100		Jaundice, 43% mortality	
Guinea Pig	3.0		Hemorrhage, adrenal atrophy, cellular depletion of lymphoid organs, 90% mortality	Gupta et al. (1973)
Rat	300		Weight loss, gastric hemorrhage, liver damage (cellular changes), jaundice	Greig et al. (1973)
Chicken	25 - 50		Pericardial edema	Greig et al. (1973)
Rat	10	Oral	Hematologic effects	Weissburg and Zinkl (1973)
Rat	0.1	Intra-peritoneal	Increased liver weights (1972)	Cunningham and Williams
Rat	50	Oral	Liver damage	Harris et al. (1973)
Mouse	50	Oral	Liver damage	Harris et al. (1973)
Rat	10		Decreased renal function	Anaizi and Cohen (1978)
Rat	10, 25, or 50		Decreased renal function	Hook et al. (1978)

Adapted from NAS (1977), NTP (1982) and Esposito et al. (1980).

of TCDD as low as 50 µg/kg were administered to male and female CD[(a)] rats, and one week after a single dose of 50 µg/kg was administered to female CD-1[(a)] mice (Harris et al. 1973). Increased liver weights were found in male Wistar rats seven days after single intraperitoneal doses of 0.1 µg/kg (Cunningham and Williams 1972).

Acute exposure to TCDF appears to cause effects similar to, but less severe than, the effects caused by TCDD. A summary of the effects of acute exposure to TCDF is presented in Table 9-4.

Acute toxicity studies show that TCDF is toxic in chickens and guinea pigs. McKinney et al. (1976) reported that Leghorn chickens given 5 µg/kg TCDF showed fluid accumulation, enlarged hearts and reduced size of thymuses and spleens. The compound also caused lethality.

Harltey guinea pigs administered TCDF showed oral LD_{50} values of 5 to 10 µg/kg, with a mean time to death of 11 days. As a comparison, TCDD was lethal to 50% of the test guinea pigs following administration of 2.0 µg/kg. Death occurred within 20.6 days (Moore et al. 1976).

Mice and rats were found to be resistant to TCDF (Moore et al. 1976). Rats administered TCDF at a dose of 1,000 µg/kg failed to show any toxic effects. Male mice administered a single oral dose of TCDF up to 6,000 µg/kg did not exhibit adverse effects, except for mild effects on the liver. Mice administered subcutaneous injections of TCDF (6,000 µg/kg) showed an increase in liver weights as well as liver-to-body-weight ratio. In contrast, TCDD caused adverse effects at levels of 0.2 µg/kg in mice (Kociba et al. 1976) and 0.1 µg/kg in rats (Vos et al. 1974).

9.2.1.2 Subchronic and Chronic Effects

Longer exposures to TCDD reportedly cause effects similar to those following acute exposure including thymic atrophy, liver damage, renal function impairment, hematological effects, hormonal alterations, immunosuppression, nervousness and irritability. A summary of major studies providing dose-response effects is presented in Table 9-5. Those studies providing dose-response data indicating the greatest sensitivity to TCDD are described below.

Doses as low as 0.1 µg/kg/day caused a slight degree of liver degeneration in rats in a subchronic 13-week (5 doses per week) study (Kociba et al. 1976). Dose levels of 1.0 µg/kg/day increased levels of serum bilirubin and alkaline phosphatase and caused pathologic changes in the livers of rats. A NOAEL of 0.01 µg/kg TCDD was reported for rats.

(a) A series of letters, numbers or a combination of both indicates a specific strain (i.e., a race or stock) of animals that all have common hereditary characteristics.

TABLE 9-4 EFFECTS OF TCDF IN ANIMALS FOLLOWING ACUTE EXPOSURE

Species	Dose (µg/kg)	Route	Effects	Reference
Chicken	5, 25	Oral	Enlarged heart, reduced size of thymus, spleen	McKinney et al. (1976)
Guinea Pig	5 to 10	Oral	LD_{50}	Moore et al. (1976)
Rat	1,000	Oral	No toxic effects	Moore et al. (1976)
Mouse	6,000	Oral	Mild liver toxicity	Moore et al. (1976)
Mouse	6,000	Subcutaneous	Increased liver weight and liver-to-body-weight-ratio	Moore et al. (1976)

Adapted from Esposito et al. (1980), NTP (1982).

TABLE 9-5 NOAEL AND LOAEL VALUES OBTAINED FROM SUBCHRONIC AND CHRONIC ORAL TOXICITY STUDIES OF 2,3,7,8-TCDD

Species	Duration of Exposure	Endpoints	NOAEL µg/kg/day	LOAEL µg/kg/day	Reference
Rat	13 weeks	Decreased body weight, liver pathology	0.01	0.1	Kociba et al. (1976)
Rat	13 weeks	Toxic hepatitis	0.07	0.14	NTP (1980a)
Rat	16 weeks	Elevated porphyrin levels	0.0014	0.014	Goldstein et al. (1982)
Rat	28 weeks	Fatty changes in the liver, decreased body weight	ND[(a)]	0.014	King and Roesler (1974)
Mouse	13 weeks	Toxic hepatitis	ND	0.014	NTP (1980a)
Monkey	36 weeks	Pancytopenia	ND	2	Allen et al. (1977)
Rat	104 weeks	Degenerative and necrotic changes in the liver	0.001	0.01	Kociba et al. (1978, 1979)
Rat	104 weeks	Toxic hepatitis	0.0014	0.007	NTP (1980a)
Mouse	104 weeks	Dermatitis and amyloidosis	ND	0.001	NTP (1980a)

(a) Not determined.

Increased mortality was observed in female Sprague-Dawley rats maintained for 2 years on a diet that provided a TCDD dose of 0.1 µg/kg/day, while no increased mortality was observed in male rats at this dose or in animals receiving doses of 0.01 or 0.001 µg/kg/day (Kociba et al. 1978, 1979). At termination of the study, gross and histologic examination indicated that the liver was the most severely affected organ, with degenerative, necrotic and inflammatory changes observed. Increases in urinary excretion rates of the metabolites, coproporphyrin and uroporphyrin, in the high and middle dose females were consistent with the observed liver damage. Primary liver injury was dose-related with the lowest dose representing a NOAEL.

When TCDD was administered by gavage (by stomach tube) in corn oil-acetone (9:1) at dose levels of 0.0, 0.5, 0.05 or 0.01 µg/kg/week, toxic hepatitis was observed in male Osborne-Mendel rats at incidences of none out of 74 tested (0/74), 14/50, 0/50 and 1/50 and in female rats at incidences of 0/75, 32/49, 1/50 and 0/50 (NTP 1980a). No other non-neoplastic lesions were observed, even though extensive histologic examinations were performed. The two preceding studies support a NOAEL for rats of ≈0.001 µg/kg/day, with a LOAEL of 0.05 µg/kg/day, and a frank effect level (FEL) for liver injury and possibly decreased survival of 0.5 µg/kg/day.

Non-neoplastic effects of chronic exposure to TCDD in mice were described in studies investigating the carcinogenic potential of TCDD. In a National Toxicology Program (NTP) (1980a) bioassay, histologic examinations were performed on B6C3F1[(a)] mice treated biweekly with TCDD by gavage in corn oil-acetone (9:1) for 104 weeks followed by an additional 3-week observation period. The doses for male animals were 0.0, 0.01, 0.05 and 0.5 µg/kg/week, and for female animals, 0.0, 0.04, 0.2 and 2.0 µg/kg/week. The only non-neoplastic adverse effect was toxic hepatitis, which occurred in males at incidences of 0/73, 5/49, 3/49 and 44/50, and in females at incidences of 0/73, 1/50, 2/48 and 34/47, respectively, in the control, low, medium and high dose groups. In another study, weekly dosing by stomach tube of TCDD at doses of 0.0, 0.007, 0.7 or 7.0 µg/kg/week for one year resulted in amyloidosis (deposition of amyloid) of the kidney, spleen and liver, and dermatitis at the time of death in male Swiss mice (Toth et al. 1978, 1979). The incidences of these effects in the control, low, medium and high dose groups, respectively, was 0/38, 5/44, 10/44 and 17/43. In the high dose group, the amyloidosis was extensive and considered to be the cause of early mortality. Severe toxic effects were observed at doses of 1 µg/kg/day (early mortality) and 0.28 to 0.07 µg/kg/day (toxic hepatitis), while a LOAEL for dermatitis and amyloidosis of 0.001 µg/kg/day was reported.

Several epidemiologic studies and case reports involving dioxin exposure in human subjects have been reported (Esposito et al. 1980). Effects observed include skin lesions (chloracne, prophyria cutanea tarda), liver function impairment and neurological disorders (polyneuropathy, peripheral nerve damage). An International Agency for Research on Cancer (IARC 1982) evaluation of human exposure data concluded that these studies are inadequate since they involve multiple chemical exposures.

The chemical TCDD has been reported to be fetotoxic and teratogenic when administered alone or in combination with other chemicals. Various studies have been identified in the available literature based on TCDD exposure alone. Effects observed were kidney anomalies, intestinal hemorrhage, general edema, cleft palate and fetal death. Adverse effects on reproduction were also reported.

Intestinal hemorrhage, general edema and a reduction in fetal weights were reported in rats following the administration of 0.125 µg/kg/day in studies by Sparschu et al. (1971). In the same studies, the number of fetuses was reduced and fetal death increased at 0.5 µg/kg/day. No structural malformations were reported at 0.03 µg/kg/day. Courtney and Moore (1971) reported cleft palate and kidney abnormalities in offspring from mice administered TCDD at doses of 1.0 µg/kg or 3.0 µg/kg. Similarly, kidney malformations were reported by the same authors in offspring from rats which received subcutaneous injections of 0.5 µg/kg/day on day 9, 10, or 13 and 14 of gestation. .

Murray et al. (1979) completed a three-generation reproduction study using Sprague-Dawley rats fed TCDD continuously in the diet (at levels of 0, 0.001, 0.01, and 0.1 µg/kg/day). Significant decreases were observed in fertility, litter size, gestation survival, postnatal survival, and postnatal body weight for the 0.01 and 0.1 µg/kg groups. No apparent adverse effect on reproduction was seen at the 0.001 µg/kg dose level.

Nisbet and Paxton (1982) reevaluated the data of Murray et al. (1979) using different statistical methods. From this reevaluation it was concluded that TCDD significantly reduced the gastational index, decreased fetal weight, and increased liver-to-body weight ratios and the incidence of dilated renal pelvis in both lower dose groups. Nisbet and Paxton (1982) concluded that the dose of 0.001 µg/kg/day was not a NOAEL but a LOAEL in this study.

Luster et al. (1980) examined bone marrow, immunologic parameters, and host susceptibility in B6C3F1[(a)] mice following pre- and postnatal exposure to TCDD. Doses of 0, 1.0, 5.0 or 15.0 µg/kg TCDD/body weight were given to dams on day 14 of gestation and to offspring on days 1, 7 and 14 following birth. Neonatal body, liver, spleen, and thymus weights were decreased and bone marrow toxicity occurred in the 5.0 and 15.0 µg/kg groups. Red blood cell counts, hematocrits, and hemoglobin were decreased at the highest dose tested.

Oishi et al. (1978) studied the subchronic toxicity of polychlorinated dibenzofurans (PCDFs) in rats. Test animals were fed diets containing 1 or 10 ppm PCDF for four weeks. The PCDF markedly depressed normal body weight gain. In rats fed diets containing 10 ppm of PCDF, significantly decreased thymus, ventral prostate, and seminal vesicle weights were found, and the animals developed chloracne-like lesions on the ears within three weeks. Hemoglobin and hematocrit values (percentage of the volume of a blood sample occupied by cells) were decreased in rats fed either diet.

9.2.2 Mutagenic and Carcinogenic Studies

Studies on the mutagenicity of TCDD have produced conflicting results. The chemicals TCDD reportedly produces mutagenic effects in various bacterial

systems. However, results were negative in tests employing other indicator test systems, including cytogenetic (chromosome analysis) tests and dominant lethal assays. Hussain et al. (1972) reported that TCDD (2 µg/mL) increased the incidence of reverse mutations in Escherichia coli. Similarly, TCDD (dose not specified) was reported to be mutagenic without metabolic activation in Salmonella typhimurium test strain TA 1532.[a] Green et al. (1977) gave 0.25, 0.5, 1.0, 2.0, or 4.0 µg/kg TCDD (dissolved in 1 part acetone: 9 parts corn oil) by gavage to male and female Osborne-Mendel rats twice weekly for 13 weeks and observed an increased incidence of chromosomal breaks in female rats dosed with 4 µg/kg and in males dosed with 2 µg/kg or 4 µg/kg.

Mutagenic effects (with or without metabolic activation) were not detected when Geiger and Neal (1981) examined the mutagenicity of TCDD (up to 20 µg/plate) using the S. typhimurium test strains[a] TA1535, TA100, TA1538, TA98, and TA1537.

The carcinogenic potential of TCDD has been studied extensively. A summary of the results of selected comprehensive studies is presented in Table 9-6. The results of these studies show that TCDD-exposed animals exhibited malignant lesions involving multiple organ systems including accessory digestive organs (liver), endocrine (thyroid, adrenal), renal, reproductive (testes), and nasal structures. Representative studies are described below.

Groups of ten male Sprague-Dawley rats were fed a diet containing TCDD for 78 weeks at dose levels ranging from 1 ppt to 500 ppt or 1 ppb to 1,000 ppb (Van Miller et al. (1977). These dose levels represent approximate weekly dose levels of 0.0003 to 0.1 µg/kg or 0.4 to 500 µg/kg. Animals exposed at 5 ppt, 50 ppt, 500 ppt or 5 ppb showed an overall incidence of neoplasm of 38% (23/60). No neoplasms were reported or observed following exposure to 1 ppt TCDD. In the 5 ppt group, 5/10 animals had six neoplasms (earduct carcinoma, lymphocytic leukemia, adenocarcinoma, malignant histiocytoma (with metastases), angiosarcoma and Leydig-cell adenoma). Neoplasm were also observed in the following groups: at 50 ppt, three in 3/10; at 500 ppt, four in 4/10; at 1 ppb, five in 4/10; at 5 ppb, ten in 7/10. Neoplasms were not observed in the controls. Rats administered TCDD at 50, 500 or 1,000 ppb exhibited 100% mortality by the fourth week.

In another study (Kociba et al. 1978), groups of 100 Sprague-Dawley rats (50 males and 50 females) received diets containing TCDD at 0, 22, 210, or 2,220 ppt (equivalent to 0.0, 0.001, 0.01 and 0.1 µg TCDD/kg/day) for two years . Administration of 0.01 µg/kg/day increased the incidence of hepatocellular hyperplastic nodules (female: 18/50 versus 8/86 controls) and focal alveolar hyperplasia in the lungs ($P<0.05$). Dietary intake of 0.1 µg/kg/day increased the incidence of hepatocellular carcinomas (female: 11/49 versus 1/86) and squamous cell carcinomas of the lung (female: 7/49 versus 0/86), hard palate/nasal turbinates (male: 4/50 versus 0/85; female: 4/49 versus 0/86), and tongue (male: 3/50 versus 0/85) ($P<0.05$). Also increased in frequency by the 0.1 µg TCDD/kg/day were adenoma of the adrenal cortex (male) and hepatocellular hyperplastic nodules (female).

TABLE 9-6 SUMMARY OF CARCINOGENIC EFFECTS OF TCDD

Species/Sex (Number)	Dose	Duration	Route	Effects	Reference
Rat/M (10)	1 ppt	78 wks	Diet	No neoplasm.	Van Miller et al. (1977)
Rat/M (10)	5-500 ppt			Ear duct carcinoma, benign tumor of the kidney and testes, lymphocytic leukemia, skin carcinomas and benign muscle tumors.	
Rat/M (10)	1-5 ppb			Cholangiocarcinoma of liver, squamous cell tumor of lung, angiosarcoma in skin, glioblastoma in brain, malignant histiocytomas in peritoneum.	
Rat/ M (50)	0.001 µg/kg	2 years	Diet	No significant increase in tumors.	Kociba et al. (1978)
F (50)	0.01 µg/kg			Liver cancer.	
	0.1 µg/kg			Liver cancer, squamous cell carcinoma of the lung, hard palate/ nasal turbinates, or tongue (P=0.05).	
Mouse/F (30)	0.015 µg/kg/wk	99-104 wks	Dermal	Fibrosarcoma in integumentary system (8/27, P=0.007).	NTP (1982b)
Rat/M (50)	0.5 µg/kg/wk	104 wks	Gavage	Follicular cell adenomas of thyroid (10/50, P=0.001).	NTP (1982a)
Rat/F (50)	0.5 µg/kg/wk	104 wks	Gavage	Neoplastic nodules of the liver (12/49, P=0.006).	

continued-

Table 9-6 - continued

Species/Sex (Number)	Dose	Duration	Route	Effects	Reference
Mouse/M&F	2.0 µg/kg/wk	104 wks	Gavage	Hepatocellular carcinoma (17/50, P=0.002 in M); (6/47, P=0.14 in F).	NTP (1982a)
Mouse/F	2.0 µg/kg/wk	104 wks	Gavage	Follicular cell adenomas of the thyroid (5/46, P=0.009)	

Adapted from Esposito et al. (1980), NTP (1982a,b).

The NTP has performed a chronic bioassay in both Osborne-Mendel rats and B6C3F1 mice to determine the carcinogenicity of a mixture containing 31% 1,2,3,6,7,8-HCDD and 67% 1,2,3,7,8,9-HCDD (NTP 1980). Other dioxins, including di-, tri-, tetra-, pentachlorodibenzo-p-dioxin, and bromopentachlorodibenzo-p-dioxin, were present at less than 0.09%. The mixture was administered to the test animals in corn oil-acetone (9:1) by gavage two times/week. The male and female rats, and the male mice received doses of 0.0, 1.25, 2.5 or 5 µg/kg/week, and the female mice received doses of 0.0, 2.5, 5.0 or 10 µg/kg/week. Treatment was continued for 104 weeks followed by a 3-4 week observation period. In both test species, exposure to HCDD produced a dose-related "toxic hepatitis" and an increased incidence of hepatocellular nodules or tumors (adenomas and carcinomas). Liver tumor incidence was statistically significant in both male and female mice and in female rats.

The NTP (1982a) conducted a study for 104 weeks using Osborne-Mendel rats and B6C3F1[(a)] mice. The rats and male mice were administered TCDD at 0, 0.01, 0.05 or 0.5 µg/kg/wk by gavage in two divided doses, and the female mice were given 0, 0.04, 0.2, or 2.0 µg/kg/wk. Incidences of follicular cell thyroid adenomas in male rats ($P<0.001$) and of neoplastic nodules in livers of female rats ($P=0.006$) increased significantly. TCDD increased the numbers of hepatocellular carcinomas in male mice ($P=0.002$) and in females ($P=0.014$). The total liver tumors (carcinomas and adenomas) were increased in males ($P<0.001$) and females ($P=0.002$). In addition, female mice had increased incidence of follicular cell thyroid adenomas. These studies indicate that TCDD is an animal carcinogen.

Epidemiologic studies on industrial workers and herbicide applicators are consistent with the conclusion from animal studies that TCDD is a carcinogen. However, since TCDD is usually a contaminant of phenoxy acids and/or chlorophenols, human exposure is always to multiple chemicals. Therefore, the evidence for human carcinogenicity from these studies is only suggestive due to the difficulty of evaluating the risk of TCDD exposure in the presence of the confounding effects of the other chemicals (USEPA 1984).

9.3 Quantitative Indices of Toxicity

9.3.1 Noncarcinogenic Effects

Recommended exposure limits to TCDD to ensure human safety have been established by many agencies. The National Academy of Sciences (NAS 1977), before TCDD was considered to be a carcinogen, suggested an ADI of 0.0001 µg/TCDD/kg/day based on a 13-week feeding study in rats (Kociba et al. 1976). The reported NOEL in that study (0.01 µg/kg) was divided by an uncertainty factor of 100 to determine the ADI. The NAS then calculated a suggested-no-adverse-effect-level in drinking water of 0.0007 µg/L, based on the average weight of a human adult (70 kg) and an average daily intake of water of 2 L, with water representing 20% of total intake.

The USEPA (1984) has calculated an ADI of 10^{-6} µg/kg/day, using a LOAEL, based on toxic effects and reduced fertility, of 0.001 µg/kg/day and employing an uncertainty factor of 1,000. Using a bioaccumulation factor of 5,000, and

assuming a daily consumption of 6.5 g of fish, a water concentration of 2.0×10^{-6} µg/L was derived. It was noted that this value may not be sufficiently low to protect against the carcinogenic effects of TCDD (USEPA 1984).

9.3.2 Carcinogenic Effects

Since there is no recognized safe concentration for a human carcinogen, and TCDD is a suspected human carcinogen, the recommended concentration of TCDD in water is zero (USEPA 1984). Assuming daily consumption of 2 L of water and 6.5 g of fish and shellfish, the concentrations of TCDD in water that correspond to excess cancer risks of 10^{-5}, 10^{-6} or 10^{-7} were calculated to be 1.3×10^{-7}, 1.3×10^{-8} or 1.3×10^{-9} µg/L, respectively (USEPA 1984). Because of the tendency for aquatic organisms to bioconcentrate TCDD, most of this risk is due to consumption of the fish or shellfish. If no contaminated fish or shellfish were eaten, the water concentrations corresponding to excess cancer risks of 10^{-5}, 10^{-6} and 10^{-7} would be 2.2×10^{-6}, 2.2×10^{-7} and 2.2×10^{-8} µg/L, respectively. These criteria are below the limit of detection of TCDD in water (approximately 3×10^{-5} µg/L) by current analytical methods.

The Food and Drug Administration (FDA) issued a health advisory stating that fish with residues of TCDD $\geq$50 ppt should not be consumed, but fish with residues of < 25 ppt pose no serious health concern (USEPA 1984). The Centers for Disease Control (CDC) has established 1 ppb TCDD as a level of concern in residential soils.

9.4 Special Concerns

The special concerns related to TCDD are its extreme toxicity and persistence in the environment. It is possibly, on a molecular basis, the most poisonous synthetic chemical known (Esposito et al. 1980). It is resistant to biodegradation and has a high affinity to soil, especially soil with a significant organic content.

The data from animal studies suggest that fetuses and newborns may be at greater risk from TCDD exposure than the general population.

9.5 References

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assuming a daily consumption of 6.5 g of fish, a water concentration of 2.0×10^{-6} µg/L was derived. It was noted that this value may not be sufficiently low to protect against the carcinogenic effects of TCDD (USEPA 1984).

9.3.2 Carcinogenic Effects

Since there is no recognized safe concentration for a human carcinogen, and TCDD is a suspected human carcinogen, the recommended concentration of TCDD in water is zero (USEPA 1984). Assuming daily consumption of 2 L of water and 6.5 g of fish and shellfish, the concentrations of TCDD in water that correspond to excess cancer risks of 10^{-5}, 10^{-6} or 10^{-7} were calculated to be 1.3×10^{-7}, 1.3×10^{-8} or 1.3×10^{-9} µg/L, respectively (USEPA 1984). Because of the tendency for aquatic organisms to bioconcentrate TCDD, most of this risk is due to consumption of the fish or shellfish. If no contaminated fish or shellfish were eaten, the water concentrations corresponding to excess cancer risks of 10^{-5}, 10^{-6} and 10^{-7} would be 2.2×10^{-6}, 2.2×10^{-7} and 2.2×10^{-8} µg/L, respectively. These criteria are below the limit of detection of TCDD in water (approximately 3×10^{-5} µg/L) by current analytical methods.

The Food and Drug Administration (FDA) issued a health advisory stating that fish with residues of TCDD $\geq$50 ppt should not be consumed, but fish with residues of < 25 ppt pose no serious health concern (USEPA 1984). The Centers for Disease Control (CDC) has established 1 ppb TCDD as a level of concern in residential soils.

9.4 Special Concerns

The special concerns related to TCDD are its extreme toxicity and persistence in the environment. It is possibly, on a molecular basis, the most poisonous synthetic chemical known (Esposito et al. 1980). It is resistant to biodegradation and has a high affinity to soil, especially soil with a significant organic content.

The data from animal studies suggest that fetuses and newborns may be at greater risk from TCDD exposure than the general population.

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10.0 SUMMARY OF TOXICOLOGICAL INFORMATION ON TRICHLOROETHYLENE

This chapter provides a summary of current toxicological information on trichloroethylene. This compound was chosen for consideration here because of its high inherent toxicity and because it is often found as a contaminant at hazardous waste sites. This chapter is similar to the sort of summary which might be prepared by a toxicologist for a hazard assessment, and therefore it is somewhat more technical and detailed than chapters 1 to 8 of this handbook. A nontoxicologist would not be expected to prepare a summary of this sort, but should, on the basis of the information presented in chapters 1 to 8 of the handbook, be able to understand and apply this information.

10.1 Chemical Properties and Environmental Stability

Trichloroethylene (TCE) is a nonflammable, colorless liquid used primarily as a solvent, in metal degreasing, in drycleaning operations and in refrigerants and fumigants. In the past, TCE has also been used as an anesthetic and as an extractant in food processing. Trichloroethylene has a sweet odor, resembling that of chloroform. Trichloroethylene is practically insoluble in water, but highly soluble in lipids.

Although TCE can be formed during the water chlorination process, the major source of environmental levels of this chemical is volatilization of TCE during manufacturing. Trichloroethylene is widely distributed in the environment; however, there is no indication that it is persistent or that it bioaccumulates in the food chain. Trichloroethylene has been detected in air, food, human tissues and in groundwater. Concentrations in groundwaters range from 18 to 22,000 ppb. An EPA finished water survey of ten cities reported TCE in half the supplies tested (levels ranged from 0.1 to 0.5 µg/L (USEPA 1975).

10.2 Summary of Health Effects Data

Exposure to TCE can produce CNS depression (e.g., unconsciousness, numbness, incoordination), minor liver damage, kidney necrosis (death of kidney cells), painful breathing and cardiac arrhythmia. The most critical endpoint for toxicological evaluation, however, is the carcinogenic potential of TCE.

The most important routes of exposure to TCE are inhalation and ingestion. Trichloroethylene is readily absorbed both orally and in the lungs. In mice and rats 95% and 98%, respectively, of an orally administered dose of TCE was absorbed from the gastrointestinal tract within 72 hours (Dekart et al. 1984). Human data concerning TCE absorption has been obtained using the inhalation route of exposure. Stewart et al. (1962) reported TCE concentrations of 4.5 to 7 mg/L in blood within two hours of exposure to a time-weighted average concentration of 1,420 mg/m^3. Estimates of retention in the lung have ranged from 28% to 24%. Dermal absorption is also rapid, but in most cases the opportunity for this route of exposure is insignificant.

The metabolism of TCE is an important factor in its toxicity. Trichloroethylene is metabolized to active intermediates (e.g., 2,2,3-trichloroxirane, dichloroacetic acid) that may be responsible for some of the long-term effects of TCE. Additionally, there are important differences in the metabolism among

different species, making interspecies comparisons difficult. Trichloroethylene does not appear to be teratogenic. Available evidence concerning mutagenicity is mixed. Trichloroethylene was mutagenic in two microbial mutagenicity assays (an Ames test and a host-mediated assay in mice). However, exposure of mouse sperm cells to TCE had no significant effect on litter size (dominant lethal assay).

10.2.1 Noncarcinogenic Studies

10.2.1.1 Animal Studies

Trichloroethylene, like most other chlorinated aliphatic hydrocarbons, produces depression of the CNS and inhibits cardiac function in animals following acute exposure. At higher doses liver damage occurs. Evidence indicates that repeated acute exposures are no more harmful to laboratory animals that a single acute exposure (i.e., there is no accumulation).

Grandjean (1960) exposed male rats to 200 and 800 ppm TCE vapor for 4 to 11 weeks. After a single three-hour exposure the animals were tested in a previously trained rope-climbing experiment. The number of spontaneous climbs to receive a reward was significantly increased in comparison to controls. The observed effect, attributed to TCE-induced excitability, was, however, not dose dependent. In a subsequent study Grandjean (1963) exposed rats to 400 or 800 ppm TCE for six hours and measured swimming performance and motor activity. At 400 ppm performance was retarded slightly; at 800 ppm performance was significantly adversely affected. One hour after termination of exposure no significant changes were observed.

Adams et al. (1951) exposed several species to various levels of TCE vapor 7 hours/day, 5 days/week for 6 months. The authors determined maximum no-effect levels of 400 ppm in monkeys, 200 ppm in rabbits and rats and 100 ppm in guinea pigs. Prendergast et al. (1967) subjected rats, rabbits, guinea pigs, monkeys and dogs to either 730 ppm TCE (5 days/week, 8 hours/day for six weeks) or 35 ppm (continuously for 90 days). In both these experiments, the only effects of TCE exposure observed were occasional, slight body weight loss or below normal body weight gain.

Kimmerle and Eben (1973) exposed male rats to 55 ppm TCE vapor for 15 weeks (8 hours/day, 5 days/week). The only observable effect seemed to be increased liver weights. Clinical hematological values were within normal ranges.

Tucker et al. (1982) exposed male and female CD-1 mice to TCE in drinking water at concentrations of 0, 100, 1,000, 2,500 or 5,000 mg/L for six months. The 100-mg/L dose produced no observed effects in the mice. The 1,000-mg/L dose produced an increase in the ratio of liver weight to total body weight in males only. At the 2,500-mg/L dose males exhibited increased ketone (an indication of diabetes) and protein levels in the urine and increased relative liver weights. These effects were not observed in the females. The highest dose produced decreased body weights, increased liver and kidney weights and increased urinary ketone and protein levels.

Stott et al. 1982 administered various doses of TCE to mice and rats by gavage (5 days/week) for three weeks. At 250 mg/kg minimal hepatotoxic effects were observed in the mice. Doses of 500 mg/kg/day or greater produced centrilobular hypertrophy (swelling of the middle of the lobe) in the mouse livers. In contrast, a dose of 1,000 mg/kg/day resulted in no liver damage in the rats.

10.2.1.2 Human Studies

In humans, acute exposure to high levels of TCE results in CNS depression such as incoordination and unconsciousness. Although there have been incidences of acute exposure, clinical analysis of these incidents rarely establishes clear-cut exposure limited to only TCE. Chronic exposure of humans to TCE in occupational settings has resulted in few reports of liver damage.

Single oral doses of TCE ranging from 7.6 to 35 g have been reported to produce adverse symptoms and eventual recovery. Feldman (1970) reported on a person exposed to TCE vapors from an overheated, degreasing unit (exposure level not reported). Symptoms included nausea, vomiting, blurred vision and facial numbness. An 18-month recovery period resulted in restoration of facial sensation and motor function. In fatal cases of acute TCE exposure, no tissue abnormalities were observed at autopsy (Kleinfeld and Tabershaw 1954).

Epidemiological evidence has been generally reported on workers exposed occupationally. These studies rarely include an unexposed control group. Demographic characteristics of the exposed group are not always provided. Another serious flaw includes lack of information about possible exposures to other chemicals in the workplace.

Bordodej and Vyskocil (1956) examined 75 persons occupationally exposed to TCE in drycleaning or degreasing establishments. The TCE concentration varied from 50 to 630 ppm; workers had been exposed from six months to 25 years. Symptoms including alcohol intolerance, shivers, giddiness, anxiety and cardiac abnormalities and sleep disturbances were found to be significantly correlated with the duration of exposure.

Takamatsa (1962) studied 50 males and female factory workers exposed to TCE for approximately 2.5 years. Airborne TCE concentrations ranged from 25 to 250 ppm. Workers exposed to less than 50 ppm TCE showed no apparent ill effects. Six workers in a degreasing room (150 to 250 ppm TCE) reported headaches, dizziness, giddiness, drunken feeling, flushing of the face, burning throat and fatigue.

10.2.2 Mutagenic and Carcinogenic Studies

10.2.2.1 Animal Studies

A number of carcinogenic studies have been performed in rats and mice that indicate the carcinogenic potential of TCE. Experimental details for these studies are provided in Table 10-1. The National Cancer Institute (NCI) (1976) conducted a 78-week carcinogenic study in rats and mice. Five-week-old

TABLE 10-1 CARCINOGENICITY OF TCE

Route	Dose	Duration of Treatment	Length of Experiment	Species (strain)	Sex	Comments	Reference
Oral	1,169 mg/kg/day 2,339 mg/kg/day	5 days/week for 78 weeks	90 weeks	mouse (B6C3F1)	M M M	Hepatocellular tumors occurred in 26/50 (low-dose) and 31/48 (high-dose) of the treated males as compared with 1/20 control male mice (P<0.05 for both dose levels).	NCI (1976)
	869 mg/kg/day 1,739 mg/kg/day	5 days/week for 78 weeks	90 weeks		F F F	Hepatocellular tumors occurred in 4/50 (low-dose) and 11/47 (high-dose) of the treated females as compared with 0/20 control female mice (P<0.05 for the high-dose level only).	
	549 mg/kg/day 1,097 mg/kg/day	5 days/week for 78 weeks	110 weeks	rat (Osborne-Mendel)	F/M F/M F/M	No effects were seen in rats.	
Oral	500 mg/kg 1,000 mg/kg	5 days/week for 103 weeks	103 weeks	rat (Fisher 344)	F/M	High-dose males showed significant increase in kidney adenocarcinomas.	NTP (1982)
Oral	1,000 mg/kg	5 days/week for 103 weeks	103 weeks	mouse (B6C3F1)	F/M	Increased incidence of hepatocellular carcinoma observed in treated males and females.	NTP (1982)
Inhalation	100 ppm 500 ppm 100 ppm 500 ppm	6 hours/day, 5 days/week for 18 months	30 months	mouse (NMRI)	F F F M M M	Histopathological examination were made on all animals. No carcinogenic effect was observed in either sex of rats or hamsters, or in male mice. In female mice, the incidence of lymphomas was higher in the low-dose (17/30) and the high-	Henschler et al. (1980)

continued-

Table 10-1 - continued

Route	Dose	Duration of Treatment	Length of Experiment	Species (strain)	Sex	Comments	Reference
	100 ppm 500 ppm	6 hours/day, 5 days/week for 18 months	36 months	rat (Wistar)	F/M F/M F/M	dose (18/28) groups of animals than in the control (9/29) group.	
	100 ppm 500 ppm	6 hours/day, 5 days/week for 18 months	30 months	hamster (Syrian)	F/M F/M F/M		
Inhalation	50 ppm 150 ppm 450 ppm	7 hours/day, 5 days/week for 104 weeks	107 weeks	mouse (IRC)	F F F F	Mice exposed to 150 and 450 ppm TCE had three times the number of lung tumors observed in the low-dose animals and the controls. A statistically significant increase was seen when the number of lung adenocarcinomas in mice exposed to 150 and 450 ppm TCE was compared with the number of lung adenocarcinomas in the low-dose and control animals.	Fukuda et al. (1983)
	50 ppm 150 ppm 450 ppm	7 hours/day, 5 days/week for 104 weeks	107 weeks	rat (Sprague-Dawley)	F F F		

mice and seven-week-old rats were gavaged 5 days/week with TCE with a high or low dose. No treatment-related effects were observed in rats. There was a significant increase in hepatocellular carcinomas in the male mice at both doses and in the females of the high-dose group. Due to suspected contamination of the original test material with epichlorohydrin (a known carcinogen), the bioassay was repeated. In the repeat bioassay, rats (F344/N)[a] and mice (B6C3F1)[a] of both sexes were administered TCE by gavage for 103 weeks. Rats received doses of 500 or 1,000 mg/kg; mice were administered 1,000 mg/kg. Trichloroethylene was not found to be carcinogenic in female F344/N[a] rats. The experiment with male rats was considered to be inadequate, since these rats received dose levels of TCE that exceeded the maximum tolerated dose. Trichloroethylene was demonstrated to be carcinogenic in both sexes of B6C3F1[a] mice, producing hepatocellular carcinomas.

The NTP (1982) completed a bioassay using Fisher 344[a] rats and B6C3F1[a] mice. Trichloroethylene was administered by gavage at doses of 500 or 1,000 mg/kg in rats and 1,000 mg/kg in mice for 103 weeks. High-dose male rats exhibited a significant increase in kidney tubular adenocarcinomas. A dose-related reduction in survival was noted in male rats. Toxic nephrosis (degeneration of the cells in the kidney tubules) was found in treated rats dying during the course of the study. In the treated mice, body weights and survival were reduced in males. In both sexes there was a significant increase in the incidence of hepatocellular carcinomas.

Henschler et al. (1980) studied the effect of chronic inhalation of TCE on the tumor incidence in NMRI[a] mice, Wistar rats and Syrian hamsters. Groups of male and female animals of each species were exposed by inhalation to 100 or 500 ppm TCE for 18 months. At 30 months (for mice and hamsters) and 36 months (for rats), no statistically significant increased tumor incidences were observed in any group except for malignant lymphomas in female mice in both treated groups.

Fukuda et al. (1983) examined the effect of chronic inhalation of reagent grade TCE (99.8% pure) on the tumor incidence in female ICR[a] mice and Sprague-Dawley rats. Groups of 49 to 51 animals of each species were exposed to 0, 50, 150 or 450 ppm TCE for 104 weeks. In mice, the incidences of pulmonary adenocarcinomas in the 150 ppm group and the 450 ppm group were significantly higher than that of controls. The average number of lung tumors per mouse in groups exposed to 150 ppm TCE and 450 ppm TCE was more than three times that of the controls. No increased tumor incidences were observed in rats.

10.2.2.2 Human Studies

Axelson et al. (1978) conducted an epidemiological study of cancer deaths among a group of 518 men occupationally exposed to TCE. Exposure was

(a) A series of letters, numbers or a combination of both indicates a specific strain (i.e., a race or stock) of animals that all have common hereditary characteristics.

estimated by measuring a TCE metabolite, trichloroacetic acid (TCA), in the urine. A TCA level of >100 mg/L was considered to be high exposure, corresponding to more than 30 ppm in air. Close agreement was found between observed and expected numbers of cancer deaths based on national Swedish death rates. Since the sample was small, the authors hesitated to rule out an increased cancer risk from TCE exposure, especially from rare tumors.

10.3 Quantitative Indices of Toxicity

A number of estimates of noncarcinogenic indices of toxicity and estimates of carcinogenic risk have been calculated for TCE. These are summarized in Table 10-2.

10.3.1 Noncarcinogenic Effects

There have been several risk estimates for TCE calculated with regard to noncarcinogenic endpoints. The National Academy of Sciences (NAS 1980) calculated a Suggested No-Adverse-Response Level (SNARL) of 105 mg/L of drinking water based on observation that the lowest oral dose of TCE reported to produce inebriation was approximately 300 mg/kg. A 100-fold safety factor was used in the calculation, recognizing that the minimum effect level for inhibition of reflexes was undoubtedly lower than 300 mg/kg. It was assumed that the sole source of TCE was drinking water and that a 70-kg human consumes 2 L/day. A seven-day SNARL of 15 mg/L was also calculated by dividing the one-day value by 7. A chronic minimum effect level was not established due to lack of appropriate data.

The EPA Office of Drinking Water (USEPA 1979) computed a one-day SNARL value of 2 mg/L. This value was based on a study in which human volunteers were exposed via inhalation to 110 ppm of TCE for an eight-hour period. The calculation considered the most sensitive subpopulation (children) and used an uncertainty factor of 100. A ten-day SNARL was conservatively estimated by dividing the one-SNARL by 10 (200 µg/L). A chronic SNARL of 74 µg/L was derived using the minimum effect level of 55 ppm observed in the Kimmerle and Eben (1973) study.

The EPA Office of Water Regulations and Standards (USEPA 1980) established an ADI of 38 mg/day based on the noncarcinogenic endpoints noted in the NCI (1976) bioassay (dose-related decreased survival, chronic kidney disease). The lowest dose (548 mg/kg/day, time-weighted average) was considered a LOEL. Assuming daily consumption of 2 L of water and 6.5 grams of contaminated fish, the level that protects against the toxic effects of TCE was calculated to be 18.3 mg/L.

The American Conference of Governmental Industrial Hygienists (ACGIH 1980) has recommended a Threshhold Limit Value (TLV) of 50 ppm and a Short-Term Exposure Limit (STEL) of 150 ppm. These levels are recommended to provide workers with adequate protection against the toxic effects of TCE. An 8 hour TLV of 100 ppm has been proposed by the National Institute for Occupational Safety and Health (NIOSH) (1973) for an eight-hour workday.

TABLE 10-2 RISK ESTIMATES FOR TCE

Organization	Noncarcinogenic	10^{-6} Excess Cancer Risk
NAS (1977)	--	6.3 µg/L
NAS (1980)	105 mg/L (one-day SNARL) 15 mg/L (seven-day SNARL)	3.0 µg/L
USEPA (1979) (Office of Drinking Water)	2 mg/L (one-day SNARL) 200 µg/L (ten-day SNARL) 74 µg/L (chronic SNARL)	--
USEPA (1980) (Office Water Regulations and Standards)	38 mg/day (ADI) 18.3 mg/L (Ambient Water Standard)	2.7 µg/L
ACGIH (1980)	50 ppm (TLV) 150 ppm (STEL)	--
NIOSH (1973)	100 ppm (TLV, 8-hour)	--

10.3.2 Carcinogenic Effects

The IARC has performed an assessment of the degree of evidence for the carcinogenicity of TCE to humans and experimental animals which was published by the World Health Organization (WHO) (WHO 1982). This assessment concluded that TCE is a Group 3 chemical (inadequate evidence of carcinogenicity in animals or humans). Nonetheless, several carcinogenic risk assessments have been performed.

A statistical assessment of human cancer risk associated with TCE in drinking water was performed by NAS (1977) based on the NCI (1976) study. For TCE at a concentration of 1 µg/L, the estimated risk was calculated to be 0.36 to 1.1×10^{-7}. The concentration corresponding to a cancer frequency of 10^{-6} is 9.1 to 27.7 µg/L. The upper bound 95% confidence limit estimate was 1.6×10^{-7} for 1 µg/L. This corresponds to a concentration of 6.3 µg/L for an excess lifetime cancer risk of 10^{-6}. Using the data from NTP (1982), another estimate was made using the multistage model (USEPA 1983). Averaging both the male and female data sets, the estimated upper 95% confidence estimate of lifetime risk per µg/L was 3.3×10^{-7}. The concentration corresponding to a 10^{-6} risk level is 3.0 µg/L. The EPA Office of Water Regulations and Standards (USEPA 1980), using the data from NCI (1976), published a recommended criterion of 2.7 µg/L for the 10^{-5} risk level.

10.4 Special Concerns

Since the toxicity of TCE is highly dependent on its metabolism, the possibility exists that there will be age and sex differences and additional risk in extrapolating results from one species to another. Additionally, there is the possibility of synergistic effects with alcohol.

10.5 References

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11.0 SUMMARY OF TOXICOLOGICAL INFORMATION ON LEAD

This chapter provides a summary of current toxicological information on lead. This compound was chosen for consideration here because of its high inherent toxicity and because it is often found as a contaminant at hazardous waste sites. This chapter is similar to the sort of summary which might be prepared by a toxicologist for a hazard assessment, and therefore it is somewhat more technical and detailed than chapters 1 to 8 of this handbook. A nontoxicologist would not be expected to prepare a summary of this sort, but should, on the basis of the information presented in chapters 1 to 8 of the handbook, be able to understand and apply this information.

11.1 Chemical Properties and Environmental Stability

Lead is a metallic element designated by the symbol Pb. Contamination of the environment with lead has increased dramatically since the industrial revolution, due primarily to lead emissions into the air. These emissions may be inhaled directly, or may settle on soil or water and be ingested. The chemical form of lead emissions and its form in soil, water or the food chain varies considerably, but is nearly always some oxide, salt or complex of the lead ion (Pb^{2+}). Lead oxides, salts and complexes may be altered by speciation reactions, but the lead ion itself is stable under normal environmental conditions.

11.2 Summary of Health Effects Data

Lead exposure produces adverse effects on many systems of the body, including the hematopoietic, cardiovascular, nervous, endocrine, renal, reproductive and digestive systems. Acute lead intoxication in humans is characterized by encephalopathy (disease of the brain), abdominal pain, hemolysis (destruction of red blood cells), liver damage, renal tubular necrosis, seizures, coma and cardiorespiratory arrest. Severe poisonings of this sort are rare, and most concern regarding lead toxicity focuses on insideous injury to the hematopoietic system, the nervous system and the cardiovascular system following chronic exposure to low levels of lead. Of particular concern are data that suggest that some of these effects may not have a threshold value.

Lead-induced effects have been extensively investigated both in animals and in humans. Studies in animals are useful since dose levels and the chemical form of administered lead are known, but they are limited by differences in lead absorption and metabolism between animals and humans. Studies in humans are, therefore, more directly useful, but accurate knowledge of exposure levels, exposure routes or chemical form are rarely known.

One means of solving this problem is to assess human exposure to lead by measuring the concentration of lead in the blood (PbB). This value reflects the magnitude of current or recent-past exposure to lead, and it is possible to calculate the exposure that produced the observed PbB value. While this approach permits the use of human data in identifying NOAEL and LOAEL values for lead, it is limited by the fact that the calculation of lead exposure from measured PbB values may not be highly accurate. Moreover, many of the toxic

effects of lead are irreversible and current or recent-past exposure levels may not reflect exposure levels at the time the injury actually occurred.

The following sections provide brief descriptions of some representative studies of the key adverse health effects of lead in animals and humans.

11.2.1 Noncarcinogenic Studies

11.2.1.1 Effects of Lead on Hematopoiesis

Lead inhibits several key enzymes involved in heme (a component of hemoglobin) biosynthesis. The activity of delta-aminolevulinic acid dehydratase (ALA-D) appears to be very sensitive to lead, and inhibition had been reported at quite low PbB values. Hernberg and Nikkanen (1970) found that ALA-D activity was inversely correlated with PbB values in a group of subjects with 50% inhibition at a PbB level of 16 µg/dL. Other reports have confirmed these observations across age groups and exposure categories.

The inhibition of ALA-D is reflected by increased levels of its substrate, delta-aminolevulinic acid (ALA), both in urine and in whole blood or plasma. The toxicological significance of an increase in cellular or plasma ALA levels is uncertain, but it appears that ALA can inhibit release of neurotransmitter from nerve cells, even at ALA levels as low as 1.0 µM (Brennan and Cantrill 1979).

Another enzyme of heme biosynthesis that is inhibited by lead is ferrochelatase. This enzyme inserts ferrous ion (Fe^{2+}) into protoporphyrin to form heme. In lead exposure, the porphyrin acquires a zinc ion in lieu of ferrous ion, forming zinc protoporphyrin (ZPP). A correlation between PbB and erythrocyte ZPP has been extensively documented, with a threshold value of about 15 to 30 µg/dL (Roels et al. 1975, Piomelli et al. 1982).

One of the most characteristic effects of chronic lead intoxication is anemia. The mechanism of lead-associated anemia appears to be a combination of reduced hemoglobin production and shortened erythrocyte survival. Reduced hemoglobin production is a consequence of inhibition of heme synthesis (as described above), coupled with a decreased production of globin. It is also clear that there is a hemolytic component to lead-induced anemia in humans, owing to shortened erythrocyte survival (Hernberg et al. 1967, Leikin and Eng 1963).

Exposure to lead results in inhibition of heme biosynthesis not only in erythrocytes, but in other tissues as well. This results in decreased activity of a number of heme-containing enzymes, including cytochrome P-450 (which is important in metabolism of many drugs, chemicals and natural compounds) and the renal enzyme 1-hydroxyglase (which mediates the final step in the synthesis of the active form of vitamin D). Inhibition of the latter enzyme impairs biosynthesis of the active form of vitamin D, and this may produce a series of adverse effects related to calcium absorption and cell development.

11.2.1.2 Effects of Lead on the Nervous System

Studies in animals indicate that the perinatal period of ontogeny is a time of particular sensitivity of the nervous system to lead exposure. In nursing female rats, ingestion of water containing levels as low as 20 mg Pb^{2+}/L (2 mg/kg/day) causes effects on neurotransmitter metabolism in the offspring, and higher exposure levels result in a number of morphologic, biochemical and electrophysical changes (Govoni et al. 1980).

Grant et al. (1980) exposed rats indirectly to lead in utero and during lactation through the mother's milk and, after weaning, directly through drinking water. There were delays in the development of surface righting and air righting reflexes in subjects given water containing 50 or 250 mg Pb^{2+}/L (5 or 25 mg/kg/day). Generally similar results were obtained when suckling or young rats were exposed to lead directly (as opposed to indirect exposure via the mother). Cory-Slechta and Thompson (1979) supplied weanling male rats with drinking water solutions containing 25, 150 or 500 mg Pb^{2+}/L (2.5, 15 or 50 mg/kg/day). Animals exposed to 50 ppm or 300 ppm lead solutions showed significantly higher response rates than matched controls during conditioned response training. Jason and Kellogg (1981) reported a developmental lag in activity around post-natal day (PND) 15 to 18, as measured in an automated activity chamber. Rat pups were dosed on PND 2 to 14 with lead at 25 or 75 mg/kg. A delay in the characteristic decrease in activity that normally occurs in pups at that age was observed, indicating that lead-exposed pups were significantly more active than control subjects at PND 18. These alterations in behavior are indicative of altered neural functioning in the CNS.

Bushnell and Bowman (1979) investigated the effect of lead on learning in rhesus monkeys. Lead acetate was fed to the animals for 12 months, resulting in PbB levels of 30 to 50 µg/dL (versus 5 µg/dL in controls). Exposed monkeys were significantly retarded in their ability to learn a visual discrimination task.

In conclusion, it appears that alterations in behavior and neural development in rats and monkeys occur as a consequence of oral exposure to lead. These alterations are presumably indicative of altered neural functioning, especially in the CNS, but whether such alterations represent biologically significant impairment in overall functioning of the lead-exposed subjects is not yet clear.

There are many reports of lead-induced nervous system injury in humans. Morgan and Repko (1974) reported deficits in hand-eye coordination and reaction time in an extensive study of behavioral functions in 190 lead-exposed workers. Similar studies (Arnvig et al. 1980, Haenninen et al. 1978, Valciukas et al. 1978) have found disturbances in visual motor performance, IQ test performance, hand dexterity, mood, nervousness and coping in lead workers with PbB values of about 50 to 80 µg/dL.

Seppalainen et al. (1975) measured nerve conduction velocity (NCV) in 26 lead workers whose PbB levels had been monitored regularly for several years. Most

PbB values ranged between 35 and 60 µg/dL with occasional values as low as 20 or as high as 70 µg/dL. There was a clear decrease in mean NCV in nerves of the arm in exposed workers compared to controls ($P<0.01$). The authors emphasized that the data showed evidence of neurophysiological effects in workers whose PbB values never exceeded 70 µg/dL.

Melgaard et al. (1976) observed a clear association between lead exposure and peripheral nerve dysfunction in 20 automobile mechanics exposed to tetraethyl lead and other lead compounds in lubricating and high-pressure oils. Half of the workers had elevated PbB levels (60 to 120 µg/dL) and showed definite electromyographic deficits (abnormal electrical activity in skeletal muscles). The mean blood lead level for the control group was 18.6 µg/dL.

Thus, considerable evidence exists that peripheral nerve dysfunction occurs in adults at PbB levels as low as 30 to 50 µg/dL. The question as to whether these reflect mild, reversible effects or are true early warning signals of progressively more serious peripheral neuropathies is still a matter of some dispute.

As in experimental animals, the developing child appears to be especially sensitive to lead-induced nervous system injury. De la Burde and Choate (1972, 1975) observed neurological dysfunctions including fine motor dysfunction, impaired concept formation and altered profiles in 70 preschool children exhibiting pica behavior (the tendency to eat dirt). These children displayed elevated PbB levels (30 to 100 µg/dL, mean = 58 µg/dL) in comparison with 70 matched control subjects not engaging in pica. Continuing CNS impairment in the lead-exposed group, as assessed by a variety of psychological and neurological tests, was observed when the children were seven to eight years old, despite the observation that many of their PbB levels had by then decreased significantly from the initial study.

The relationship between low-lead exposure, psychometric function and electrophysiological response in children aged 13 to 78 months was explored in studies by Milar et al. (1980, 1981), Otto et al. (1981) and Benignus et al. (1981). Psychometric evaluation revealed lower scores for children with PbB levels of 30 µg/dL or higher compared to children with PbB levels under 30 µg/dL, but the observed IQ deficits were confounded by poor home caregiver environment scores in children with elevated PbB levels. Electrophysiological assessments, including analyses of slow potentials during sensory conditioning and electroencephalogram (EEG) spectra, did provide evidence of CNS effects of lead in the same children. A significant linear relationship between PbB (ranging from 7 to 59 µg/dL) and slow wave (SW) voltage was observed (Otto et al. 1981). Analyses of quadratic and cubic trends in SW voltage did not reveal any evidence of a threshold for this effect.

Beattie et al. (1975) identified 77 retarded children and 77 normal children matched for age, sex and geography. Of 64 matched pairs, 11 of the retarded children came from homes with high concentration of lead in the water. By contrast, none of the control children came from such homes. In a follow-up study, PbB values of the mental retardates, measured during the second week of life, were found to be significantly higher than those of control subjects (25.5 ± 8.9 versus 20.9 ± 7.9 µg/dL) (Moore et al. 1977). When compared with

studies of children suffering neurobehavioral deficits produced by direct exposure to lead, these studies suggest that the brain of the fetus is considerably more sensitive to the toxic effects of lead than the brain of the young child.

11.2.1.3 Effects of Lead on Blood Pressure

Several epidemiological studies have indicated that chronic lead exposure may be associated with increased blood pressure in humans (Dingwall-Fordyce and Love 1963, Beevers et al. 1980), although other studies have not detected such an association (Cramer and Dahlberg 1966).

More recently, Harlan et al. (1985) examined the relationship between PbB and blood pressure by statistical analysis of the data base obtained during the second National Health and Nutrition Examination Survey (NHANES-II). This survey collected information on a representative cross-section of over 20,000 members of the U.S. population. Using simple regression analysis, a direct, nearly linear relationship was found between PbB and blood pressure in both men and women aged 12 to 74 years. Since blood pressure is known to be related to factors such as age and body mass, multiple regression analyses were performed to separate confounding factors. After accounting for these and other variables, PbB was found to retain a statistically significant relationship to blood pressure in males ($P<0.05$) but not in females. The authors cautioned that causal inferences about effects of PbB on blood pressure should not be drawn from this cross-sectional survey, although the results obtained were consistent with a direct effect.

Pirkle et al. (1985) employed the NHANES-II data base to perform a detailed statistical analysis of the relation between PbB and blood pressure in white males aged 40 to 59 years. This sub-population was selected because, in this age range, the effects of age on blood pressure are small, and confining the analyses to white males obviated the confounding effects of sex and race. Regression analyses correcting for age and body mass indicated that PbB values correlated to both systolic and diastolic blood pressure. Segmented regression analyses indicated there was no threshold below which blood pressure was not related to PbB.

11.2.2 Mutagenic and Carcinogenic Studies

Studies of cytogenetic (chromosome appearance) abnormalities in persons exposed to lead have yielded mixed results. For example, O'Riordan and Evans (1974) reported no significant chromosomal damage in male workers with PbB values from 40 to 120 µg/dL. However, Forni et al. (1976) found the incidence of abnormal metaphases doubled ($P\leq0.05$) in workers exposed for one month to air lead levels of about 0.8 mg/m^3.

A number of ingestion studies on the carcinogenic potential of various lead salts in animals have been reported. The most common observation was increased frequency of renal tumors, although evidence of tumors in other tissues has been noted. The doses of lead producing these effects were quite high, generally 0.1% to 1% in the diet (equivalent to about 50 to 500 mg Pb/kg/day). For example, Azar et al. (1973) reported dose-dependent

increases in renal tumor frequency in male rats fed 500 to 2,000 ppm Pb in the diet. It should be noted that these doses are associated with moderate to severe non-carcinogenic effects in rats.

A number of epidemiological studies of industrial workers with elevated lead exposure have been conducted to evaluate the role of lead in the induction of human neoplasia (Cooper 1976, Dingwall-Fordyce and Lane 1963, Lane 1964, McMichael and Johnson 1982). In general, these studies made no attempt to consider the types of lead compounds to which workers were exposed, or to determine probable routes of exposure. While a number of these studies found an association between lead exposure and the frequency of various cancer types, no study was sufficiently free of confounding factors to permit a clear conclusion.

The IARC has performed an assessment of the degree of evidence for the carcinogenicity of lead and lead compounds in humans and experimental animals (WHO 1982). This assessment concluded that lead and lead compounds are Group 3 compounds (sufficient evidence for carcinogenicity of some lead salts in animals, but inadequate evidence for carcinogenicity in humans).

11.3 Quantitative Indices of Toxicity

Many studies in animals indicate that adverse effects occur in pups born to dams exposed to doses of lead from 5 to 150 mg/kg/day. Similarly, studies in young animals exposed to lead directly indicate that doses of 5 to 500 mg/kg/day cause behavioral or neurological effects.

Studies in humans suggest that PbB values of around 25 µg/dL or higher are associated with adverse effects in adults, and even lower values may be associated with adverse effects in children. Some effects may occur without a threshold value. Equations have been developed which describe the relationship between PbB values and lead exposure via inhaled air and ingested food, water or soil. While there is some variability between different studies, application of these equations makes clear that daily ingestion or inhalation of several hundred micrograms or less of Pb could yield these PbB values.

The current MCL for lead in drinking water is 50 µg/L (USEPA 1976). This corresponds to a total ADI of 100 µg/day. The present TLV for inorganic lead compounds in inhaled air is 0.15 mg/m^3, and the STEL is 0.45 mg/m^3 (ACGIH 1980). There is growing awareness that present exposure limits may not provide an adequate margin of safety (e.g., see NAS 1977), and several agencies are evaluating recent data on lead toxicity to determine if revisions in existing guidelines and standards are appropriate.

11.4 Special Concerns

There are a number of special concerns associated with lead toxicity. First, most adverse effects develop only slowly, but are basically irreversible. Thus, by the time injury is recognized, permanent harm may already have been done. Second, the fetus *in utero* and the young child are especially sensitive. Third, as analytical techniques and testing protocols become more

powerful and sophisticated, adverse effects of lead are being detected at levels previously thought to be safe. Indeed, some researchers feel there may be no threshold for some lead-induced effects.

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