CHAPTER 22

Forensic and Clinical Toxicology

STACY BRANCH

22.1 INTRODUCTION

Forensic toxicology refers to the use of toxicology for the purposes of the law. It is considered a hybrid of analytical chemistry and fundamental toxicology. The efforts or activities conducted to effectuate this purpose include but are not limited to the following:

- Urine testing to detect drug use
- Regulatory toxicology
- Occupational disease
- Identification of causative agents causing death or injury in humans and animals
- Courtroom testimony and consultation concerning toxicoses

Analytical toxicology in a clinical setting plays a role similar to that in forensic toxicology. Therefore, this chapter will be divided into forensic and clinical toxicology.

22.2 FOUNDATIONS OF FORENSIC TOXICOLOGY

Until the 1700s convictions associated with homicidal poisoning were based only on circumstantial evidence rather than the identification of the actual toxicant within the victim. In 1781, Joseph Plenic stated that the detection and identification of the poison in the organs of the deceased was the only true sign of poisoning. Years later, 1813, Mathieiv Orfila (considered the father of toxicology) published the first complete work on the subject of poisons and legal medicine. By 1836, James M. Marsh developed a test for the presence of arsenic in tissue. Then, in 1839, Orfila successfully used Marsh's test to identify arsenic extracted from human tissues. Fifty years later, Ernst Wilhelm Heinrich Gutzeit developed a method (Gutzeit test) to quantitate arsenic in tissues. In this process arsenic compounds are reduced by hydrogen produced when zinc and sulfuric acid react. The hydrogen then reduces the arsenic compounds to arsine, which is exposed to paper that has been treated with mercuric chloride solution. This

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produces a color range from yellow to brown depending on the arsenic concentration. By 1918, the Medical Examiner's Office and Toxicology Laboratory was established in New York. The chief forensic toxicologist was Alexander O. Gettler who is considered the father of American toxicology.

22.3 COURTROOM TESTIMONY

Reports provided by forensic toxicology personnel and expert consultants may ultimately be introduced as evidence in a court of law. These reporting individuals may be asked to interpret and substantiate their findings and any associated opinions. It is therefore necessary that the forensic toxicologist be thoroughly knowledgeable or familiar with legal practices and be professionally comfortable in a courtroom environment.

Expert Witness. An expert witness is one who possessed the knowledge or experience in subject matters beyond the range of ordinary or common knowledge or observation. The court considers the following when qualifying an expert witness:

- Education
- Work experience
- Job training
- Academic appointments
- Publications
- Acceptance of witness by other courts
- Professional board certifications and memberships

22.4 INVESTIGATION OF TOXICITY-RELATED DEATH/INJURY

The basic phases in conducting an investigation of a suspected toxicant-induced/related death can be viewed as follows:

Collection of information and specimens
Toxicological analysis
Data interpretation

The primary questions to be answered are when conducting an investigation include:

- What was the route of administration?
- What was administered dose?
- Is concentration enough to have caused death or injury or altered the victim's behavior enough to cause death or injury?

Collection of Information and Specimens. As much information as possible concerning the facts of the case must be collected. Due to the often limited amount of physical material available for analysis, it is essential to obtain as much historical information as possible. In addition to any witness accounts of events, one must accurately record

information such as the age, sex, and weight of the victim, his medical history, identification of any medications or other drugs/substances taken before death, and the time interval that elapsed between the intake of these substances and death.

When collecting the specimens, many different body fluids and organs should be collected since xenobiotics have different affinities for body tissues and therefore multiple extractions (for specific analyses) may be needed. Specimens should be collected before applying processes that may destroy evidence, that is, before embalming. The process of embalming, for example, may destroy or dilute the xenobiotic and may yield a false positive result, for example, for the presence of ethanol (which is a constituent of embalming fluid). It is possible to obtain useful specimens from burned or burial remains. The tissue often collected under these circumstances include bone marrow, skeletal muscle, vitreous humor, hair, and maggots. For example, from hair samples, it is possible to detect the presence of antibiotics, antipsychotics, and drugs of abuse. However, the information is primarily qualitative in nature. From maggots, barbiturates, barbiturates, benzodiazepines, phenothiazines, morphine, and malathion can be detected.

It is often necessary to add preservatives to specimens to protect against postmortem changes. For example, the addition of sodium fluoride to a tissue specimen can prevent the production of bacterial ethanol (which can potentially yield a false positive result for the presence of ingested ethanol).

22.4.1 Documentation Practices

Labeling and all handling documentation must exist from the beginning of data/specimen collection to analysis. Figure 22.1 is a sample of a typical toxicology worksheet. The name of the victim (if known) is recorded along with the name of the medical examiner. The condition of the body when found is described and the date of death is recorded. Additionally the date of request of toxicological analyses is also stated on the report. Each collected specimen is identified as well as the tests to be performed. In the results section it is necessary that the analyst for each test signs the form identifying the actual results for the tissue tested and the date the results were obtained.

22.4.2 Considerations for Forensic Toxicological Analysis

The decision concerning which analytical methods to employ depend greatly on a number of factors. One can imagine that a given method may need more sample volume or weight than another method. Therefore the amount of specimen available is a critical determinant of methods to chose for proper toxicological analysis. It is necessary to know the nature of the toxicant to test. In a particular case, is it relevant to detect the parent compound, its metabolites, or all of these? Furthermore toxicant biotransformation must be taken into account when doing the analyses and making interpretations. A low concentration of a toxic parent compound may reflect biotransformation as opposed to a low level of exposure. Conversely, a low-level presence of a nontoxic parent compound may be associated with a sufficient concentration of a biotransformation product that was high enough to cause the insult. Furthermore both forms (parent and metabolites) may have contributed to the adverse outcome.

TOXICOLOGY WORK SHEET

Name - Johnston, Gato T. **Date of Death** - 8/08/2002

Medical Examiner - Bahiyah Sushaunna Ngozi, M.D. **Date of Request** - 8/09/02

Reference number - 38470-38973-2 **Decomposed Corpse**: ~~Yes~~ No

Brief History - Subject was found with a syringe, white power at scene, a small transparent glass bottle, and 0.5 liters of alcohol.

SPECIMENS	#OF SAMPLES	TEST
Blood (Heart)	1	Alcohol X
Blood (Femoral)	1	Acidic Drug
Blood (Other: cephalic)	1	Basic Drug
Urine	1	Other: analyze unknown white powder
Bile		
Vitreous	1	
Kidney		
Brain		
Other: ( )		

RESULTS:

ANALYSIS	DATE	TEST
Estacia Sullivan	8/9/02	Heart Bld Alc 44.12 mmol/L
Francisco Ruiz	8/10/02	Femoral Bld Alc 33.22 mmol/L
Alejandra Santiago	8/10/02	White Powder Heroine - pos. GC/MS
Estacia Sullivan	8/11/02	Femoral Bld Heroine 12.08 μmol/L; GC/MC

Figure 22.1 Toxicology work sheet.

22.4.3 Drug Concentrations and Distribution

As a rule, the highest concentrations of a poison are found at the site of administration. A large quantity of drug in the GI tract and liver indicates oral ingestion. The gastrointestinal (GI) tract may contain large amounts of unabsorbed toxicant. Cases that involve the oral administration of toxicants indicate analysis of GI contents. However, the presence of toxic material in the GI tract does not provide sufficient evidence that the agent is the cause of death. Absorption and transport of the toxicant to the site of action must be demonstrated. Blood and tissue analysis is necessary and would still be paramount.

Higher concentrations of drug or toxicant in the lungs compared to other tissues may indicate inhalation, while compounds located in tissue surrounding an injection

site indicates a fresh intramuscular or intravenous injection. Detection of drug combustion breakdown products within fluids/tissues reveals that smoking was the route of drug administration. For example, the primary pyrolysis product of "crack" cocaine is anhydroecgonine methylester. A high concentration of this compound and the parent cocaine indicates smoking as the route of the cocaine administration. Urine analysis is also of great value since the kidney is the major organ of excretion for most toxicants. The liver is usually the first internal organ to be analyzed. After GI tract absorption, xenobiotics are transported to the liver. This is a major center of compound biotransformation. Finally blood specimens must be collected with care and thought. When collecting blood, it is advantageous to collect both heart and peripheral blood specimens. Postmortem blood drug concentrations are site-dependent. This site dependency is referred to as "anatomical site concentration differences" or "postmortem redistribution."

22.5 LABORATORY ANALYSES

The nonspecific initial tests in a series are valuable for determining the presence or absence of a particular class of compounds. Colorimetric tests to detect the presence of phenothiazines would give initial information about a drug class present. This would be followed by more specific tests to identify the actual compound as well as provide quantitative data. Another example of a type of initial test would be an immunoassay that determines the presence of barbiturates. Confirmatory tests are mandatory to identify the particular drug within the class detected.

22.5.1 Colorimetric Screening Tests

These tests require little sample preparation and are usually performed directly on the specimen. This is a rapid procedure but requires confirmation.

22.5.2 Thermal Desorption

In addition to the analysis of arson crime scene evidence, thermal desorption has been used for the analysis of residual volatile agents in street drugs and the analysis of stains on forensic evidence. Samples are heated to volatilize water and organic compounds. The organic analytes may then be separated by gas chromatography (Figure 22.2).

22.5.3 Thin-Layer Chromatography (TLC)

An extract of a specimen is spotted on a TLC plate, the plate is placed in a mobile phase. The solvent travels up plate via capillary action and the compounds separate depending on compound solubility. Detection is by observing color changes or by using UV light to observe bands. The R_f value is calculated (the distance traveled by the compound divided by distance traveled by solvent). This value along with color reactions are used as qualitative results.

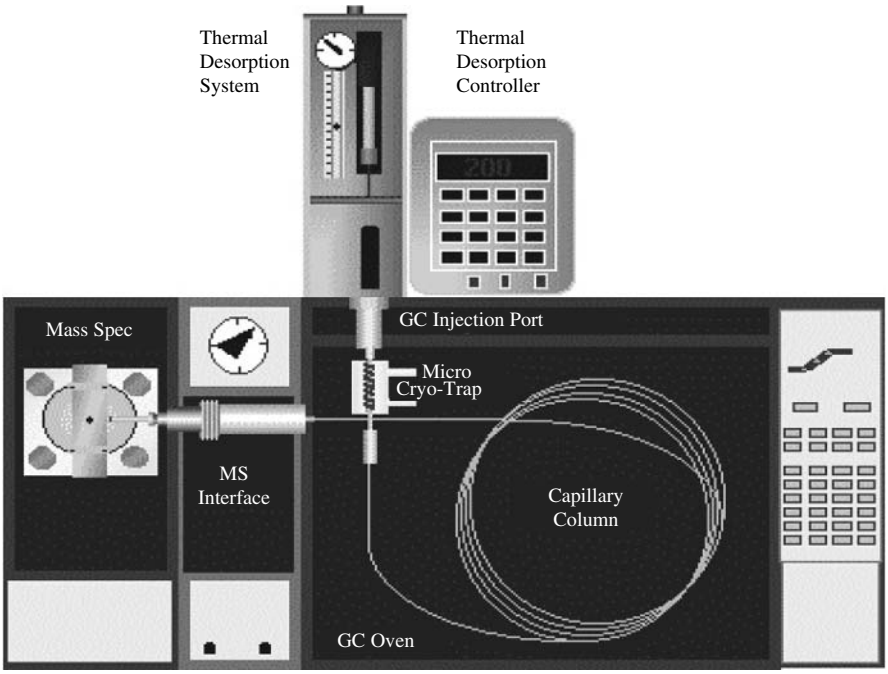

Figure 22.2 Thermal desorption system.

22.5.4 Gas Chromotography (GC)

Involves a sample being vaporized and injected into the head space of the chromatographic column. The sample is transported through the column by the flow of an inert gas (mobile phase). The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid. Retention time with detection techniques (spectrophotometer, mass spectrometry, fluorescence) identifies the compound.

22.5.5 High-Performance Liquid Chromatography (HPLC)

The mobile phase is a solvent that is pumped at high pressure through a packed column. As described for GC, retention time with various detection techniques identifies the compound.

22.5.6 Enzymatic Immunoassay

An enzyme-linked drug derivative is added to the specimen to be tested. This competes with the drug in question for antibody. The more drug that binds to antibody, the less is bound to enzyme-linked drug. Enzyme activity is proportional to the amount of the drug that was already in the specimen.

22.6 ANALYTICAL SCHEMES FOR TOXICANT DETECTION

The circumstances surrounding the case will usually determine the types of toxicological tests that are required. There are different screens specific for the type of substance

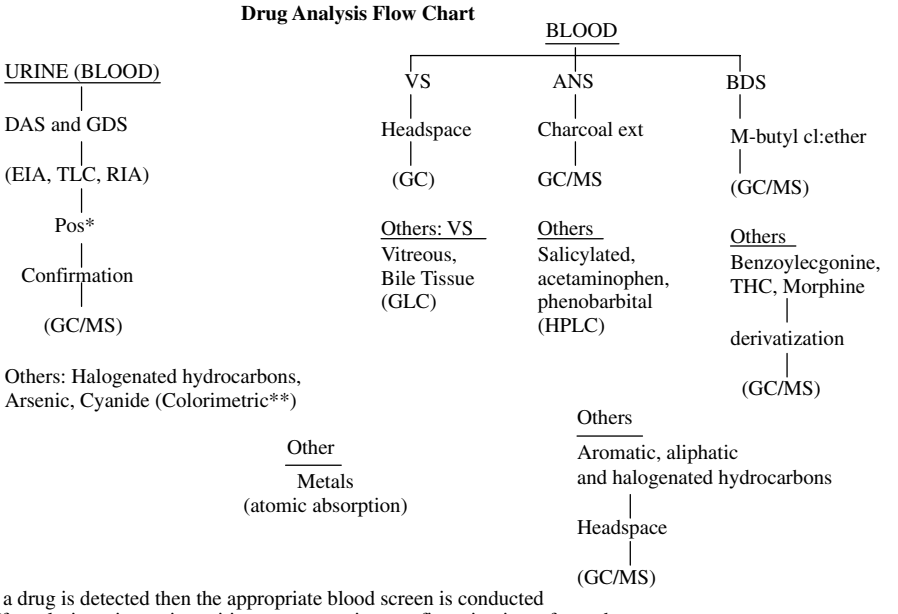

Figure 22.3 Drug analysis flowchart.

to be assayed. A given laboratory will follow an algorithm to handle the analysis. The Volatile Screen (VS) is frequently used for the detection of ethanol. A Drugs of Abuse Screen (DAS) is commonly used for amphetamines, cocaine, marijuana, and so on. When the cause of death is unclear, a General Drug Screen (GDS) is employed. Acidic/Neutral Screen (ANS) is primarily used to detect barbiturates, muscle relaxants, and so on. Basic Drug Screens (BDS) are more specific for the detection of drugs such as cocaine and antidepressants.

It is recommended that the presence of a drug or toxicant be verified in more than one specimen. However, if only one specimen is available, replicate analyses on different occasions should be performed with adequate concurrent positive and negative controls. However, it should be recognized that the compound in question may not necessarily be present in all specimen types. Figure 22.3 shows a typical drug analysis flowchart. The algorithmic approach is employed to adequately identify the drug (or at least drug class) that may be present in a tested specimen.

22.7 CLINICAL TOXICOLOGY

As was pointed out at the beginning of the chapter, the analytical toxicology approaches used in forensic toxicology play important roles in a clinical setting. The methods and instrumentation used in a clinical toxicology laboratory are similar to those used in forensic toxicology laboratories. The described approaches have the following benefits in clinical toxicology:

- Aids the diagnosis and treatment of toxicoses
- Allows the monitoring of treatment effectiveness

- Identification of the nature of exposure
- Quantification of toxicant

22.7.1 History Taking

Taking thorough general history aids in the effective treatment of an intoxicated patient. Often it may not be possible to communicate directly with the patient due to a lack of consciousness, altered mental state leading to inaccurate information, or deliberate submission of misleading information. The type of information that is essential and helpful include:

- Identifying what was taken and when, how much, and by what route
- Presence of preexisting conditions or allergies
- Whether the patient is currently using any medications or substances of any kind
- Whether the patient is pregnant

Historical information can include information obtained from family, friends, law enforcement and medical personnel, and any observers.

22.7.2 Basic Operating Rules in the Treatment of Toxicosis

The following concepts are central to approaching a toxicosis patient:

- Ensure airway so that breathing and circulation are adequate
- Remove unabsorbed material
- Limit the further absorption of toxicant
- Hasten toxicant elimination

Reducing further exposure to a toxicant is crucial and may include removal of the patient from a toxic environment and the application of decontamination procedures. Immediate decontamination reduces absorption of toxic compounds and represents a primary essential aspect of the treatment regimen.

External/Skin Decontamination. This entails the complete removal of clothing and gentle washing of the victim with copious amounts of lukewarm water. Mild soaps are often useful and may increase effectiveness of the removal of the offending substance.

Internal Decontamination. The most recommended methods of internal decontamination Include gastric lavage, whole bowel irrigation and administration of activated charcoal.

Lavage. This is utilized if a patient has ingested a life-threatening toxicant. It is recommended for use within a few short hours after ingestion and involves the use of a nasogastric or orogastric tube to flush the gastrointestinal tract. A large bore tube is inserted into the stomach and the contents removed with sequential administration and aspiration of small quantities of warm water or saline. This technique is contraindicated

in cases of ingestion of corrosives (i.e., acids, bases) and hydrocarbons (i.e., fuels, essential oils).

Whole-Bowel Irrigation. This involves the infusion by nasogastric or orogastric tube of a lavage solution consisting of an isosmotic electrolyte (polyethylene glycol electrolyte solution is currently recommended). This procedure is indicated after ingestion of metals (e.g., iron, lithium), controlled release medications (terms to describe formulations that do not release the active compound immediately after oral ingestion), ingestion of a large amount of an anticholinergic drug (e.g., tricyclics, carbamazepine), and after ingestion of large numbers of tablets. A polyethylene glycol electrolyte solution is administer per hour per os (P.O.) or via a nasogastric tube. Antiemetics may be required to control vomiting. This is continued until the rectal effluent is clear (approximately 3 to 6 hours). The goal is to completely irrigate the gastrointestinal tract to prevent or decrease toxicant absorption. The use of an isosmotic compound such as polyethylene glycol results in minimal electrolyte loss and fluid changes.

Activated Charcoal. This is considered the most useful agent for the prevention of absorption of toxicants. Repeated administration (multiple dose activated charcoal) can impair the enteroenteric-enterohepatic circulation of drugs by binding to drugs that undergo significant enterohepatic or enteroenteric recycling, including carbamazepine, digoxin, phenobarbitone, theophylline, and verapamil. The prescribed amount of activated charcoal is administered every hour P.O or via nasogastric tube.

Emesis and Catharsis. Emesis is not recommended as a treatment measure for the toxicosis patient. The danger of aspiration of the gastric contents is great (leading to asphyxiation or aspiration pneumonia). Also a concern is the damage to esophageal and related tissue by ingested corrosive substances. Sorbitol is a commonly used cathartic. Often used in charcoal formulations, it increases the gut motility to improve excretion of poison-charcoal complexes. It is not recommended in poisonings by compounds that cause profuse diarrhea (e.g., organophosphates, carbamates, and arsenic). The use of a cathartic alone has no value in the management of the poisoned patient. Its use is even controversial as a treatment in combination with activated charcoal. Its use is contraindicated in hypotensive patients, when dehydration or electrolyte balance is present, when corrosive substances have been ingested and in cases of abdominal trauma or surgery, and intestinal perforation or obstruction.

22.7.3 Approaches to Selected Toxicoses

A number of antidotes are effective by altering the distribution or metabolism of a toxicant. Reducing the distribution of toxic substances to their sites of action can be achieved by a variety of methods including blocking access of specific poisons to their receptors with compounds that can compete with these receptors and by using chelating agents to bind the toxicants (e.g., dimercaprol for arsenic). Biotransformation of a toxic compound into a less toxic form can be achieved by certain agents. For example, thiosulphate is used to increase the conversion of cyanide into thiocyanate.

Ethylene Glycol Toxicosis. Ethylene glycol is commonly used as an antifreeze. It is metabolized by alcohol dehydrogenase to mixed aldehydes, carboxylic acids, and

oxalic acid. Toxicosis results in nephrotoxicosis (kidney damage). Administration of fomepizole (4-methylpyrazole) or ethanol is effective due to their competition with ethylene glycol for the alcohol dehydrogenase enzyme. Although ethanol has a long-term history of clinical experience and is less costly to acquire, fomepizole treatment is associated with less adverse effects, predictable pharmacokinetics and has a validated efficacy. Hemodialysis is indicated in patients with severe kidney failure.

Ethanol Toxicosis. Absorption of ethanol by the GI tract absorption is rapid. Peak levels can be reached 30 to 60 minutes after ingestion. As a rule of thumb, 1 ml of absolute ethanol per kilogram weight results in a level of 100 mg/100 ml (0.1%) in 1 hour. Supportive treatment is directed toward the control of acidosis and hypoglycemia.

Organophosphates and Carbamates. The adverse effects of organophosphorous and carbamate pesticides are mediated through the inhibition of the cholinesterase enzymes. One form, acetylcholinesterase, is located at neurosynaptic junctions while butyryl cholinesterase is primarily located in the plasma and pancreas. Organophosphate pesticides inhibit cholinesterase by forming covalent bonds via phosphorylation. Enzymatic regeneration half-lives are long, taking days to months. Organophosphates affect both red blood cell and plasma cholinesterase activity. Carbamates primarily affect only the plasma derivative. Carbamate insecticides inhibit cholinesterase activity in reversible manner. Since carbamates interact with cholinesterase by weak, ionic bonding, the cholinesterases can regenerates itself more readily in matter of minutes to hours.

Since organophosphate toxicosis results in respiratory failure, the treatment approach for must include the maintenance of a patent airway. Artificial respiration may also need to be employed. The first pharmacological approach is the administration of atropine. Atropine competes with acetylcholine for its receptor site, thus reducing the effects of the neurotransmitter. *N*-methylpyridinium 2-aldoxime (2-PAM) is used in with atropine therapy as an effective means to restore the covalently bound enzyme to a normal state. It reacts with the phosphorylated cholinesterase enzyme removing the phosphate group. As previously mentioned, carbamates interact with cholinesterase by weak, ionic bonding; thus 2-PAM is of no use to combat toxicosis caused by these compounds. However, atropine is effective to prevent the effects on respiration.

Arsenic Toxicosis. Urine arsenic is the best indicator of current or recent exposure. Atomic absorption spectrophotometry is preferred as the detection method. Hair or fingernail sampling may also be helpful. Use of blood is useful if analyzed soon after exposure or in cases of continuous chronic exposure. After acute exposure, chelation therapy is instituted utilizing either (1) Dimercaprol BAL (British Anti-Lewisite) and analogues:

DMSA (dimercaptosuccinic acid)
DMPS (dimercaptopropane succinate)

or (2) d-penicillamine. Supportive/symptomatic therapy is also necessary. A higher protein diet and the alleviation of dehydration due to diarrhea and vomiting are beneficial.

Chronic Exposure (Arsenic). Primarily symptomatic treatment is chosen. Chelation therapy is practiced, but its usefulness in cases of chronic exposure is still questionable.

Supportive treatment is an essential component of the management of the intoxicated patient. Monitoring and assessment of all organ systems in conjunction with the use of appropriate pharmaceutical agents/antidotes increases therapeutic success. The nature of this care will depend on the toxicant in question and the patient's condition upon presentation.

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CHAPTER 23

Prevention of Toxicity

ERNEST HODGSON

23.1 INTRODUCTION

It is obvious, but often forgotten, that toxicity is always a consequence of exposure and that no matter what the results of hazard assessment, without exposure there cannot be a toxic effect. However, if both hazard and exposure are verified, and the risk appears to be significant, there are a range of possible actions available to reduce that risk. These actions range from outright banning, from both production and use, of the chemical in question, through measures to restrict exposure, to measures to restrict effect. Exposure can be restricted by prevention of manufacture, control of use patterns, control of application techniques, environmental manipulation, and education. Effects can be restricted by prophylactic and therapeutic methods and by education. Many of these approaches are controlled in whole, or in part, by legislation. All of them, taken together, comprise the subject matter of this chapter.

Laws and regulations provide the framework for organized efforts to prevent toxicity, and sanctions are necessary to prevent those without social conscience from deliberately exposing their fellows to risks from toxic hazards. That is not enough, however, without a population educated to toxic hazards and their prevention, the laws could never be administered properly. Moreover, in many circumstances, and particularly in the home, wisdom dictates courses of action not necessarily prescribed by law. The key to toxicity prevention lies in information and education with legislation, regulation, and penalties as final safeguards. In all probability, the better the general population is educated and informed, the less likely are laws to be necessary.

23.2 LEGISLATION AND REGULATION

In the best sense, legislation provides an enabling act describing the areas to be covered under the particular law and the general manner in which they are to be regulated, and designating an executive agency to write and enforce specific regulations within the intent of the legislative body. For example, the Toxic Substances Control Act (TSCA) was passed by Congress to regulate the introduction of chemicals into commerce, to

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determine their hazards to the human population and the environment, and to regulate or ban those deemed hazardous. The task of writing and enforcing specific regulations was assigned to the Environmental Protection Agency (EPA).

Legislative attempts to write specific regulations into laws usually fail. The resultant laws lack flexibility and, because they are written by lawyers rather than toxicologists, seldom address the problems in a scientifically rigorous manner.

It should be borne in mind that legislation is a synthesis of science, politics, and public and private pressure. It represents a society's best estimate, at that moment, of the risks it is prepared to take and those it wishes to avoid, as well as the price it is prepared to pay. Such decisions properly include more than science. The task of the toxicologist is to see that the science that is included is accurate and is interpreted logically.

This section is based primarily on regulations in the United States, not because these are the best but because, in toto, they are the most comprehensive. In many respects they are a complex mixture of overlapping laws and jurisdictions, providing unnecessary work for the legal profession. At the same time few, if any, toxic hazards in the home, workplace, or environment are not addressed.

23.2.1 Federal Government

Following is a summary of the most important federal statues concerned in whole or in part with the regulation of toxic substances.

Clean Air Act. The Clean Air Act is administered by the EPA. Although the principal enforcement provisions are the responsibility of local governments, overall administrative responsibility rests with EPA. This act requires criteria documents for air pollutants and sets both national air quality standards and standards for sources that create air pollutants, such as motor vehicles, power plants, and so on. Important actions already taken under this law include standards for the now complete phased-out elimination of lead in gasoline, and the setting of sulfuric acid air emission guidelines for existing industrial plants.

Clean Water Act. The Clean Water Act, which amends the Federal Water Pollution Control Act, is also administered b the EPA and provides for funding of municipal sewage treatment plants. However, with respect to toxicity prevention, it is more important that the act regulates emissions from municipal and industrial sources. It has as its goal the elimination of discharges of pollutants and the protection of rivers so that they are "swimmable and fishable" and applies to "waters of the United States" subsequently defined to include all waters that reach navigable waters, wetland, and intermittent streams. Some of the more important actions taken under this statute include setting standards for emissions of inorganics from smelter operations and publishing priority lists of toxic pollutants. This act allows the federal government to recover cleanup and other costs as damages from the polluting agency, company, or individual.

Safe Drinking Water Act. (1974, 1986, 1996). Specifically applied to water supplied for humans consumption, this act requires the EPA to set maximum levels for contaminants in water delivered to users of public water systems. Two criteria are established for a particular contaminant: the *maximum containment level goal* (MCGL) and the *maximum contaminant level* (MCL). The former, the MCLG, is the level at which no

known or anticipated adverse effects on the health of persons occur and within an allowed adequate margin of safety. The latter, the MCL, is the maximum permissible level of a contaminant in water that is delivered to any user of a public water system. MCLs are expected to be as close to the MCLG as is feasible.

Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). This is an attempt to deal with the many waste sites that exist across the nation. It covers remedial action, including the establishment of a National Priorities List to identify those sites that should have a high priority for remediation. This act authorizes the cleanup of hazardous waste sites, including those containing pesticides, that threaten human health or the environment. If they can be identified, the US EPA is authorized to recover cleanup costs from those parties responsible for the contamination. CERCLA provides a fund to pay for the cleanup of contaminated sites when no other parties are able to conduct the cleanup. The Superfund Amendments and Reauthorization Act (SARA) (1986) is an amendment to CERCLA that enables the US EPA to identify and cleanup inactive hazardous waste sites and to recover reimbursement of cleanup costs. One section of CERCLA authorizes the EPA to act whenever there is a release or substantial threat of release of a hazardous substance or "any pollutant or contaminant that may present an imminent or substantial danger to the public health or welfare" into the environment.

Consumer Products Safety Act and Consumer Products Safety Commission Improvements Act. Administered by a Consumer Products Safety Commission, the Consumer Products Safety Act is designed to protect the public against risk of injury from consumer products and to set safety standards for such products.

Controlled Substances Act. The Controlled Substances Act not only strengthens law enforcement in the field of drug abuse but also provides for research into the prevention and treatment of drug abuse.

Federal Food, Drug, and Cosmetic Act. The Federal Food, Drug, and Cosmetic Act is administered by the Food and Drug Administration (FDA). It establishes limits for food additives and cosmetic components, sets criteria for drug safety for both human and animal use, and requires the manufacturer to prove both safety and efficacy. The FDA is authorized to define the required toxicity testing for each product. This act contains the Delaney clause, which states that food additives that cause cancer in humans or animals at any level shall not be considered safe and are therefore prohibited from such use. This clause has recently been modified to permit the agency to use more flexible risk-benefit based guidelines. Under the Food Quality Protection Act of 1966 (see below) the Delaney clause is no longer applied to pesticide residues in food. This law also empowers the FDA to establish and modify the generally recognized as safe (GRAS) list and to establish good laboratory practice (GLP) rules.

Occupational Safety and Health Act. Administered by the Occupational Safety and Health Administration (OSHA), the Occupation Safety and Health Act concerns health and safety in the workplace, OSHA sets standards for worker exposure to specific chemicals, for air concentration values, and for monitoring procedures. Construction and environmental controls also come under this act. This act provides for research, information, education, and training in occupational safety and health.

By establishing the National Institute for Occupational Safety and Health (NIOSH), the act provided for appropriate studies to be conducted so that regulatory decisions could be based on the best available information.

National Environmental Policy Act. The National Environmental Policy Act is an umbrella act covering all US government agencies, requiring them to prepare environmental impact statements for all federal actions affecting the quality of the human environment. Environmental impact statements must include not only an assessment of the effect of the proposed action on the environment, but also alternatives to the proposed action, the relationship between local short-term use and enhancements of long-term productivity, and a statement of irreversible commitment of resources. This act also created the Council on Environmental Quality, which acts in an advisory capacity to the president on matters affecting or promoting environmental quality.

Resource Conservation and Recovery Act. Also administered by the EPA, the Resource Conservation and Recovery Act (RCRA) is the most important act governing the disposal of hazardous wastes including pesticide formulations, containers, and rinsates; it promulgates standards for identification of hazardous wastes, their transportation, and their disposal. Included in the latter are siting and construction criteria for landfills and other disposal facilities as well as the regulation of owners and operators of such facilities. The three principal areas covered are hazardous wastes, nonhazardous solid wastes, and underground storage tanks. Farmers and commercial pesticide applicators are subject to penalties if they fail to store or dispose of pesticides and pesticide containers properly. The Agency is responsible for enforcement.

Toxic Substances Control Act. Administered by the EPA, the TSCA is mammoth, covering almost all chemicals manufactured in the United States for industrial and other purposes, excluding certain compounds covered under other laws such as FIFRA. The EPA may control or stop production of compounds deemed hazardous. Producers must give notice or intent to manufacture new chemicals or increase significantly the production of existing chemicals. They may be required to conduct toxicity and other tests. This law is as yet incompletely applied due to the enormous number of existing chemicals that must be evaluated. Once fully applied, it will be the most important statute affecting toxicology.

Statutes Affecting the Manufacture and Use of Agricultural Chemicals. Because of the intense interest and concern over the use of agricultural chemicals, especially pesticides, and their possible effects on human health, these statutes are perhaps the most overregulated group of commercial xenobiotics in use today. A number of laws deal almost exclusively with this use class while several others also deal with them, to a greater or lesser extent. The first law directed specifically toward pesticides in the United States was the Insecticide Act of 1910. This act was passed to ensure that the percentages of ingredients were as stated and that the product was efficacious.

Surprisingly, it was 37 years before a law was written to replace the 1910 Act. This replacement was the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). First passed in 1947 and amended many times since, this act is now administered by the EPA. FIFRA regulates all pesticides and other agricultural chemicals, such as plant growth regulators, used in the United States. Establishing the requirement "that the

burden of proof of a product's acceptability rested with the manufacturer," it includes the authority to establish registration requirements, with appropriate chemical and toxicological tests prescribed by the agency. This act also permits the agency to specify labels, to restrict application to certified applicators, and to deny, rescind, or modify registration. Under this act the EPA also establishes tolerances for residues on raw agricultural products. FIFRA was amended in 1988 requiring a re-evaluation of all pesticides manufactured prior to 1984. The purposes of the 1988 amendment were to remove hazardous pesticides and to require additional testing, primarily toxicity tests that were not available when these early compounds were registered. Section 19 of the 1988 FIFRA amendments greatly expanded the Agency's authority to regulate pesticide storage, transport and disposal of pesticides, containers, and rinsates of containers.

The Food Quality Protection Act (FQPA) of 1996 is an amendment to FIFRA and provides a new standard for evaluating pesticides applied to food crops, in that there be "reasonable certainty of no harm" from residues found on food. US EPA is required to perform an aggregate risk assessment that combines dietary risk from a specific pesticide with those from resides in drinking water and from residential exposure. As a result of this law, the US EPA is required to reevaluate all existing food tolerance residue levels based on a number of criteria. One of these is to determine the cumulative (combined) risk of exposure to classes of pesticides having the same mechanism of toxicity, with special emphasis on infants and children. In some instances this has required adding an additional safety factor to the tolerance of between 3 and 10 for certain compounds to ensure the safety of children. This factor is in addition to the safety factor of 100 covering differences due to species and individual variation. Thus, if typical residue levels on a food crop are 1.0 ppm, then a tolerance of 0.01 ppm could be established, and if the additional factor of 10 were added, the tolerance could be set at 0.001 ppm. Currently organophosphate insecticides and several other classes are undergoing this reassessment process.

The Act established the Tolerance Reassessment Advisory Committee (TRAC), composed of individuals with a variety of backgrounds and interests to consult and make recommendations to both the EPA and USDA. When this committee went out of existence in 1999, the EPA and USDA established a new advisory committee, the Committee to Advise on Reassessment and Transition (CARAT) to provide strategic advice on issues raised by this Act.

An Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) was established to develop a comprehensive screening and testing program for pesticides and other compounds to determine potential estrogenic effects on both humans and on wildlife. FQPA is one of the most significant amendments ever made to FIFRA and continues to generate considerable controversy as it is put into effect.

The Worker Protection Standard for Agricultural Pesticides (1994) was written to protect workers from pesticide exposures. Responsibility lies with the employer and involves two types of employees: agricultural workers (e.g., harvesters) and pesticide handlers (e.g., mixers). It requires that these people be provided safety training and access to labels, and that medical treatment be made available prior to and 30 days after the REI has expired. The types of protection offered include notification prior to applying pesticides, exclusion during applications and during an REI, and monitoring the worker's PPE. In addition the employer is required to provide a decontamination site equipped with water and a clean change of clothes.

The Act was written to cover pesticide use on farms, forests, nurseries, and greenhouses. It does not include applications to pastures, golf courses, parks, livestock, right-of-way, or home gardens, nor does it cover treatments for mosquito abatement and rodent control.

Many other legislative acts impact in whole or in part on pesticide use. They include the Endangered Species Act of 1973, an act written to protect endangered wildlife; it regulates pesticide use around wildlife sanctuaries. Pesticides might injure or kill endangered species if allowed to drift onto habitat, or runoff into streams, lakes, or wetlands might be found to significantly degrade endangered wildlife habitat. Also included are the Clean Water Act, the Safe Drinking Water Act, RCRA, CERCLA, and SARA, all discussed above.

Other Statutes with Relevance to the Prevention of Toxicity. It should be noted that some of these statutes have been superseded by others, either in whole or in part.

- Comprehensive Employment and Training Act
- Dangerous Cargo Act
- Federal Coal Mine Safety and Health Amendment Act
- Federal Caustic Poison Act
- Federal Railroad Safety Authorization Act
- Hazardous Materials Transport Act
- Lead-Based Paint Poison Prevention Act
- Marine Protection Research and Sanctuaries Act
- Poison Prevention Packaging Act
- Ports and Waterways Safety Act

23.2.2 State Governments

Within the United States, states are free to adopt legislation with toxicological significance, although their jurisdiction does not extend beyond their geographic boundaries. In other cases the states may enforce federal statutes under certain circumstances. For example, if state regulations concerning hazardous waste disposal is neither less comprehensive nor less rigorous than the federal statute, enforcements is delegated to the states. Similarly certain aspects of FIFRA are enforced by individual states. In some cases (California is notable in this respect) states have passed laws considerably more comprehensive and more rigorous than the corresponding federal stature.

23.2.3 Legislation and Regulation in Other Countries

It would serve little purpose to enumerate all the laws affecting toxicology, toxicity testing, and the prevention of toxicity that have been promulgated in all countries that have such laws. Legislation in this area has been adopted in most countries of western Europe and in Japan. Although the laws in use in the United States are a complex mixture of overlapping statutes and enforcement agencies, they are probably the most comprehensive set of such laws in existence. Most other industrialized countries have legislation in the same areas, although the emphasis varies widely from one country to

another. Many underdeveloped countries, due to the lack of both trained workers and financial resources, are unable to write and enforce their own code of regulations, and instead, many adopt the regulatory decisions of either the United States or some other industrialized nation. For example, they will permit the use, in their own territory, of pesticides registered under FIRA by the US EPA and will prohibit the use of pesticides not so registered.

23.3 PREVENTION IN DIFFERENT ENVIRONMENTS

Humans spend their time in many environments. Homes vary with climate, family income, and personal choice. The workplace varies from pristine mountains to industrial jungles, and the outdoor environment from which recreation, food, and water are derived varies through the same extremes. Each of these environments has its own specific complex of hazards, and thus requires its own set of rules and recommendations if these hazards are to be avoided.

23.3.1 Home

Approximately 50% of all accidental poisoning fatalities in the United States involve preschool children. Thus prevention of toxicity is particularly important in homes with young children.

Prescription drugs should always be kept in the original container (in the United States and some other countries, these are now required to have safety closures). They should be taken only by the person for whom they were prescribed, and excess drugs should be discarded safely when the illness is resolved. When children are present, prescription drugs should be kept in a locked cabinet because few cabinets are inaccessible to a determined child. Although nonprescription drugs are usually less hazardous, they are frequently flavored in an attractive way. Thus it is prudent to follow the same rules as for prescription drugs.

Household Chemicals such as lye, polishes, and kerosene should be kept in locked storage if possible; if not, they should be kept in as secure a place as possible, out of the reach of children. Such chemicals should never be stored in anything but the original containers. Certainly they should never be stored in beverage bottles, kitchen containers, and so on. Unnecessary materials should be disposed of safely in appropriate disposal sites.

Certain Household Operations such as interior painting, and so on, should be done only with adequate ventilation. Insecticide treatment should be done precisely in accordance with instructions on the label.

Increasing fuel costs have caused several changes in lifestyle, and some of these changes carry potential toxic hazards. They include more burning of wood and coal and the construction of heavily insulated houses with a concomitant reduction in ventilation. In the latter circumstances, improperly burning furnaces can generate high levels of CO and aromatic hydrocarbons, whereas even those burning properly may still generate oxides of nitrogen (NO_x) at levels high enough to cause respiratory tract irritation in sensitive individuals. These effects can be avoided by ensuring that all

heating equipment (e.g., furnaces, wood stoves, heaters) is properly ventilated, maintained, and checked regularly. In addition some ventilation of the building itself should always be provided. Less ventilation is needed when the temperature is either excessively high or excessively low, and more is needed when the temperature is in the midrange, but under no circumstances should the homeowner strive for a completely sealed house.

23.3.2 Workplace

Exposure levels of hazardous chemicals in the air of work environments are mandated by OSHA as exposure limit values. The studies necessary to establish these limits are carried out by NIOSH. However, the more complete list of the better-known threshold limit values (TLVs) is established by the American Conference of Governmental Industrial Hygienists. Although TLVs are not binding in law, they are an excellent guide to the employer. In fact they are often adopted by OSHA as exposure limit values. The concentrations thus expressed are the weighted average concentrations normally considered safe for an exposure of 8 h/day, 5 days/week. Absolute upper limits (excursion values) may also be included. Some exposure limits are shown in Table 23.1.

Concentrations at or lower than those normal or working exposures are usually maintained by environmental engineering controls. Operations that generate large amounts of dusts or vapors are conducted in enclosed spaces that are vented separately or under hoods. Other spaces are ventilated adequately, and temperature and humidity controls are installed where necessary.

Table 23.1 Some Selected Threshold Limit Values (1991)

Chemical	TLV-TWA[a] (ppm)	TLV-STEL[b] (ppm)	TLV-C[c] (ppm)
Acetaldehyde	100	150	—
Boron trifluoride	—	—	1
o-Dichlorobenzene	—	—	50
p-Dichlorobenzene	75	110	—
N-Ethylmorpholine	5	20	—
Fluorine	1	2	—
Phosgene	0.1	—	—
Trichloroethylene	50	200	—

[a]TLV-TWA, threshold limit value: time-weighted average, concentration for a normal 8-hour workday and 40-hour workweek to which nearly all workers may be repeatedly exposed without adverse effect.

[b]TLV-STEL, threshold limit value: short-term exposure limit, concentration. This time-weighted 15-minute average exposure should not be exceeded at any time during a workday even if the TLV-TWA is within limits. Intended as supplement to TLV-TWA.

[c]TLV-C, threshold limit value–ceiling, concentration that should not be exceeded at any time.

Other precautions must be taken to prevent accidental or occasional increases in concentrations. Materials should be transported in "safe" containers, spilled material removed rapidly, and floor and wall materials selected to prevent contamination and allow easy cleaning.

Additional methods for the prevention of toxicity in the workplace include the use of personal safety equipment—protective clothing, gloves, and goggles are the most important. In particularly hazardous operations, closed-circuit air masks, gas masks, and so on, may also be necessary.

Pre-employment instruction and pre-employment physical examinations are of critical importance in most work situations involving hazardous chemicals. The former should make clear the hazards involved, the need to avoid exposure under normal working conditions, and the mechanisms by which exposure is limited. Furthermore employees should understand how and when to contain spills and how and when to evacuate the area around the spill. Locations and use of emergency equipment, showers, eye washes, and so on, should also be given, and the most important procedures should be posted in the work area.

23.3.3 Pollution of Air, Water, and Land

The toxicological significance of pollution of the environment may be work related, as in the case of agricultural workers, or related to the outside environment encountered in daily life. In the case of agricultural workers, numerous precautions are necessary for the prevention of toxicity. For example:

- Pesticides and other agricultural chemicals should be kept only in the original container, carrying the labels prescribed by EPA under FIFRA.
- Empty containers and excess chemicals should be disposed of properly in safe hazardous waste disposal sites, incinerated when possible, or, in some cases, decontaminated.
- Workers should not re-enter treated areas until the safe re-entry period has elapsed.
- Certain workers such as applicators and those preparing tank mixes should wear appropriate protection clothing, gloves, face masks, and so forth. The development of closed systems for mixing pesticides should help protect mixers and loaders of pesticides from exposure.
- Spraying operations should be carried out in such a way as to minimize drift, contamination of water, and so on.

Pesticides have caused a number of fatalities in the past. The current practice in some countries of restricting the most hazardous chemicals for use only by certified operators should greatly minimize pesticide poisoning in these locations.

Individuals can do little to protect themselves from poisoning by chemicals that pollute the air and water except to insist that discharge of toxicants into the environment be minimized. The exposure levels are low compared with those in acute toxicity cases, and the effects may be indirect, as in the increase in preexisting respiratory irritation during smog. Thus these effects can be determined only at the epidemiologic level. Because many persons are not affected or may not be affected for years, it is often argued that environmental contamination is not very important. However, a

small percentage increase may represent a large number of people when the whole population is considered. Furthermore chronic toxicity is not often reversible. Because in most industrialized countries laws already exist to control emission problems, if such problems exist in these countries, they are usually problems of enforcement.

One of the most critical areas for the prevention of toxicity caused by environmental contamination is that of disposal of hazardous wastes. It is now apparent that past practices in many industrialized countries have created large numbers of waste sites in which the waste is often unidentified, improperly stored, and leaching into the environment. The task of rectifying these past errors is an enormous one just now being addressed.

The ideal situation for current and future practices is to reduce chemical waste to an irreducible minimum and then to place the remainder in secure storage. Waste reduction can be accomplished in many ways.

- Refine plant processes so that less waste is produced.
- Recycle waste into useful products.
- Concentrate wastes.
- Incinerate. The technology is available to incinerate essentially all waste to inorganic slag. Unfortunately, the technology is sophisticated and expensive. Inadequate incineration is itself a hazard because of the risk of generating dioxins and other toxicants and releasing them into the environment. Less complex and more easily maintained incinerators will be essential if this technology is to play a prominent role in waste reduction.

Safe storage for the remaining waste may be in dump sites or in above-ground storage. In either case such storage ideally should be properly sited, constructed, maintained, and monitored.

Because of the nature of commerce, probably none of these measures will be successful unless the laws, penalties, and incentives are manipulated in such a way as to make safe disposal more attractive economically than unsafe disposal.

23.4 EDUCATION

Because chemicals, many of them hazardous, are an inevitable part of life in industrialized countries, education is probably the most important method for the prevention of toxicity. Unfortunately, it is also one of the most neglected. In a typical public debate concerning a possible chemical hazard, the principle protagonists tend to fall into two extreme groups. The “everything is OK” protagonists and the “ban it completely” protagonists. The media seldom seem to educate the public, usually serving only to add fuel to the flames.

The educational role of the toxicologist should be the voice of reason, presenting a balanced view of risks and benefits, and outlining alternatives whenever possible. The simple lesson that science deals not in certainty, but rather degrees of certitude, must be learned by all involved.

In terms of ongoing educational programs, there should be opportunities at all levels: elementary schools, high schools, university, adult education, and media education. Several approaches can be used to educate the general public in ideal situations:

- *Elementary school.* Teach the rudiments of first aid and environmental concerns, proper disposal, and so on.
- *High school.* Teach concepts of toxicology (dose response, etc.) and environmental toxicology (bioaccumulation, etc.), as these concepts can be introduced into general science courses.
- *University.* In addition to toxicology degrees, general courses for nontoxicology and/or nonscience majors should stress a balanced approach, with both responsible use and toxicity prevention as desirable end points. General Toxicology should be a required course in all chemically related academic programs such as chemistry and chemical engineering.
- *Media.* Encourage a balanced approach to toxicity problems. Toxicologists should be available to media representatives and, where appropriate, should be involved directly.

SUGGESTED READING

American Conference of Governmental Industrial Hygienists. *TLVs—Threshold Limit Values for Chemical Substances and Physical Agents in the Work Environment with Intended Changes*. Cincinnati (published annually).

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CHAPTER 24

Human Health Risk Assessment

RONALD E. BAYNES

24.1 INTRODUCTION

We often perform toxicological research to better understand the mechanism and associated health risk following exposure to hazardous agents. Risk assessment is a systematic scientific characterization of potential *adverse health effects* following exposure to these hazardous agents. Risk assessment activities are designed to *identify, describe*, and *measure qualities and quantities* from these toxicological studies, which are often conducted with homogeneous animal models at doses and exposure duration not encountered in a more heterogeneous human population. Herein lie the challenge of risk assessment. The use of default assumptions because of some level of uncertainty in our extrapolations across species, doses, routes, and interindividual variability, the risk assessment process is often perceived as lacking scientific rigor. This chapter will cover traditional practices as well as new and novel approaches that utilize more of the available scientific data to identify and reduce uncertainty in the process. The advent of powerful computers and sophisticated software programs has allowed the development of quantitative models that better describe the dose-response relationship, refine biologically relevant dose estimates in the risk assessment process, and encourage departure from traditional default approaches (Conolly et al., 1999). Although the focus of this chapter is on current and novel risk assessment methods that are scientifically based, it is critical that the reader be aware of the differences between risk assessment and risk management, which are summarized in Table 24.1.

Results from the risk assessment are used to inform *risk management*. The risk manager uses the risk information in conjunction with factors such as the social importance of the risk, the social acceptability of the risk, the economic impacts of risk reduction, engineering, and legislative mandates when deciding on and implementing risk management approaches.

The risk assessment may be perceived as the source of a risk management decision, when in fact, social concerns, international issues, trade, public perception, or other non-risk considerations may be taken into consideration. Finally there is one activity known as *risk communication* that involves making the risk assessment and risk

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Table 24.1 Comparison of Risk Assessment and Risk Management Activities

Risk Assessment	Risk Management
Nature of effects	Social importance of risk
Potency of agent	Acceptable risk
Exposure	Reduce/not reduce risk
Population at risk	Stringency of reduction
Average risk	Economics
High-end risk	Priority of concern
Sensitive groups	Legislative mandates
Uncertainties of science	Legal issues
Uncertainties of analysis	Risk perception
Identify	*Evaluate*
Describe	*Decide*
Measure	*Implement*

management information comprehensible to lawyers, politicians, judges, business and labor, environmentalists, and community groups.

24.2 RISK ASSESSMENT METHODS

According to the National Research Council of the National Academy of Science, risk assessment consists of four broad but *interrelated* components: hazard identification, dose-response assessment, exposure assessment, and risk characterization, as depicted in Figure 24.1. The reader should, however, be aware that these risk assessment activities can provide research needs that improve the accuracy of estimating the "risk" or probability of an adverse outcome.

24.2.1 Hazard Identification

In this first component of risk assessment, the question of causality in a qualitative sense in addressed; that is, the degree to which evidence suggests that an agent elicits

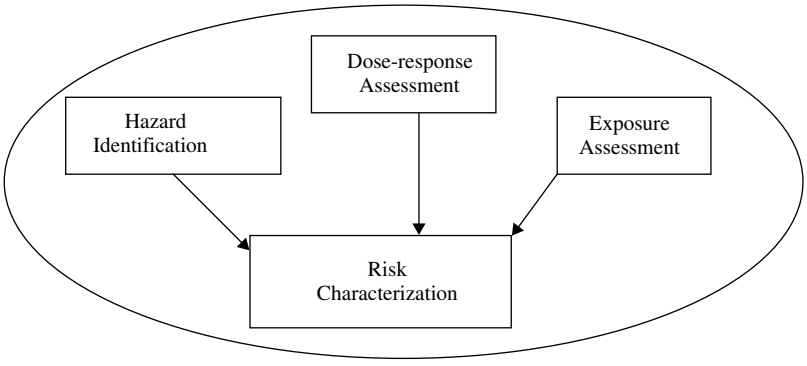

Figure 24.1 Risk assessment paradigm as per NAS and US EPA.

a given effect in an exposed population. Among many factors the quality of the studies and the severity of the health effects should be evaluated at this stage. The following are evaluated: (1) validity of the toxicity data, (2) weight-of-evidence summary of the relationship between the substance and toxic effects, and (3) estimates of the generalizability of data to exposed populations. Where there are limited in vivo toxicity data, *structural activity relationships* (SARs) and *short-term assays* may be indicative of a chemical hazard. Key molecular structures such as *n*-nitroso or aromatic amine groups and azo dye structures can be used for prioritizing chemical agents for further testing. SARs are useful in assessing relative toxicity of chemically related compounds, but there are several limitations. For example, toxicity equivalent factors (TEFs) based on induction of Ah receptor by dioxins demonstrated that SARs may not always be predictive. In vitro short-term inexpensive test such as bacterial mutation assays can help *identify* carcinogens, and there are other short-term tests that can help identify chemicals that potentially can be associated with neurotoxicity, developmental effects, or immunotoxicity. Many of these in vitro studies can provide some insight into mechanism(s) of action, but there may be some *false positives* and *false negatives*. Animal studies are usually route-specific and relevant to human exposure, and animal testing usually involves two species, both sexes, 50 animals/dose group, and near-lifetime exposures. Doses are usually 90, 50, and 10 to 25% of the maximum tolerated dose (MTD). In carcinogenicity studies, the aim is to observe significant increases in number of tumors, induction of rare tumors, and earlier induction of observed tumors. However, rodent bioassays may not be predictive of human carcinogenicity because of mechanistic differences For example, renal tumors in male rats is associated with $\alpha_{2\mu}$-globulin-chemical binding and accumulation leading to neoplasia; however, $\alpha_{2\mu}$-globulin is not found in humans, mice, or monkeys. There are differences in susceptibility to aflatoxin-induced tumors between rats and mice that can be explained by genetic differences in expression of cytochrome P450 and GST isoenzymes. Whereas humans may be as sensitive as rats to AFB_1-induced liver tumors, mice may not be predictive of AFB_1-induced tumors in humans. Epidemiological data from human epidemiological studies are the most convincing of an association between chemical exposure and disease, and therefore can very useful for hazard identification. Exposures are not often well defined and retrospective, and confounding factors such as genetic variations in a population and human lifestyle differences (e.g., smoking) present a further challenge. The three major types of epidemiological studies available are (1) *cross-sectional studies*, which involve sampling without regard to exposure or disease status, and these studies identify risk factors (exposure) and disease but not useful for establishing cause-effect relationships; (2) *cohort studies*, which involve sampling on the basis of exposure status, and they target individuals exposed and unexposed to chemical agent and monitored for development of disease, and these are *prospective studies*; (3) *case-control studies*, which involve sampling on the basis of disease status. These are retrospective studies, where diseased individuals are matched with disease-free individuals.

24.2.2 Exposure Assessment

This process is an integral part of the risk assessment process. However this will be introduced only briefly in this chapter, and the reader is encouraged to consult Chapter 28 in this text as well as numerous other texts that describe the process in

more depth. In brief, exposure assessment attempts to identify potential or completed exposure pathways resulting in contact between the agent and at-risk populations. It also includes demographic analysis of at-risk populations describing properties and characteristics of the population that potentiate or mitigate concern and description of the magnitude, duration, and frequency of exposure. The reader should be aware that exposure may be aggregate (single event added across all media) and/or cumulative (multiple compounds that share a similar mechanism of toxicity). Various techniques such as biomonitoring, model development, and computations can be used to arrive at an estimate of chemical dose taken up by humans, that is, chemical exposure. For example, the lifetime average daily dose (LADD) is a calculation for individuals exposed at levels near the middle of the exposure distribution:

$$\text{LADD} = \frac{(\text{Conc. in media}) \times (\text{Contact rate}) \times (\text{Contact fraction}) \times (\text{Exposure duration})}{(\text{Body weight}) \times (\text{Lifetime})}.$$

Biological monitoring of blood and air samples represent new ways of reducing uncertainty in these extrapolations. For occupational exposures there are occupational exposure limits (OELs) that are guidelines or recommendations aimed at protecting the worker over their entire working lifetime (40 years) for 8 h/day, 5 days/week work schedule. Most OELs are presented as a time-weighted average concentration for an 8-hour day for a 40-hour work week. There are threshold limit values (TLVs) that refer to airborne concentrations and conditions under which workers may be exposed daily but do not develop adverse health effects. The short-term exposure limit (STEL) are recommended when exposures are of short duration to high concentrations known to cause acute toxicity.

24.2.3 Dose Response and Risk Characterization

Dose response is a quantitative risk assessment process, and primarily involves characterizing the relationship between chemical potency and incidence of adverse health effect. Approaches to characterizing dose-response relationships include effect levels such as LD50, LC50, ED50, no observed adverse effect levels (NOAELs), margins of safety, therapeutic index. The dose-response relationship provides an estimation of the relationship between the dose of a chemical agent and incidence of effects in a population. Intuitively, a steep dose-response curve may be indicative of a homogeneous population response, while less steep or almost flat slope may be indicative of greater distribution in response. In extrapolating from relatively high levels of exposure in experimental exposures (usually animals) to significantly lower levels that are characteristic of the ambient environment for humans, it is important to note the shape of the dose-response function below the experimentally observable range and therefore the range of inference. The shape of the slope may be linear or curvilinear and, it should be noted that the focus of risk assessment is generally on these lower regions of the dose-response curve (Figure 24.2).

There is a class of curvilinear dose-response relationships in toxicological and epidemiological studies that may be described as *U-shaped* or *J-shaped curves*. Other terms such as biphasic, and more recently *hormesis*, have been used to refer to paradoxical effects of low-level toxicants. In brief, these dose-response curves reflect an apparent improvement or reversal in the effect of an otherwise toxic agent. These

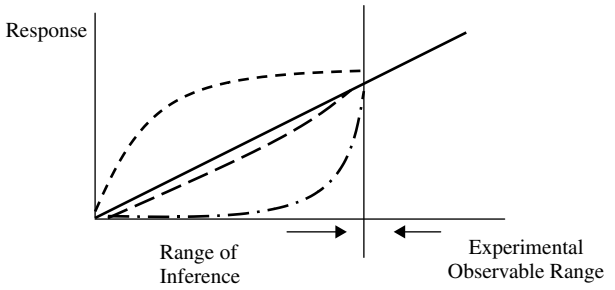

Figure 24.2 Dose-response curve, with emphasis on the shape of the dose-response function below the experimentally observable range and therefore the range of inference where people are realistically exposed.

U-shaped effects can be explained in terms of homeostatic adjustments or overcorrections in the operation of feedback mechanisms. Examples of studies with data fitting a U-shaped curve include the hormetic effect of organic lead on body growth in rats (Cragg and Rees, 1984) and peripheral nerve conduction velocity in children at low doses (Ewert et al., 1986). Similar relationships have been observed with alcohol and nicotine in humans. It has been proposed that because thresholds are inherent in U-shaped dose-response curves, the linear no-threshold extrapolation method is not an appropriate approach for regulating hormetic agents. The current risk assessment paradigm used by US EPA and other federal agencies does not conflict with the concept of hormesis, but it has been proposed that the risk assessor's analyzes make an active consideration of the data and the application of that data in the low dose portion of the dose-response curve for hormetic agents.

24.3 NONCANCER RISK ASSESSMENT

The noncancer risk assessment process assumes a *threshold*. For many noncarcinogenic effects, protective mechanisms are believed to exist that must be overcome before an adverse effect is manifested. At the cellular level for some toxicant, a range of exposures exists from zero to some finite value that can be tolerated by the organism with essentially no chance of expression of adverse effects. The aim here in risk assessment is to identify the upper bound of this tolerance range (i.e., the maximum subthreshold level). This approach involves obtaining the no observed adverse effect level. NOAEL is the highest dose level that *does not produce a significant* elevated increase in an adverse response. Significance refers to biological and statistical criteria and is dependent on dose levels tested, number of animals, background incidence in the unexposed control groups. Sometimes there is insufficient data to arrive at a NOAEL, and a LOAEL (lowest observed adverse effect level) is derived. The NOAEL is the key datum obtained from the study of the dose-response relationship. The NOAEL is used to calculate reference doses (RfD) for chronic oral exposures and reference concentrations (RfC) for chronic inhalation exposures as per EPA. Other agencies, such as the ATSDR and WHO, use the NOAEL to calculate *minimum risk levels* (MRLs) and *acceptable daily intakes* (ADI). The US EPA describes the RfD as an estimate, with uncertainty spanning an order of magnitude, of a daily exposure to the human population, including

sensitive subgroups, that is likely to be without appreciable deleterious effects during a lifetime. In deriving reference doses, ADIs, or MRLs, the NOAEL is divided by uncertainty factors (UF) as per EPA (EPA, 1989) and ATSDR (ATSDR, 1993) and by modifying factors (MF) as per EPA:

$$\text{RfD} = \frac{\text{NOAEL}}{(\text{UF} * \text{MF})}, \qquad \text{US EPA;}$$

$$\text{MRL} = \frac{\text{NOAEL}}{\text{UF}}, \qquad \text{ATSDR.}$$

The calculated RfD or RfC is based on the selected critical study and selected critical end point. The risk assessor may obtain numerous studies where the toxicant may have more than one toxic end point, and thus there may be many NOAELs to choose from the literature. In some instances poor data quality may be used to exclude those end points from consideration. Also at issue is the determining what is considered an adverse effect, and this has been summarized with a few examples in Table 24.2. In sum, the MRL or RfD is based on the less serious effects and no serious effects. The following are example effects not used in obtaining a NOAEL: decrease in body weight less than 10%, enzyme induction with no pathologic changes, changes in organ weight with no pathologic changes, increased mortality over controls that is not significant ($p > 0.05$), and hyperplasia or hypertrophy with or with out changes in organ weights.

24.3.1 Default Uncertainty and Modifying Factors

Most extrapolations from animal experimental data in the risk assessments require the utilization of uncertainty factors. This is because we are not certain how to extrapolate across species, with species for the most sensitive population, and across duration. To account for variations in the general population and to protect sensitive subpopulations, an uncertainty factor of 10 is used by EPA and ATSDR. The value of 10 is derived from a threefold factor for differences in toxicokinetics and for threefold factor for toxicodynamics. To extrapolate from animals to humans and account for interspecies variability between humans and other mammals, an uncertainty factor of 10 is used by EPA and ATSDR, and as with intraspecies extrapolations, this 10-fold factor is assumed to be associated with in toxicodynamics and toxicokinetics. An uncertainty

Table 24.2 Comparison of Less Serious Effects and Serious Effects

Less Serious	Serious
Reversible cellular changes	Death
Necrosis, metaplasia, or atrophy	Cancer
	Clinically significant organ impairment
Delayed ossifciation	Visceral or skeletal abnormalities
Alteration in offspring weight	Cleft palate, fused ribs
Altered T-cell activity	Necrosis inn immunologic components
Auditory disorders	Visual disorders
50% Reduction in offspring	Abnormal sperm

factor of 10 is used when a NOAEL derived from a subchronic study instead of a chronic study is used as the basis for a calculation of a chronic RfD (EPA only). Note that ATSDR does not perform this extrapolation but derive chronic and subchronic MRLs. An uncertainty factor of 10 is used in deriving an RfD or MRL from a LOAEL when a NOAEL is not available. It should be noted that there are no reference doses for dermal exposure, however when there is insufficient dermal absorption data, the EPA uses a default factor of 10% to estimate bioavailability for dermal absorption. A modifying factor ranging from 1 to 10 is included by EPA only to reflect a qualitative professional assessment of additional uncertainties in the critical study and in the entire data base for the chemical not explicitly addressed by preceding uncertainty factors.

Refinements of the RfC have utilized mechanistic data to modify the interspecies uncertainty factor of 10 (Jarabek, 1995). The reader should appreciate that with the inhalation route of exposure, dosimetric adjustments are necessary and can affect the extrapolations of toxicity data of inhaled agents for human health risk assessment. The EPA has included dosimetry modeling in RfC calculations, and the resulting dosimetric adjustment factor (DAF) used in determining the RfC is dependent on physiochemical properties of the inhaled toxicant as well as type of dosimetry model ranging from rudimentary to optimal model structures. In essence, the use of the DAF can reduce the default uncertainty factor for interspecies extrapolation from 10 to 3.16.

The 1996 Food Quality Protection Act (FQPA) now requires that an additional safety factor of 10 be used in the risk assessment of pesticides to ensure the safety of infants and children, unless the EPA can show that an adequate margin of safety is assured with out it (Scheuplein, 2000). The rational behind this additional safety factor is that infants and children have different dietary consumption patterns than adults and infants, and children are more susceptible to toxicants than adults. We do know from pharmacokinetics studies with various human pharmaceuticals that drug elimination is slower in infants up to 6 months of age than in adults, and therefore the potential exists for greater tissue concentrations and vulnerability for neonatal and postnatal effects. Based on these observations, the US EPA supports a default safety factor greater or less than 10, which may be used on the basis of reliable data. However, there are few scientific data from humans or animals that permit comparisons of sensitivities of children and adults, but there are some examples, such as lead, where children are the more sensitive population. It some cases qualitative differences in age-related susceptibility are small beyond 6 months of age, and quantitative differences in toxicity between children and adults can sometimes be less than a factor of 2 or 3.

Much of the research efforts in risk assessment are therefore aimed at reducing the need to use these default uncertainty factors, although the risk assessor is limited by data quality of the chemical of interest. With sufficient data and the advent of sophisticated and validated physiologically based pharmacokinetic models and biologically based dose-response models (Conolly and Butterworth, 1995), these default values can be replaced with science-based factors. In some instances there may be sufficient data to be able to obtain distributions rather than point estimates.

24.3.2 Derivation of Developmental Toxicant RfD

Developmental toxicity includes any detrimental effect produced by exposures during embryonic development, and the effect may be temporary or overt physical malformation. Adverse effects include death, structural abnormalities, altered growth, and

functional deficiencies. Maternal toxicity is also considered. The evidence is assessed and assigned a weight-of-evidence designation as follows: category A, category B, category C, and category D. The scheme takes into account the ratio of minimum maternotoxic dose to minimum teratogenic dose, the incidence of malformations and thus the shape of the dose-response curve or dose relatedness of the each malformation, and types of malformations at low doses. A range of uncertainty factors are also utilized according to designated category as follows: category A = 1–400, category B = 1–300, category C = 1–250, and category D = 1–100. Developmental RfDs are based a short duration of exposure and therefore cannot be applied to lifetime exposure.

24.3.3 Determination of RfD and RfC of Naphthalene with the NOAEL Approach

The inhalation RfC for naphthalene was 0.003 mg/m^3, and this RfC was derived from a chronic (2-year) NTP inhalation study in mice using exposures of 0, 10, or 30 ppm (NTP, 1992). Groups of mice were exposed for 5 days a week and 6 hours a day. This study identified a LOAEL of 10 ppm. A dose-related incidence of chronic inflammation of the epithelium of the nasal passages and lungs was observed. This LOAEL concentration was normalized by adjusting for the 6-hour-per-day and 5-day-per-week exposure pattern. A LOAEL of 9.3 mg/m3 was obtained was derived by converting 10 ppm first to mg/m^3 and then duration-adjusted levels for 6 h/day and 5 days/week for 103 weeks. An UF of 3000 was used, where 10 was for the interspecies (mice to humans) extrapolations, 10 for intraspecies variation in humans, 10 for using a LOAEL instead of a NOAEL, and 3 for database deficiencies.

The oral RfD for naphthalene was 0.02 mg/kg/day, and a study by Battelle (1980) was used to calculate the RfD. Decreased body weight was the most sensitive end point in groups of Fischer 344 rats given 0, 25, 50, 100, 200, or 400 mg/kg for 5 days/week for 13 weeks. These doses were also duration-adjusted to 0, 17.9, 35.7, 71.4, 142.9, and 285.7 mg/kg/day, respectively. The NOAEL for $a > 10\%$ decrease in body weight in this study was 71 mg/kg/day. The UF of 3000 was based on 10 for rats to humans extrapolation, 10 for human variation, 10 to extrapolate from subchronic to chronic, and 3 for database deficiencies including lack of chronic oral exposure studies.

24.3.4 Benchmark Dose Approach

There are several problems associated with using the NOAEL approach to estimate RfDs and RfCs. The first obvious constraint is that the NOAEL must by definition be one of the experimental doses tested. Once this dose is identified, the rest of the dose-response curve is ignored. In some experimental designs where there is no identifiable NOAEL but LOAEL, the dose-response curve is again ignored, and the NOAEL is derived by application of uncertainty factors as described earlier. This NOAEL approach does not account for the variability in the estimate of the dose response, and furthermore experiments that test fewer animals result in larger NOAELs and thus larger RfDs and RfCs.

An alternative approach known as the benchmark dose (BMD) approach has been developed and implemented by risk assessors as an alternative to the NOAEL approach to estimate RfDs and RfCs. This approach is not constrained by experimental design

as the NOAEL approach, and it incorporates information on the sample size and shape of the dose-response curve. In fact this approach can be used for both threshold and nonthreshold adverse effects as well as continuous and quantal data sets. This requires use of Benchmark Dose Software where the dose-response is modeled and the lower confidence bound for a dose at a specified response level (benchmark response) is calculated. The benchmark response is usually specified as a 1–10% response; that is, it corresponds to a dose associated with a low level of risk such as 1–10%.

Figure 24.3 shows how an effective dose that corresponds to a specific change of effect/response (e.g., 10%) over background and a 95% lower confidence bound on the dose is calculated. The latter is often referred to as the BMDL or LBMD, as opposed to the BMD, which does not have this confidence limited associated with it.

Because the benchmark represents a statistical lower limit, larger experiments will tend, on average, to give larger benchmarks, thus rewarding good experimentation. This is not the case with NOAELs, as there is an inverse relationship between NOAEL and size of experiments. For example, poorer experiments possessing less sensitivity for detecting statistically significant increases in risk inappropriately result in higher NOAELs and RfDs, which may have an unknown unacceptable level of risk. In essence, the NOAEL is very sensitive to sample size, and there can also be high variability between experiments. With the benchmark dose approach, all the doses and slopes of the curve influence the calculations, variability of the data is considered, and the BMD is less variable between experiments. In the BMD approach quantitative toxicological data such as continuous data (organ weights serum levels, etc.) and quantal or incidence data (pathology findings, genetic anomalies, etc.) are fitted to numerous dose-response models described in the literature. The resulting benchmark dose that, for example, corresponds to a tumor risk of 10% generally can be estimated with adequate precision and not particularly dependent on the dose-response model used to fit the data. Note that dose intervals are not required for BMD estimation. This will be greatly appreciated in the cancer risk assessment section of this chapter.

24.3.5 Determination of BMD and BMDL for ETU

The BMD method has been quite extensively in assessing quantal data, and very often this has involved analysis of data from developmental and reproductive toxicity

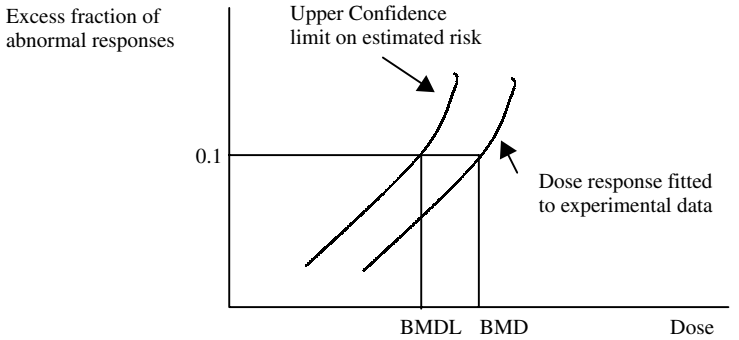

Figure 24.3 Benchmark dose determination from dose response relationship with the BMDL corresponding to the lower end of a one-sided 95% confidence interval for the BMD.

studies. In this study example (Crump, 1984), rats were exposed to ethylenethiourea (ETU) at 0, 5, 10, 20, 40, and 80 mg/kg doses, and the number affected with fetal anomalies per number of rats were 0/167, 0/132, 1/138, 14/81, 142/178, and 24/24, respectively. The benchmark dose computation can involve utilization of any given dose-response probability model, but in this example the quantal Weibull model was used and the specified effect was set at 0.01 (1%) with confidence level of 0.95. The BMD was determined to be 8.9 mg/kg, and the BMDL was 6.9 mg/kg. This value is close to the NOAEL, which is 5 mg/kg, but it does demonstrate that the NOAEL approximates a lower confidence limit on the BMD corresponding to an excess risk of about 1% for proportions of fetal anomalies. In fact an empirical analysis of some 486 developmental toxicity studies has demonstrated that the NOAEL can result in an excess risk of 5% for proportions of dead or malformed fetuses per litter. The reader should at this stage recognize that the BMD approach can also be used in cancer risk assessment as we are often times working with quantal data that are ideally suited for BMD modeling.

24.3.6 Quantifying Risk for Noncarcinogenic Effects: Hazard Quotient

The measure used to describe the potential for noncarcinogenic toxicity to occur is not expressed as the probability. Probabilistic approach is used in cancer RA. For noncancer RA, the potential for noncarcinogenic effects is evaluated by comparing an exposure level (E) over a specified time period with a reference dose (RfD). This ratio is called a hazard quotient:

$$\text{Hazard quotient} = \frac{\text{E}}{\text{RfD}}.$$

In general, the greater the value of E/RfD exceeds unity, the greater is the level of concern. Note that this is a ratio and not to be interpreted as a statistical probability.

24.3.7 Chemical Mixtures

Human populations are more likely to be exposed simultaneously or sequentially to a mixture of chemicals rather than to one single chemical. Standard default approaches to mixture risk assessment consider doses and responses of the mixture components to be additive. However, it should also be recognized that components in the mixture can also result in synergistic, antagonistic, or no toxicological effect following exposure to a chemical mixture. Therefore mixture toxicity cannot always be predicted even if we know the mechanisms of all toxic components in a defined mixture. Furthermore tissue dosimetry can be complicated by interactions at the route of entry (e.g., GIT, skin surface) and clearance mechanisms in the body. In essence, there are considerable uncertainties involved in trying to extrapolate effects following exposure to chemical mixtures. Several PBPK models have been used to quantitate these effects and also provide some information useful for risk assessment of chemical mixtures (Krishnan et al., 1994; Haddad et al. 2001).

The 1996 FQPA has also mandated that the EPA should also consider implementing cumulative risk assessments for pesticides. Cumulative risk assessments usually involve

integration of the hazard and cumulative exposure analysis, and it primarily involves cumulative nonoccupational exposure by multiple routes or pathways to two or more pesticides or chemicals sharing a common mechanism of toxicity.

Calculation procedures differ for carcinogenic and noncarcinogenic effects, but both sets of procedures *assume dose additivity* in the absence of information on mixtures:

$$\text{Cancer risk equation for mixtures}: \quad \text{Risk}_\text{T} = \Sigma\text{Risk}_\text{I},$$

$$\text{Noncancer hazard index} = \frac{\text{E}_1}{\text{RfD}_1} + \frac{\text{E}_2}{\text{RfD}_2} + \cdots + \frac{\text{E}_i}{\text{RfD}_\text{i}}.$$

This hazard index (HI) approach as well as other indexes (e.g., relative potency factors) are applied for mixture components that induce the same toxic effect by identical mechanism of action. In cases where there are different mechanisms, separate HI values can be calculated for each end point of concern. As the equation above indicates, the HI is easy to calculate, as there is simply scaling of individual component exposure concentrations by a measure of relative potency such as the RfD or RfC, and adding scaled concentrations to get an indicator of risk from exposure to the mixture of concern. However, as noted above, this additivity approach does not take into account tissue dosimetry and pharmacokinetic interactions. Recent published risk assessments have utilized mixture PBPK models to account for multiple pharmacokinetic interactions among mixture constituents. These interaction-based PBPK models can quantify change in tissue dose metrics of chemicals during exposure to mixtures and thus improve the mechanistic basis of mixture risk assessment. Finally the reader should be aware that this HI is different from the a term known as the margin of safety (MOS), which is the ratio of the critical or chronic NOAEL for a specific toxicological end point to an estimate of human exposure. MOS values greater than 100 are generally considered protective if the NOAEL is derived from animal data.

24.4 CANCER RISK ASSESSMENT

For cancer risk assessment an assumption is held that a threshold for an adverse effect does not exist with most individual chemicals. It is assumed that a small number of molecular events can evoke changes in a single cell that can lead to uncontrolled cellular proliferation and eventually to a clinical state of disease. This mechanism is referred to as "nonthreshold" because there is believed to be essentially no level of exposure to such a chemical that does not pose a finite probability, however small, of generating a carcinogenic response. That is, no dose is though to be risk free. Therefore, in evaluations of cancer risks, an effect threshold cannot be estimated. For carcinogenic effects, the US EPA uses a two-part evaluation: (1) the substance is first assigned a weight-of-evidence classification and then (2) a slope factor is calculated.

1. Assigning a weight-of-evidence. The aim here is to determine the likelihood that the agent is a human carcinogen. The *evidence* is characterized separately for human studies and animal studies as *sufficient, limited, inadequate, no data*, or *evidence of no effect*. Based on this characterization and on the extent to which the chemical has been shown to be a carcinogen in animals or humans or both, the chemical is given a provisional *weight-of-evidence* classification. The US EPA classification system (EPA,

Table 24.3 Weight of Evidence Designation Based on EPA (1986) Guidelines

Group	Description
A	Human carcinogen
B1 or B2	Probable human carcinogen
C	Possible human carcinogen
D	Not classifiable as to human carcinogenicity
E	Evidence of noncarcinogenicity for humans

Note: B1 indicates that limited human data are available; B2 indicates sufficient evidence in animals and inadequate or no evidence in humans.

1986) shown in Table 24.3 has been revised in the EPA (1996) proposed guidance and more recent draft guidance (EPA, 1999).

This system was also adapted from the approach taken by the International Agency for Research on Cancer (IARC). This alphanumeric classification system has been replaced with a narrative and the following descriptor categories: *known/likely, cannot be determined*, or *not likely*. These EPA (1996) guidelines indicate that not only are tumor findings an important consideration, but also structure-activity relationships, modes of action of carcinogenic agents at cellular or subcellular level and toxicokinetic and metabolic processes. These revised guidelines also indicate that the weighing of evidence should address the conditions under which the agent may be expressed. For example, an agent may "likely" be carcinogenic via inhalation exposure but "not likely" via oral exposure. The narrative will summarize much of this information as well as the mode of action information.

2. Quantifying risk for carcinogenic effects. In the second part of the evaluation, the EPA (1986) guidelines required that quantitative risk be based on the evaluation that the chemical is a known or probable human carcinogen, a toxicity value that defined quantitatively the relationship between dose and response (slope factor) is calculated. Slope factors have been calculated for chemicals in classes A, B1, and B2. Sometimes a value is derived for those in class C on a case-by-case basis. The slope factor is a plausible upper-bound estimate of the probability of a response per unit intake of chemical over a lifetime. Slope factors have been accompanied by the weight-of-evidence classification to indicate the strength of evidence that the chemical is a human carcinogen.

Development of a slope factor entails applying a model to the available data set and using the model to extrapolate from high doses to lower exposure levels expected for human contact. There are a number of low-dose extrapolation models that can be divided into distribution models (e.g., log-probit, Weibull) and mechanistic models (e.g., one-hit, multi-hit, and *linearized multistage*). EPA 1986 guidelines for carcinogen risk assessment are currently being revised, and it is very likely that the new guidelines will encourage the use of biologically based models for cancer risk assessment. The previous guidelines (EPA, 1986) recommended that the linearized multistage model, which is a mechanistic model, be employed in as the default model in most cases. Most of the other models are less conservative. The proposed biologically based models attempt to incorporate as much mechanistic information as possible to arrive at an estimate of slope factors. In essence, after the data are fit to the selected model, the

upper 95th percent confidence limit of the slope of the resulting dose response curve is calculated. *This represents the probability of a response per unit intake over a lifetime*, or that there is a 5% chance that the probability of a response could be greater than the estimated value on the basis of experimental data and model used. In some cases, the slope factors based on human dose-response data are based on "best" estimate instead of upper 95th percent confidence limit. The toxicity values for carcinogenic effects can be expressed in several ways.

The slope factor is expressed as q_1^*:

$$\begin{aligned}\text{Slope factor} &= \text{Risk per unit dose} \\ &= \text{Risk per mg/kg-day.}\end{aligned}$$

The slope factor can therefore be used to calculate the upper bound estimate on risk (R)

$$\text{Risk} = q_1^*[\text{risk} \times (\text{mg/kg/day})^{-1}] \times \text{exposure (mg/kg/day)}.$$

Here risk is a unitless probability (e.g., 2×10^{-5}) of an individual developing cancer and exposure is really chronic daily intake averaged over 70 years: mg/kg/day. This can be determined if we can determine the slope factor and human exposure at the waste site or occupational site. The EPA usually sets a goal of limiting lifetime cancer risks in the range of 10^{-6} to 10^{-4} for chemical exposures, while the FDA typically aims for risks below 10^{-6} for general population exposure. It is therefore quite likely for very high exposures for the accepted EPA range of risk to be exceeded. The EPA range is considered protective of the general and sensitive human population. It should be noted that these orders of magnitude are substantially greater than those used in estimating RfD and RfCs in noncancer risk assessment.

Because relatively low intakes (compared to those experienced by test animals) are most likely from environmental exposure at Superfund hazardous waste sites, it generally can be assumed that the dose-response relationship will be linear on the low-dose portion of the multistage model dose-response curve. The equation above can apply to these linear low-dose situations. This linear equation is valid only at low risk levels (i.e., below the estimated risk of 0.01). For risk above 0.01 the one-hit equation should be used:

$$\text{Risk} = 1 - \exp(\text{-exposure} \times \text{slope factor}).$$

As indicated above, biologically based extrapolation models are the preferred approach for quantifying risk to carcinogens, although it is possible that all the necessary data will not be available for many chemicals. The EPA (1986) guidelines have been modified to include the response data on effects of the agent on carcinogenic processes in addition to data on tumor incidence. Precursor effects and tumor incidence data may be combined to extend the dose response curve below the tumor data; that is, below the range of observation. Thus a biologically based or case-specific dose-response model is developed when there is sufficient data, or a standard default procedure is used when there is insufficient data to adequately curve-fit the data. In brief, the dose-response assessment is considered in two parts or steps, range of observation and range of extrapolation, and the overriding preferred approach is to use the biologically based or case-specific model for both of these ranges. In the first

step of this process, the lower 95% confidence limit on a dose associated with an estimated 10% increase in tumor or nontumor response (LED_{10}) is identified. When human real world exposures are outside the range of the observed or experimental data, this serves as the point of departure or marks the beginning for the extrapolating to these low environmental exposure levels. Note that these procedures are very similar to the benchmark procedure for quantitating risk to noncarcinogenic chemicals. In the second step, the biologically based or case-specific model is preferred for use in extrapolations to lower dose levels provided that there are sufficient data. If the latter is not the case, then default approaches consistent with agent chemical mode of action are implemented with the assumption of linearity or nonlinearity of the dose-response relationship. The linear default approach is a departure from the 1986 guidelines, which used the linearlized multistage (LMS) procedure, but is based on mode of action or alternatively if there is insufficient data to support a nonlinear mode of action. In brief, it involves drawing a straight line from the point of departure (LED_{10}) to the origin (i.e., zero). When there is no evidence of linearity or there is a nonlinear mode of action, the default approach is the margin of exposure (MOE) analysis. The MOE approach computes the ratio between the LED_{10} and the environmental exposure, and the analysis begins from the point of departure that is adjusted for toxicokinetic differences between species to give a human equivalent dose.

Finally it should be noted that prior to the FQPA in 1996, the Delaney clause prohibited the establishment of tolerances or maximum allowable levels for food additives if it has been shown to induce cancer in human or animal. This is an important change in regulations because pesticide residues were considered as food additives. Because of the FQPA, pesticide residues are no longer regarded as food additives, and there is no prohibition against setting tolerances for carcinogens.

24.5 PBPK MODELING

Physiologically based pharmacokinetic (PBPK) modeling has been used in risk assessment to make more scientifically based extrapolations, and at the same time to help explore and reduce inherent uncertainties. Historically pharmacokinetics has relied on empirical models, and in many instances this process offers little insight into mechanisms of absorption, distribution, and clearance of hazardous agents and does not facilitate translation from animal experiments to human exposures. For example, dose scaling using by body weight or size may often time overestimate or underestimate toxicant levels at the target tissue. PBPK models can help predict tissue concentrations in different species under various conditions based on *independent* anatomical, physiological, and biochemical parameters. In these analyzes physiological parameters such as organ volumes, tissue-blood partition coefficients, and blood flow to specific tissue compartments described by the model, are calculated or obtained from the literature and integrated into the model. Monte Carlo analysis, a form of uncertainty analysis, can now be performed, and this allows for the propagation of uncertainty through a model that results in estimation of the variance of model output. This can be achieved by randomly sampling model parameters from defined distributions; some parameters such as cardiac output, metabolic, and log P parameters, may have a lognormal distribution, while other parameters may be normal or uniform. In essence, the Monte Carlo analysis when coupled with PBPK characterizes the distribution of potential risk

in a population by using a *range* of potential values for each input parameter (not single values) as well as an estimate of how these values are distributed (Clewell and Andersen, 1996). By these approaches, uncertainty is identifiable and quantifiable, and can reduce inappropriate levels of concern in reporting the risk of chemical exposure. These mathematical modeling approaches also help identify areas of potential scientific research that could improve the human health assessment.

In recent years there have been significant efforts at harmonizating noncancer and cancer risk assessments (Barton et al., 1998; Clewell et al., 2002), and in this respect PKPD modeling can be a very useful tool in the risk assessment process. For example, recall that noncancer risk assessment addresses variability in a population by dividing the NOAEL by 10, whereas the cancer risk assessment does not address this quantitatively. PBPK modeling coupled with Monte Carlo analysis is one approach as described in the previous paragraph that will help address this level of uncertainty in the risk assessment. In conclusion, it should be noted that PBPK modeling has been utilized with very few toxicants. It is hoped that risk assessment policy will encourage the use of this tool as well as other appropriate models to integrate mechanistic information and the pharmacokinetics (dosimetry), and pharmacodynamics (dose response) of toxicants. Improved quantitative risk assessments will ultimately provide scientifically sound information that will influence the risk management decision process.

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