Toxicology and Risk Assessment

edited by
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Preface

The presence of chemicals in our environment presents a multi-dimensional world involving toxicological evaluations, risk assessment, risk management, and risk communication relating to these chemicals. These elements provide the basis for risk analysis for policy decisions. This book provides state-of-the art discussions on various aspects of chemical toxicology and health risk assessment, and some of the associated international risk management and risk communication activities.

The book starts with a description of the general principles and practices in risk assessment for cancer and non-cancer toxicological endpoints, followed by a description of various methodologies used in risk assessment, including recent advances in benchmark dose (BMD) modeling, structure activity relationship (SAR), physiologically based pharmacokinetic (PBPK) modeling, dose–response assessment, and epidemiological methods. The book provides unique coverage of new advances in cancer risk assessment, special considerations for different age groups such as infants, children, and older adults, and use of uncertainty factors considering pharmacokinetic and pharmacodynamic characteristics.

The book includes developing, conducting, and interpreting toxicological evaluations and risk assessments for major chemicals or chemical groups of current concern. It emphasizes the need for sufficient scientific background and knowledge to enable the understanding of toxicity testing and the science underlying the key issues in exposure to environmental chemicals and the associated health risks, such as in the discussion of asbestos, trichloroethylene, nickel, fumigants, organophosphate and carbamate pesticides, endosulfan, and phthalates. The same also applies to emergency response, drug development and food safety assessment. It provides case studies using specific chemicals and situations to demonstrate how to execute chemical- and situation-specific human health risk assessment, how science is used in deciding or providing health-based criteria for environmental management and regulations, and how risk assessment influences risk-based decision making, such as in the

discussion of trichloroethylene, aluminum, melamine, diethylhexyl phthalate, cholinesterase-inhibiting pesticides (cumulative risk), and multiple chemicals in drinking water (incorporating multi-route exposure estimation). It discusses current issues and emerging science such as in the discussion of age sensitivity, nanotoxicology, and mode of action (MOA) to evaluate the developmental toxicity of endosulfan. It emphasizes the importance of coordination and communication among scientific entities, integration with public health policies, critical thinking, and exposure databases such as those providing data on food and drinking water consumption rates for the associated assessments. It considers the current use of animal testing and the future of less resource-intensive toxicity testing in the 21st century as described by the U.S. National Academy of Sciences and U.S. Environmental Protection Agency.

The book is illustrated with excellent figures and references. It is a handbook and reference book covering in-depth evaluations of current topics in toxicology and risk assessment and of selected major chemicals of interest for professionals. It is suitable as a graduate-level textbook or for training courses on risk assessment.

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Chapter 1

Principles and Approaches for Human Health Risk Assessment of Environmental Contaminants

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The views expressed are those of the author and do not necessarily represent those of the New Jersey Department of Environmental Protection.

1.1 Introduction

Since its introduction more than 30 years ago, human health risk assessment continues to be an essential component of evaluation and decision making about environmental contaminants to which humans are exposed. This chapter provides an overview of the general risk assessment process, including recent refinements and advances, as well as future directions as currently envisioned. The

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information presented here is intended to provide a useful starting point for understanding of the subsequent chapters which discuss specific aspects relating to chemical toxicity and risk assessment.

Human health risk assessment is defined by the United States Environmental Protection Agency (USEPA) as "the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future" [1]. Risk assessment, along with other types of information, is used in the evaluation and regulation of environmental contaminants. This chapter provides a brief introduction to the principles and approaches used for human health risk assessment of environmental contaminants, focusing primarily on approaches used by the USEPA. Specific aspects of risk assessment and case studies are discussed in detail in subsequent chapters, as well as in the references cited herein. Approaches used by states (e.g., California [2] and New Jersey [3]), and other US federal agencies (such as the Agency for Toxics Substances and Disease Registry [ATSDR] [4]), are generally based on those of the USEPA. Discussion of a related process, ecological risk assessment [5], which addresses ecological impacts of environmental contaminants, is beyond the scope of this chapter. Stern [6] provides useful perspectives on many of the human health risk assessment issues mentioned in this chapter.

Human health risk assessment methodologies used in other nations are largely similar in concept to those of the USEPA, although they may differ in specific details [7]. An alternative approach to decision making about human exposure to environmental contaminants is based on the precautionary principle, generally meaning that protective measures should be taken if there is uncertainty about potential risks of serious or permanent effects even when these risks have not been definitively demonstrated. The precautionary principle has been considered in the development of some policies related to human exposure to potentially harmful substances, particularly by the European Union [7].

Human health risk assessment seeks to address issues about environmental contaminants such as the nature of the health effects that they may cause, the levels of human exposure from various environmental media, the impacts of human activity patterns on exposures, the doses and exposure durations at which health effects may occur, the probability of health effects from different levels of

exposure, and factors that may result in greater exposures and/or greater susceptibility to health effects in specific subpopulations.

The results of human health risk assessments are used, along with other information, in many different types of decision making related to environmental contamination. Some examples include advice for action in emergency situations such as industrial accidents or spills of transported materials, the human health basis of chemical-specific regulatory standards and guidance levels, decisions about manufacture and use of pesticides and other toxic substances, and assessment of potential health risks at contaminated sites. The duration of exposure of interest for a risk assessment (acute, intermediate, or chronic) depends on the situation to which the results will be applied. For example, many regulatory standards are based on the assumption of lifetime exposure, cleanup of residential sites is often based on the assumption of 30 years of residence, and shorter-term exposures (e.g., a few days) may be the relevant time period for exposure to water or air contaminants from an accident or other nonrecurring release that will quickly dissipate.

Definitive information on health effects in human populations exposed to contaminants at the levels that are found in the environment is the most directly relevant for risk assessment, but such data are rarely available. To be protective of public health, exposures to environmental contamination must be addressed within a reasonable time frame. Therefore risk assessments must often be developed on the basis of the information that is available even if it is incomplete. Data from animal toxicology studies, or less commonly, epidemiology studies of workers with exposures far above environmental levels, are most often used as the primary basis for risk assessment. Uncertainties are involved in the interspecies extrapolation of data from experimental animals to potential effects in humans, as well as the extrapolations from the higher doses usually used in the animal studies or typically present in the workplace to environmental exposures that are generally much lower. In the absence of chemical-specific data, default assumptions are used to address these uncertainties and data gaps. Since the risk assessment process is intended to be protective of public health, the assumptions used to address these uncertainties are intended to be reasonable but conservative so that risk is not likely to be underestimated [8]. If data becomes available to address sources of uncertainty, the risk

assessment can be revised to replace the assumption with chemical-specific information based on this data.

The level of uncertainty that is considered to be acceptable may vary depending on the purpose of the risk assessment. When a risk assessment is urgently needed to provide advice in an emergency situation, it must be based on whatever information is readily available, even if there is a great deal of uncertainty due to data gaps. A higher degree of uncertainty may be acceptable in risk assessments used for screening purposes or for site-specific guidance than in a risk assessment that is the basis of an enforceable regulatory standard. When there is a high level of uncertainty because of data gaps for an important risk assessment (e.g., a risk assessment used as the basis for regulatory standards with large economic consequences), a decision may be made to await finalizing of the risk assessment until additional research to provide key data has been conducted.

Risk assessment is only one component in the decision-making process used in addressing environmental contaminants of human health concern. Other scientific, technical, and policy factors, in addition to the results of human health risk assessment, are also considered in making risk management decisions about the contaminant levels at which regulatory standards, discharge permits, or remediation goals are set. Some examples of technical and scientific considerations are the levels to which the chemical can be reliably quantitated by analytic methods, availability of treatment removal technology, and natural background levels of contaminants. Examples of other considerations include legal requirements, economic factors that may be evaluated through cost-benefit analysis, regulatory requirements such as a specified target cancer risk level (e.g., 10^{-6}), and social and policy considerations including environmental justice issues. A detailed discussion of the role of the of risk management in environmental decision making is found in a recent National Research Council (NRC) report [9].

General information on the USEPA risk assessment is found at its risk assessment website [1]. The USEPA's documentation of its risk assessment process [10] began in the 1970s with *Quantitative Risk Assessment for Community Exposure to Vinyl Chloride* [11] and *Interim Procedures and Guidelines for Health Risk and Economic Impact Assessments of Suspected Carcinogens* [12]. In 1980, the USEPA used a quantitative risk assessment approach to develop human health–based water quality criteria for 64 contaminants [13].

The overall framework for the risk assessment approach used by the USEPA and other agencies was developed in 1983 by the NRC [14], and this basic framework remains generally applicable to risk assessment today. The five steps of this approach, discussed in more detail in the following text, are:

1. *Scoping*: The USEPA [1] has recently placed increased emphasis on planning the risk assessment prior to the four steps outlined earlier, as recently recommended by the NRC [9]. In this planning process, the purpose, scope, and approaches to be used in the risk assessment are defined.
2. *Hazard identification*: Qualitative determination of the adverse effects caused by a contaminant is done.
3. *Dose–response assessment*: Quantitative evaluation of the effects that a chemical may cause at different doses is done.
4. *Exposure assessment*: Characterization of the nature and magnitude of exposure to the contaminant is done.
5. *Risk characterization*: A description of the results of the risk assessment, including the underlying assumptions, and uncertainties is made.

Chemical-specific risk assessments representing the consensus of the USEPA programs are developed by the USEPA's Integrated Risk Information System (IRIS) [15], which was initiated in the 1980s. Many guidance documents providing details on specific aspects of risk assessment are linked from the IRIS website.

1.2 General Types of Risk Assessments

Results of a risk assessment can take several forms that fall into two general types, chemical specific and site specific.

Results of chemical-specific risk assessments include toxicity factors and health-based criteria. Toxicity factors for chronic exposures are usually presented as oral reference doses (RfDs) (mg/kg/day) or inhalation reference concentrations (RfCs) ($\mu g/m^3$) for noncarcinogenic effects and as oral slope (or potency) factors $(mg/kg/day)^{-1}$ or inhalation unit risk factors $(\mu g/m^3)^{-1}$ for carcinogenic effects. Health-based criteria for specific environmental media, such as water, air, or soil, are developed by combining chemical-specific toxicity factors with media-specific exposure assumptions, as well

as target cancer risk levels in the case of carcinogens. Health-based criteria, along with other factors mentioned earlier, are considered in developing chemical-specific benchmarks such as guidance values, screening levels, and regulatory standards.

Examples of site-specific risk assessments include assessment of the risks of contaminants from a spill or other accident, evaluation of the risks of contaminants present at a contaminated site, and fish consumption advisories based on the levels of contaminants found in fish from specific water bodies. In such cases, the contaminant levels at the site are compared to risk-based benchmarks. These benchmarks are developed from toxicity factors for the duration of exposure of concern and exposure assumptions that may be defaults or site specific. Combined risks from multiple chemicals that are present, or from exposure to the same chemical from multiple media, may be evaluated as part of a site-specific risk assessment.

1.2.1 Consideration of Sensitive Subpopulations in Risk Assessment

Risk assessments include consideration of specific subgroups within the human population that may be at greater risk than the general population due to greater exposure and/or susceptibility to toxic effects. These subpopulations include the developing fetus, children, the elderly, populations with specific susceptibilities such as poor nutrition, and individuals with certain genetic variants, diseases, or medical conditions.

The developing fetus is an obvious example of specific susceptibility to chemicals that cause teratogenic effects. Additionally, other types of effects occurring during fetal development or infancy (e.g., inhibition of thyroid function) may cause permanent damage, while the same effect in older individuals may have less serious consequences that are reversible when exposure ceases. The ability to metabolize or excrete certain toxic chemicals can be diminished in the elderly, increasing their sensitivity to these chemicals.

Infants and young children may also have greater exposures than adults, for example, because they consume more water on a body weight basis [16] and ingest more soil and house dust than adults due to time spent playing on the ground and the floor and in activities such as hand-to-mouth behaviors [16]. Behaviors of certain ethnic or other demographic groups can also lead to increased exposures.

For example, members of certain ethnic groups may consume much greater quantities of locally caught fish from contaminated water bodies than the general population [17].

1.3 Hazard Identification

In the hazard identification step of the risk assessment, all relevant data are considered in determining of the weight of evidence for whether a chemical can causes effect(s) in humans. This includes studies in experimental animals, human epidemiology data, in vitro studies, and evaluation of the relevance of health effects information for related chemicals. Examples of issues evaluated in this step include the nature of the effects reported to be caused by the chemical, weight of evidence for carcinogenicity and/or other effects, identification of key studies and endpoints, evaluation of the mode(s) of action of toxicity, relevance of effects observed in animals to humans, and relevance of effects from a given exposure route to other exposure routes. Studies of metabolites of the chemical, as well as the chemical itself, may be relevant to hazard identification [18].

Studies involving routes of administration that are relevant to environmental exposures (oral, inhalation, dermal) are usually used as the primary basis of risk assessments, while data from other routes (e.g., injection or implantation) may provide useful supporting information. Endpoints that occur only at the point of contact for a specific exposure route may not be relevant to other exposure routes. For example, toxicity to nasal tissue from inhalation is not considered relevant to oral exposure, while systemic effects (e.g., kidney toxicity) from a given exposure route (e.g., oral) are also considered relevant to other routes of exposure (e.g., inhalation). Acute or short-term studies are generally not used as the primary basis for risk assessment of risk from chronic exposure but can provide useful supporting information. Exceptions are instances in which developmental effects that occur during a sensitive time window, which may be of short duration, are the most sensitive endpoints of toxicity (see following text). In these cases, it is appropriate to use the developmental effects resulting from a short exposure period as the basis for both short-term and chronic risk assessments.

In vitro studies from cellular and subcellular assay systems provide data on mutagenicity, genotoxicity, gene activation profiles,

metabolism to reactive intermediates and detoxification products, receptor activation, and other parameters that are important for understanding a chemical's mode of action. Current efforts to develop in vitro methods that could be used instead of animal testing to quickly assess the risks of large numbers of chemicals are discussed in the Future Directions section later.

Considerations for evaluation of the quality of epidemiology and toxicology studies are discussed by the USEPA in Ref. [18], and considerations for epidemiology studies are further discussed in Chapter 5 and Ref. [19]. Efforts are currently underway by the USEPA to further formalize this process through approaches for "systematic review" of studies being considered for use in risk assessment [20].

In addition to factors relevant to the evaluation of scientific studies in general (e.g., study design, reporting of data, statistical power, parameters evaluated, and others), an important consideration in toxicology studies used in risk assessment is whether the experimental animal is a relevant model for effects in humans of the chemical under evaluation. The default assumption in risk assessment is that effects observed in experimental animals are relevant to humans. However, important toxicokinetic (extent of absorption, patterns of distribution to specific tissues, excretion rates, metabolic pathways leading to toxic intermediates) or toxicodynamic (factors affecting toxicological response at site of action, such as interaction with cellular receptors) differences may exist between the animal model and humans. As an example of toxicokinetic dissimilarities, female rats excrete perfluorooctanoic acid (PFOA) and some other perfluorinated compounds rapidly, while these chemicals are persistent in other animal models and in humans [21]. An example of toxicodynamic differences is the kidney toxicity due to increased α-2-microglobulin production from certain hydrocarbons that occurs only in male rats and is not relevant to humans [22].

A chemical's classification as a carcinogen or a noncarcinogen in the hazard identification step is a key decision in the risk assessment process, since different dose–response approaches are generally used for noncarcinogenic and carcinogenic effects (see following text). For a given chemical, several relevant endpoints may be evaluated in the risk assessment, including both noncancer and cancer endpoints for carcinogens. For example, trichloroethylene was recently classified as a human carcinogen by the USEPA, and

both cancer and noncancer endpoints were assessed [23]. The RfD for noncancer effects of trichloroethylene is based on the midpoint of "candidate RfDs" for several endpoints considered to be "critical effects" (developmental immunotoxicity and decreased thymus weights in mice and cardiac malformations in rats).

1.3.1 Noncarcinogenic Effects

Many systemic (noncancer) endpoints may be considered in developing a risk assessment, including mortality, body weight, absolute and relative organ weights, gross and microscopic pathological changes, neurologic function, effects on the immune system, behavioral changes, changes in hormone levels, and hematology and clinical chemistry parameters, among others. Risk assessments may also be based on effects seen from short-term exposures during sensitive time periods, such as effects on reproductive parameters, including fertility, birth weight, and survival of offspring, and developmental effects, including teratogenicity and neurobehavioral effects after early life exposure. Prenatal and early life exposure as a cause of health effects that are not evident until later in life is a current focus of toxicology [24], but these potentially important endpoints are not yet routinely used as the basis for risk assessment.

An additional consideration is the nature of the effects chosen as the basis for the risk assessment. Important issues include whether the effect under evaluation is adverse (or a precursor to an adverse effects) as opposed to an adaptive response that is not considered to be adverse, the severity of the effect, and whether it is reversible or irreversible.

1.3.2 Carcinogenic Effects

Only a small subset of the environmental contaminants that are treated as carcinogens for risk assessment purposes have been definitively shown to cause cancer in exposed human populations. However, increased rates of human cancer from specific chemical exposures at an incidence generally considered to be of public health concern (e.g., 1 in 10,000 to 1 in 1,000,000) may not be noticeable against the much higher background cancer incidence in the same tissues and organs. The evidence for the majority of chemicals

treated as carcinogens is from animal data, with or without supporting human data. The use of epidemiology data in assessing a chemical's carcinogenic potential is discussed in Chapter 5 and Ref. [19]. Information on the mode of action for carcinogenicity, and whether the mode of action in animals is relevant to humans, is also considered. Mutagenicity and genotoxicity assays and other mechanistic studies provide information on the mode of action of carcinogens. This is important in determining the appropriate dose–response approach (linear low-dose extrapolation or threshold; see following text).

Traditionally, the protocols for chronic bioassays conducted by the National Toxicology Program and other organizations require dosing of male and female rats and mice for two years, beginning in young adulthood. To assess the greater sensitivity to carcinogens that may occur during critical developmental time periods, a perinatal exposure protocol that includes exposure during gestation and lactation has more recently been developed [25].

A detailed discussion of the many issues related to interpretation of the results of chronic bioassays in regard to evaluation of the chemical's human carcinogenic potential is beyond the scope of this chapter. Examples of such issues are whether the maximum tolerated dose was reached and/or exceeded, appropriate statistical tests and statistical significance levels, and consideration of background rates of tumors in concurrent and historical controls.

The 2005 USEPA *Guidelines for Carcinogen Risk Assessment* [26] present the scientific basis for the default assumptions currently employed in cancer risk assessment. These default assumptions are used unless chemical-specific information indicates that they are not applicable. Some of the assumptions related to the evaluation of animal data in the hazard identification step include the following [26]:

- Positive results in animal studies indicate human carcinogenic potential.
- Negative findings in two or more animal species indicate lack of human carcinogenic potential.
- Carcinogenicity that occurs as a result of excessive toxicity at high doses is not relevant to human carcinogenic potential.
- Target organs for carcinogens are not necessarily concordant in animals and humans.

- Benign tumors that can progress to malignancy are relevant to carcinogenic potential.

As part of the hazard identification component of the risk assessment, contaminants are classified as to their weight of evidence for carcinogenicity according to categorization schemes used by the USEPA or other agencies such as the International Agency for Research on Cancer (IARC) [27]. The weight of evidence approach presented in the current USEPA *Guidelines for Carcinogen Risk Assessment* [26] replaced the approach included in the earlier USEPA *Risk Assessment Guidelines of 1986* [28] (Table 1.1). However, the USEPA risk assessments that were developed under the 1986 risk assessment guidelines are still in place for many chemicals that have not been reassessed under the newer USEPA (2005) *Guidelines for Carcinogen Risk Assessment*. Efforts are currently underway at the USEPA to determine if similar standardized schemes can be developed for noncancer effects [18].

Table 1.1 Carcinogenicity classifications/descriptors used by the USEPA

Classifications from the USEPA *Risk Assessment Guidelines of 1986* [28]	**Descriptors from the USEPA 2005 *Guidelines for Carcinogen Risk Assessment* [26]***
Known human carcinogen (group A)	Carcinogenic to humans
Probable human carcinogen (groups B1 and B2)	Likely to be carcinogenic to humans
Possible human carcinogen (group C)	Suggestive evidence of carcinogenic potential
Not classifiable as to human carcinogenicity (group D)	Inadequate information to assess carcinogenic potential
Evidence of noncarcinogenicity for humans (group E)	Not likely to be carcinogenic to humans

*Under the 2005 USEPA guideline [26], the weight of evidence narrative summarizes the results of the hazard assessment and includes a descriptor that represents the overall conclusion about human carcinogenic potential. The USEPA emphasizes the importance of considering all information in the weight of evidence narrative rather than just the descriptor.

1.4 Dose–Response Assessment

The dose–response assessment step involves determination of the nature and shape of the dose–response curve. Regardless of the overall dose–response approach used, the doses used in the animal study of interest must be converted to an equivalent human dose. The appropriate dose metric may differ depending on the endpoint of interest. For example, the lifetime average dose would be used to assess effects from chronic exposure but not for effects resulting from exposure during a critical time period, such as during gestation. The standard USEPA approaches for interspecies dose extrapolation are a comparison of humans and animals on the basis of body weight$^{3/4}$ for oral exposures, depending on allometric scaling of metabolic and physiological processes between species [29], and application of dosimetry models for inhalation exposures that differ for point-of-contact and systemic toxicants [17]. For studies with intermittent dosing (such as 8 out of 24 hours per day for inhalation studies or 5 out of 7 days per week for studies using oral gavage dosing), the average daily dose is calculated by linear adjustment of the exposure concentration or administered dose. Approaches and assumptions have also been developed for extrapolation between different routes of administration (e.g., oral to inhalation or vice versa).

If possible, it is preferable to compare animals to humans on the basis of internal dose (such as serum level), particularly when interspecies toxicokinetic differences result in very different internal doses from the same administered dose. The relationship between administered dose and internal dose (e.g., serum level) in humans and animals is sometimes known from experimental data or can be estimated using toxicokinetic modeling, but this information is often not available.

Human exposure to environmental contaminants is usually below the levels for which information on effects in humans or animals is available. Therefore, the assumed shape of the dose–response curve at these lower doses is important for risk assessment, particularly whether a threshold assumption (an assumption that there is a dose below which effects do not occur) or a nonthreshold assumption (an assumption that some level of risk of an effect results from any dose above zero) is used. A chemical's classification as a carcinogen or

a noncarcinogen is a key decision in the risk assessment process, since different dose–response assumptions are generally used for noncarcinogenic and carcinogenic effects.

An important principle of classical toxicology is that there is a clear dose–response relationship for toxic effects, with effects increasing over a certain dose range below which there is a threshold below which no adverse effects occur (Fig. 1.1). A threshold is assumed for most noncarcinogenic effects, although a threshold (i.e., no-observed-adverse-effect level [NOAEL]) is not necessarily observed in a given study (see following text). However, for some noncarcinogenic effects, it has not been possible to identify a threshold below which effects do not occur. A well-known example is the neurodevelopmental effects of lead in children [30].

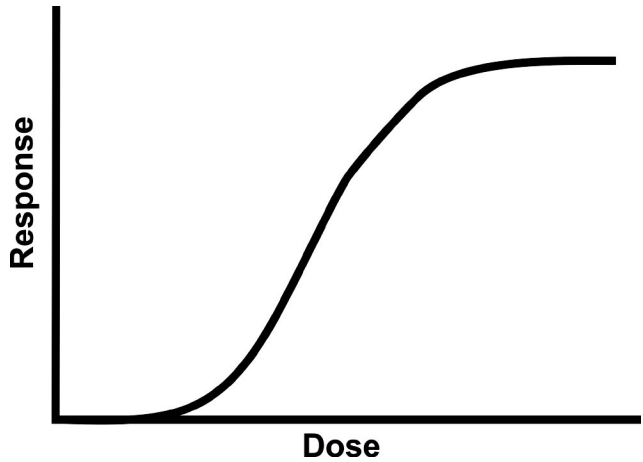

Figure 1.1 Typical dose–response curve for a chemical with a threshold for response.

In contrast, in assessing the risks of exposure to chemicals that cause cancer, the default assumption is that some risk of cancer results from exposure to any amount of the chemical [26] (Fig. 1.2). This default assumption, that there is no threshold below which no risk exists, is used unless a mode of action indicating a threshold has been clearly demonstrated. In most cases, the mode of action of carcinogenicity is unknown, and the public health protective assumption is made that any dose level may cause the initiating event(s), such as DNA damage, that lead to cancer.

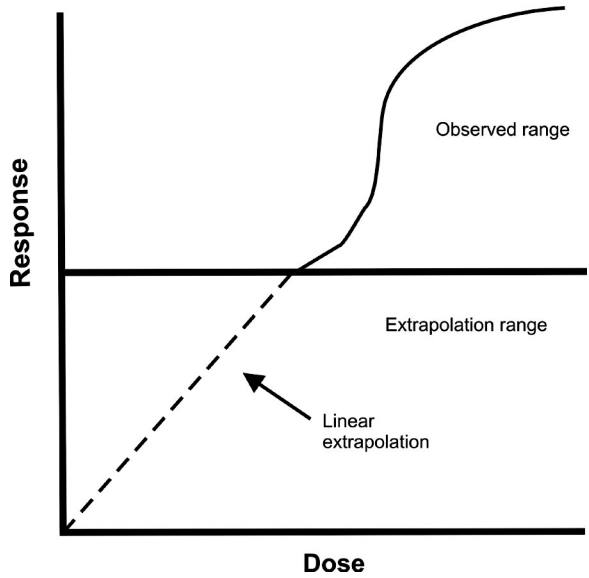

Figure 1.2 Low-dose linear (nonthreshold) dose–response curve.

For some chemicals, it has been shown that cancer occurs secondary to systemic effects. In these cases, it is assumed that a threshold for carcinogenicity exists and a threshold dose–response approach similar to that described in the following text for noncarcinogens is appropriate. Examples of this approach are chloroform, for which tumors in rodents appear to occur only after cell damage and regenerative growth [31], and antithyroid agents that cause sustained elevations in levels of thyroid-stimulating hormone (TSH), resulting in continued stimulation of the thyroid and the development of thyroid tumors [32]. Harmonization of dose–response approach applicable to both carcinogens and noncarcinogens has been proposed [9] but has not been incorporated into standard risk assessment practice as yet.

Nonmonotonic (U-shaped or inverted U–shaped) dose–response relationships (Fig. 1.3), in which lower doses cause greater effects than higher doses over a portion of the dose range, have also been observed, particularly for some endocrine and neurotoxic effects. Vitamins can also exhibit this type of dose–response relationship, as toxicity can result both from deficiency at low doses and from excessive exposure at high doses. The significance of these types of

dose–response curves, and development of approaches to address them, is a current issue for risk assessment of environmental contaminants [33, 34].

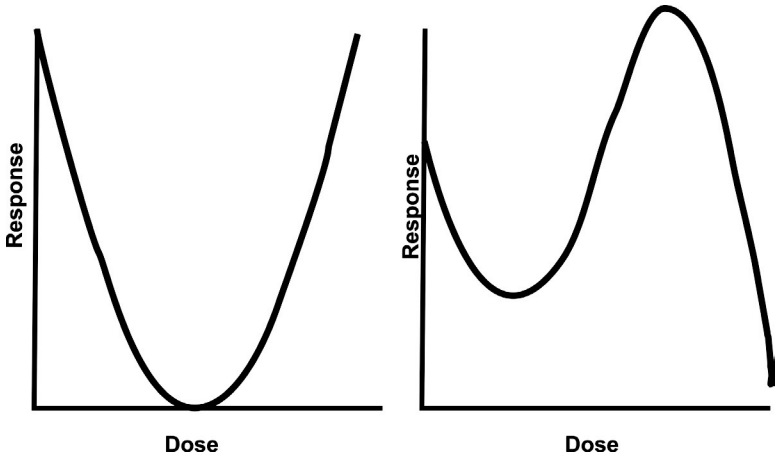

Figure 1.3 Examples of nonmonotonic dose–response curves.

1.4.1 Point of Departure

In risk assessments based on the threshold or nonthreshold approach, a "point of departure" is identified. The point of departure is the dose level used as the starting point for the dose–response extrapolation to doses below those used in the study. When a threshold approach is used (usually for noncancer endpoints), the point of departure can be the highest dose at which no adverse effects have been observed (NOAEL), the lowest dose at which adverse effects have been observed (lowest-observed-adverse-effect level, or LOAEL), or a benchmark dose (BMD)/benchmark dose lower confidence level (BMDL). A BMD is the dose producing a predetermined change in response rate of an adverse effect (e.g., 1%, 5%, or 10%). It is derived by modeling the dose–response data to predict a dose at which such a response rate for the parameter of interest will occur [35] (Fig. 1.4). Under the current USEPA risk assessment guidelines [26], cancer risk assessments based on the default nonthreshold assumption also use linear extrapolation from a BMD for tumor incidence data or the tumor precursor endpoint of interest.

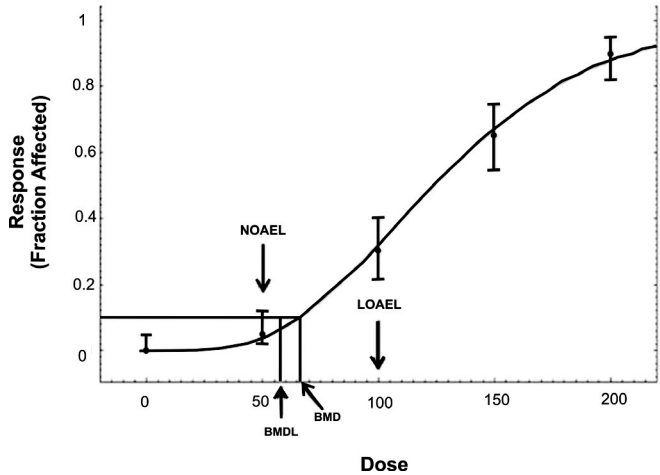

Figure 1.4 Example of the relationship of BMD and BMDL to NOAEL and LOAEL.

1.4.2 Dose Response for Noncarcinogenic Effects

For most noncarcinogenic effects, it is assumed that there is a threshold dose below which no adverse effects occur. The oral RfD, in units of mg/kg/day, and the inhalation RfC, in units of $\mu g/m^3$, represent levels at which no adverse effects are expected from lifetime exposure. RfDs and RfCs are developed from animal data or, less commonly, from human data by applying appropriate uncertainty factors to a dose or concentration (NOAEL, LOAEL, BMDL) chosen as the point of departure (see preceding text) [36]. The uncertainty factors (formerly called "safety factors") used in the derivation of the RfD development account for uncertainties such as:

- Interindividual variability in sensitivity within the human population, including susceptible subpopulations
- Interspecies differences in sensitivity between humans and experimental animals
- Less-than-lifetime duration of a study (or less-than-full critical period for reproductive or developmental effects).
- Extrapolation to a NOAEL from a LOAEL when a NOAEL has not been identified
- Gaps in the toxicology database for the chemical

Uncertainty factors of 10 each are often used, but factors of 3 or other values can be chosen if warranted by the data. The interspecies uncertainty factor is usually assumed to be based equally on toxicokinetic and toxicodynamic factors of $10^{0.5}$, or about 3, each. When comparisons of human animal and human are based on allometric scaling, species-specific dosimetry, or toxicokinetic modeling, it is assumed that interspecies toxicokinetic differences have been adequately considered, and an interspecies factor of 3 for toxicodynamic differences is used. It is recommended that the total uncertainty factor used to develop an RfD or RfC not exceed 10,000 (or 3,000 when interspecies toxicokinetics differences are otherwise accounted for) [36].

The RfD or RfC is derived as follows:

$$\text{RfD (mg/kg/day)} = \frac{\text{Point of departure (NOAEL, LOAEL, or BMD; g/kg/day)}}{\text{Product of appropriate uncertainty factors}}$$

$$\text{RfC } (\mu g/m^3) = \frac{\text{Point of departure (NOAEL, LOAEL, or BMD; } \mu g/m^3)}{\text{Product of appropriate uncertainty factors}}$$

1.4.3 Estimation of Cancer Risk

The doses used in chronic animal studies designed to evaluate carcinogenic potential are generally much greater than the doses to which humans are exposed in the environment. These higher doses are used, in part, because the lowest statistically significant tumor incidence observable in the dose groups (50 per sex per dose) typically used in these studies is about 1%–10% [26], several orders of magnitude higher than the lifetime cancer risk levels generally considered to be significant for public health (1 in 10,000 to 1 in 1,000,000). Under the current USEPA risk assessment guidelines [26], cancer risk is estimated from laboratory animal data for tumor incidence or (less commonly) precursor effects for tumor formation, or, in some cases, human epidemiological data. The carcinogenic potential of a chemical is expressed quantitatively as a slope (or potency) factor (in units of the inverse of daily dose: per milligrams per kilogram per day [mg/kg/day] $^{-1}$) for oral exposure, and the unit risk factor (in units of inverse of air concentration: per microgram

per cubic meter, [μg/m^3]$^{-1}$) for inhalation exposure. The slope factor or unit risk factor used in quantitative risk assessment is derived from linear extrapolation through the origin from the point of departure, which is a BMDL or other estimated dose near the lower end of the observed range (generally the lower 95% confidence limit on the lowest dose level that can be supported for modeling by the data) [26].

The slope (or potency) factor is related to the dose (in mg/kg/day) and the lifetime cancer risk, which is unitless, as follows:

$$\text{Risk (unitless)} = \text{Dose (mg/kg/day)} \times \text{Slope factor (mg/kg/day)}^{-1}$$

Similarly, the unit risk factor is related to the air concentration (in μg/m^3) and the lifetime cancer risk, as follows:

$$\text{Risk (unitless)} = \text{Air concentration (μg/m}^3\text{)} \times \text{Unit risk factor (μg/m}^3\text{)}^{-1}$$

Because of data suggesting greater susceptibility to carcinogens with a mutagenic mode of action in early life than in adulthood, the USEPA recommends the application of age-dependent adjustment of cancer slope factors when assessing risks from exposures that begins in early life [37]. The recommended slope factor adjustments (tenfold for birth to <2 years of age and threefold for 2 years to <16 years of age) must be combined with appropriate age-specific exposure factors for the medium of concern (e.g., drinking-water consumption, incidental soil ingestion; see following text) to determine the media-specific adjustment to the estimated cancer risk.

In the risk characterization portion of the risk assessment, cancer slope factors and unit risk factors, along with exposure factors (see following text), are used to develop health-based criteria based on a specified lifetime cancer risk level or to estimate cancer risks from the concentrations of carcinogenic contaminants found in environmental media.

1.4.3.1 Dose–response approach for suggestive or possible human carcinogens

For certain contaminants, some evidence for carcinogenicity exists, but the weight of evidence is not sufficient for classification as "likely to be carcinogenic to humans" [26] or "probable human carcinogen" (group B2) [28]. These contaminants are classified as "suggestive

carcinogens" [26] or "possible human carcinogens" (group C under the previous 1986 USEPA guidelines) [28]. The risk assessment approach used for such chemicals is a science policy decision. The USEPA Office of Water generally addresses drinking-water contaminants in these categories on the basis of the RfD for noncarcinogenic effects, with the incorporation of an additional uncertainty factor to account for the evidence of possible carcinogenicity. The uncertainty factor is usually 10, but other values from 1 to 10 may be used, as appropriate. Less commonly, if there are insufficient data to develop an RfD, the Office of Water Risk Assessment is based on the cancer slope factor and a lifetime risk level of 10^{-5} (1 in 100,000) to 10^{-6} (1 in 1,000,000) [38]. In contrast, in the USEPA Superfund program for cleanup of contaminated sites, health-based criteria for these chemicals are preferentially based on the cancer slope factor, when one can be developed, or on the RfD without additional adjustment to account for possible carcinogenicity if no slope factor is available [39].

1.5 Exposure Assessment

The exposure assessment step includes characterization of exposure scenarios, routes of exposure, exposed populations (including sensitive subpopulations), and range of exposure levels, including typical and high-end exposures. Exposure metrics of interest include external dose, absorbed dose, and doses to sites within the body that are the targets for toxicity.

Routes of exposure to environmental contaminants include ingestion, inhalation, and dermal absorption. Such exposures can occur from a variety of environmental media through many different types of human activity. Examples of environmental media and exposure pathways include tap water (ingestion as a beverage or in prepared food), inhalation of volatile contaminants released from water into indoor air, surface water (incidental ingestion of water and dermal absorption from water during recreational activities), fish (consumption of recreationally caught or commercially available fish), soil (incidental ingestion, dermal absorption, inhalation of

volatile contaminants or dust particles), and outdoor air (inhalation of air toxics from point or nonpoint sources).

The exposure parameters used in risk assessments often are default values but may be site specific if the needed information is available. Since average daily exposure on a body weight basis is generally the basis for risk assessment, information is needed about body weight and exposure frequency (hours per day, days per week, weeks per year) and duration (number of years) of exposure, as well as the daily exposure to the medium of interest (e.g., liters per day of water ingested, grams per day of fish or soil ingested, cubic meters of air inhaled per day). Extensive data on human exposure parameters to many environmental media during a wide range of human activities are available for children and adults [16, 17]. These data sources include the distribution of exposure parameters in the general population, as well as exposures by age group, gender, and other relevant subgroups. To be protective of public health, reasonable upper-percentile exposure parameters, such as 90th percentile values, rather than median or average, are usually used in risk assessments. Probabilistic approaches, such as Monte Carlo simulations, which estimate the overall exposure distributions by combining the distribution ranges of each of the parameters that impact exposure (e.g., body weight, exposure frequency, daily intake) have been developed, but these have not been widely incorporated into risk assessment practice [6].

Exposure assumptions for adults (e.g., body weight of 70 kg and ingestion rate of 2 L/day for drinking-water assessments) are often used to develop health-based criteria intended to be protective for lifetime exposure. When a risk assessment is based on effects in a sensitive subpopulation, the exposure parameters for that subgroup are used in the risk assessment. For example, the USEPA drinking-water standard for nitrate is based on methemoglobinemia in infants and uses exposure parameters (body weight and daily water consumption) for infants instead of adults [40]. Similarly, fish consumption advisories based on prevention of neurodevelopmental effects resulting from fetal exposure use the body weight of a pregnant women instead of average adult. Exposure assumptions for infants or children may also be generally used for health-based levels protective for shorter-term exposure durations, such as the USEPA One Day and Ten Day Drinking Water Health Advisories

[41]. Additionally, separate risk-based criteria applicable to chronic exposures of different subpopulations can be developed using exposure assumptions appropriate for each group. For example, residential and nonresidential soil remediation criteria are based on exposures of children and outdoor workers, respectively [42].

Finally, exposure parameters for different life stages may be integrated to provide time-weighted average exposures for the entire time period of interest. For example, residential soil remediation criteria assume 30 years of residence at a contaminated site. The soil criteria based on oral exposure integrate the greater default daily incidental soil ingestion rate (200 mg/day) and lower average body weight (15 kg) from ages 1–6 years and the lower default soil ingestion rate (100 mg/day) and higher average body weight (70 kg) from ages 7–31 years [42]. A similar approach has been developed for integration of drinking-water ingestion rates during different age periods [43]. The values used are 0.137 L/kg-day for up to 2 years of age, 0.047 L/kg-day for 2–16 years of age, and 0.039 L/kg-day 16 years of age and older), depending on the time-weighted average of the 95th percentile intake rate for each age range.

Slope factors for carcinogens estimate risks of lifetime exposures, typically assumed to be 70 years. Cancer risks from chronic exposures (often defined as greater than 10% of a lifetime) that are less than a full lifetime in duration (such as the 30-year period often assumed to be the length of residence in a particular location), are generally assumed to be proportional to the exposure durations (e.g., 30/70 years). However, estimation of cancer risks from exposures for less-than-chronic time periods representing a much smaller fraction of the total lifespan (e.g., 1 year or less) on the basis of chronic slope factors is considered to be highly uncertain.

1.5.1 Multiple Exposure Routes

Exposure to a given environmental contaminant usually does not occur through a single route but rather through multiple pathways and sources. In some cases, risk assessments consider only the risks from one exposure route, while in other instances, multiple routes are considered. For example, health-based drinking-water criteria for noncarcinogenic contaminants consider contributions from non-drinking-water sources of exposure, including air and food,

to ensure that total exposure from all sources does not exceed the RfD. This is accomplished through application of a relative source contribution (RSC) factor of between 20% and 80% to the drinking-water equivalent level (DWEL; the concentration based on exposure through drinking-water ingestion alone) [44]. When the data on air and food exposures needed to develop a contaminant-specific RSC are unavailable, as is the case for most contaminants, a default value for the RSC of 20% is used. If drinking water contributes 80% or more of total exposure, a "ceiling" RSC value of 80% is used to protect individuals whose nonwater exposures may be higher than that indicated by available data. If drinking water contributes less than 20% of total exposure, a "floor" RSC value of 20% is generally used. In such cases where drinking water contributes a relatively small portion of total exposure, it is presumed that efforts are best directed toward reducing the exposures from other sources, as further reductions of the drinking-water standard or guidance based on use of an RSC of less than 20% will not result in a significant decrease in total exposure to the contaminant.

Another example of multiroute exposure is human health–based surface water criteria that consider all designated uses of a water body. If a freshwater body is used both as a drinking-water source and as a source of consumed fish, exposures from drinking water (2 L/day) and fish consumption (17.5 g/day) are considered in developing the criteria [44].

1.5.2 Bioavailability

Bioavailability refers to the fraction of a substance that is absorbed and/ or becomes available to the target tissue after administration or exposure. An implicit assumption in most risk assessments in which bioavailability is not explicitly considered is that the fraction absorbed in the animal or human study that forms the basis for the risk assessment is the same as from the environmental exposure of concern. For some contaminants, bioavailability (fraction absorbed) is known to differ among environmental media, and the exposure assessment component of the risk assessment can be adjusted to account for this. For example, the RfD for cadmium in the diet is higher than for cadmium in drinking water due to bioavailability differences [45]. Bioavailability may also vary on a site specific

basis, particularly for metals in soil. If it is known that the human bioavailability of a soil contaminant at a specific site is lower than the default value, due to the geochemical characteristics of the soil at the site, it is appropriate to adjust the assumed exposure from the soil accordingly. Approaches have been developed to assess bioavailability of some soil contaminants on a site-specific basis [46].

1.6 Risk Characterization

In the risk characterization step of the risk assessment, the information from the earlier steps (hazard identification, dose response, and exposure assessment) is combined and synthesized. Risk characterization includes description of the risk assessment results as well as discussion of the underlying assumptions, uncertainties, and overall confidence in the risk assessment.

Results of a risk assessment may be expressed in several forms. For example, a health-based criterion may be developed that is protective for short-term, intermediate, or lifetime exposure to a contaminant in a particular medium, such as air, water, or soil. As discussed in the preceding text, exposure assumptions appropriate for specific populations (e.g., children, workers) may be used, depending on the purpose of the criterion. To develop health-based criteria for carcinogenic contaminants, a target risk level (typically in the range of 10^{-4} to 10^{-6}) must be selected. It is important to recognize that there is no scientific basis for the choice of a particular cancer risk level and that the selection of the risk level is a policy decision.

As examples that are representative of the derivation of criteria for environmental media in general, the equations used to derive health-based criteria for noncarcinogenic and carcinogenic drinking-water contaminants are as follows:

Health-based drinking-water criterion for noncarcinogens (mg/L)
= RfD (mg/kg/day) × Body weight (kg)
× RSC factor (unitless) /Ingestion rate (L/ day)

Health-based drinking-water concentration for carcinogens (mg/L)
= Target cancer risk level (10^{-x}, unitless)
× Body weight (kg)/Slope factor (mg/kg/day) $^{-1}$
× Ingestion rate (L/day)

The results of a risk assessment may also be presented as an evaluation of the potential risks from exposure to one or more contaminant(s) from a contaminated site or other situation, such as a chemical release or spill. In this type of risk assessment, the contaminant levels to which people may be exposed are estimated from site-specific data and compared to risk-based criteria for the environmental media and contaminants of concern.

Multiple chemicals are often present at contaminated sites or other situations of environmental contamination. Prediction of the toxicity of mixtures is complicated because chemicals can interact to cause additive, as well as synergistic (greater than additive) or antagonistic (less than additive) effects [47]. Toxic equivalency factors (TEFs) or relative potency factors (RPFs) have been developed for assessing the risks of mixtures of a few groups of structurally related chemicals that are known to cause toxicity through a common mode of action but with varying potencies. This approach has been applied to mixtures of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and dioxin-like polychlorinated biphenyls (PCBs) [48], and to polycyclic aromatic hydrocarbons (PAHs) [49]. In this approach, each member of the group is assigned a TEF on the basis of its relative potency as compared to an index compound with a TEF or an RPF of 1 [e.g., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for dioxins, benzo(a)pyrene for PAHs]. The risk assessment is based on the toxicity-weighted concentrations of all members of the group, expressed as toxic equivalents (TEQs) of the index compound.

Approaches have also been developed to estimate the combined risks of other mixtures of contaminants, depending on simplifying assumptions. For carcinogens, the total cancer risk can be estimated by summing the cancer risks for the individual contaminants on the basis of the assumption that there are no synergistic or antagonistic interactions related to carcinogenicity. For noncarcinogens, an approach has been developed in which the estimated exposure, expressed as fraction of the RfD (hazard quotient) is calculated for each chemical of concern. The hazard quotients for each chemical are totaled to obtain a hazard index [39, 47]. A hazard index of less than 1 is assumed to be without risk of health effects, while a hazard index exceeding 1 may pose a risk. Although the hazard index approach is scientifically supportable only for chemicals that cause

the same type of toxicity through the same mechanism of action, it is often applied, at least as an initial screen, to RfDs for all chemicals that are present at the site being evaluated, without an evaluation of whether they cause similar toxicity.

1.6.1 Characterization of Uncertainty

Uncertainty in risk assessment is most often expressed qualitatively but can be presented quantitatively, for example, through sensitivity analysis, if sufficient data are available for the parameters of interest. Uncertainties can relate to the completeness of the health effects database, the quality of the key studies, and the consistency of the health effects reported in animals and/or humans. If studies of key toxicological endpoints such as developmental effects or cancer have not been conducted, there is uncertainty about whether a risk assessment based only on other endpoints for which data are available is protective for these unstudied potential effects. Additionally, there is growing awareness of important health effect endpoints that may not be detected by standard toxicity study protocols. Two examples of such endpoints are effects resulting from prenatal or early-life exposures that do not become evident until adulthood and subtle neurobehavioral effect detected only through specialized protocols. Another example of uncertainties in the hazard identification component is the level of confidence about the relevance to low-dose human environmental exposures of modes of action that have been characterized in animals and/or highly exposed humans.

Uncertainties in the dose–response portion of the risk assessment (discussed in the preceding text) relate to the magnitude of the total uncertainty factor used in the development of a RfD or RfCs and uncertainties about cancer risk estimates based on extrapolations over several orders of magnitude from experimental doses causing tumors in several percent of exposed animals to exposures relevant to low target risk levels (usually 1 in 10,000 to 1 in 1,000,000). More generally, it is usually not known by how much the RfD can be exceeded before effects begin to occur. For cancer risk assessment, the primary uncertainty is the actual shape of the dose–response curve at doses below the observed range.

Uncertainties in the exposure assessment component may include uncertainties about the completeness of the data used to

develop default exposure parameters and the relevance of exposure parameters based on the general population to subpopulations with higher exposures. Additionally, the use of reasonable upper percentile (e.g., 90th percentile) estimates for multiple factors, when compounded, may result in an overall exposure estimate representative of the extreme high end of the exposure distribution.

The overall confidence in the risk assessment is generally based on professional judgment that considers all of the issues mentioned in the preceding text and any other factors specific to the risk assessment being evaluated.

1.7 Future Directions

The NRC's vision and recommendations for improving both the technical basis and the "utility" of risk assessment within the USEPA are presented in a 2009 report [9]. Some of the major recommendations related to the technical aspects of risk assessment include improvement in analysis of uncertainty and variability and development of a unified approach that can be used to develop risk estimates for both cancer and noncancer endpoints.

Additionally, a 2007 recent report by the NRC of the National Academy of Sciences [50], commissioned by the USEPA, envisaged a shift from the current whole animal-based testing systems to high-throughput testing founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components. The overall goal is to develop the ability to rapidly test large numbers of chemicals, while reducing the necessity for animal testing, which is expensive and time consuming and raises concerns related to animal rights and animal welfare. However, many questions and issues must be addressed for this vision to become a reality, and it is unclear whether the in vitro methods advocated by this report are capable of predicting most or all of the toxic effects that can occur within the complex biochemical and physiological systems of vertebrates. Future use of high-throughput testing data as the basis for the dose–response component of risk assessment has been proposed, but at the present time, these approaches appear to be most appropriate as screening tools that can contribute to the hazard identification step of the risk assessment process [51].

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Chapter 2

Dose–Response Assessment: Significance in Risk Assessment

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2.1 Introduction

The risk assessment approach is based on the risk assessment paradigm developed by the National Research Council of the National Academy of Sciences [1]. This paradigm divides the process of human health risk assessment into four components: hazard identification, dose–response assessment, exposure assessment, and risk characterization. The first step, hazard identification, is a qualitative assessment that determines whether a given chemical is causally linked to particular health effects. It involves the review of relevant scientific data to determine if exposure to a chemical substance is causally related to increased incidence of adverse health effects in humans, the nature of those effects, and the biological

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significance and relevance of those observed effects. The second step, dose–response assessment, is a quantitative evaluation that determines the relationship between the magnitude of exposure and the probability of occurrence of the health effects in question. It involves determination of the doses at which various effects are observed, with the goal of identifying the critical effect (the most sensitive adverse effect) and estimating the quantitative relationship between the amount of exposure and the risk of a particular adverse effect at that dose. The exposure assessment involves identification of the routes of exposure (i.e., oral, inhalation, dermal), estimation of the amount of a chemical an individual is exposed to, and estimation of the number of individuals likely to be exposed. The last step, risk characterization, integrates dose–response and exposure assessment to determine the likelihood of a response under specific exposure conditions. The risk characterization step also identifies limitations and uncertainties in the derived risk values to provide a comprehensive estimate of potential risk to exposed populations.

Dose–response assessment plays a central role in the risk assessment paradigm. For the purpose of health protection, regulatory agencies are interested in identifying potential health effects caused by exposure to a particular agent, doses to which humans might be exposed, and a level that could cause potential deleterious effects. To achieve this goal, we ideally need to have a comprehensive understanding of the dose–response relationships for the chemical of concern as well as the level of human exposure that could be at very low levels from the environment. In some instances, epidemiologic data are sufficient to define a dose–response relationship based on observations of exposure and health effects in humans. In such cases, the only necessary extrapolations to the low-level environmental exposure would be to account for population differences in sensitivity. In most cases, there is a lack of such epidemiologic data, and controlled animal studies are conducted to provide needed information or supplement available human toxicity data. Animal studies allow rigorous study design to control experimental factors such as the number and composition (age, gender, species) of test subjects, the levels of dose tested, and the measurement of specific responses; therefore, they often provide reliable observations. Use of a designed study typically leads to more meaningful statistical conclusions than does an uncontrolled

observational study, where additional confounding factors must also be considered for their impact on the conclusions. However, dose–response relationships observed from controlled animal studies are often at much higher doses than would be anticipated for human environmental exposure; thus, an extrapolation to lower doses is necessary. The dose–response relationship observed in animal studies must also be extrapolated from animals to humans in order to predict the relationship for humans. These extrapolations, among others, would introduce uncertainty into the dose–response analysis.

2.2 Dose–Response Relationship

Dose–response assessment includes two processes. The first process is an assessment of all data that are available or can be gathered through experiments to document the dose–response relationship(s). Frequently this range of data points may not include sufficient information to identify a critical region where the adverse effect starts to occur (i.e., the dose that is low enough not to cause the effect or reference dose shown in Fig. 2.1) in the human population. The second process consists of extrapolation to estimate the risk of potential adverse effect beyond the lower range of available data to make inferences about the critical region where the dose level begins to cause the adverse effect in the human population. The extrapolation may involve a high- to low-dose extrapolation and/or animal-to-human extrapolation.

The initial step of dose–response assessment is to evaluate the scientific information for a better biological understanding of how each type of toxicity or response (adverse effect) occurs (i.e., a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in the effect) also known as mode of action. Depending on the chemical's mode of action, different approaches (nonlinear or linear dose–response assessment) are used to estimate the potential risk posed by a chemical substance. For example, many regulatory organizations assume that noncarcinogenic and nonmutagenic effects have a threshold, a dose level below which an empirically observable response is unlikely because homeostatic

compensation and adaptive mechanisms in the exposed tissue protect against or effectively repair toxic effects. In contrast, chemicals that cause cancer by a mutagenic or unknown mode of action are assumed not to have a threshold. On the basis of this mode of action, the risk assessor determines the nature of the extrapolation used in the second process discussed in the preceding text, either through nonlinear or through linear dose–response assessment.

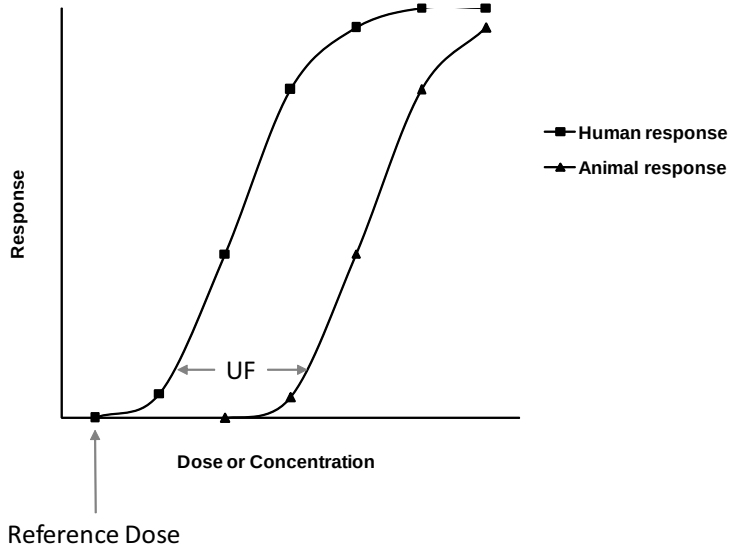


Figure 2.1 An example of dose–response relationship between humans and animals.

2.3 Nonlinear Dose–Response

The assumption of a threshold noncarcinogenic response allows for the estimation of a risk value for humans. It is generally based on an estimated response level that marks the beginning of a low-dose extrapolation (i.e., the point of departure [POD]). The POD is derived from well-conducted epidemiologic studies if they are available or, more commonly, animal toxicity studies with subsequent application of uncertainty factors (UFs). Traditionally, a no-observed-adverse-effect level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL) is used as the POD. Recently, dose–response modeling

(i.e., calculating a benchmark dose [BMD] or a lower-bound benchmark dose [BMDL]) has been used to identify the POD in place of a NOAEL.

2.3.1 NOAEL and LOAEL

The NOAEL is the highest exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control. Due to the nature of methods used to define an adverse effect, some effects (increased frequency or severity) may be produced at this level, but they are not considered to be biologically significant or statistically significant. The NOAEL is commonly used as an estimate of a subthreshold dose for the adverse effect of interest and as the POD to derive risk values. The LOAEL is the lowest exposure level at which there are statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control. In the absence of a NOAEL, a LOAEL is used as the POD and a LOAEL-to-NOAEL extrapolation UFs would be applied to estimate a risk value.

2.4 Dose–Response Modeling

One of the most recent significant improvements in dose–response assessment in chemical risk assessment is the application of BMD modeling methodology. The BMD (for oral exposure) or the benchmark concentration (BMC; for inhalation exposure) is defined by the United States Environmental Protection Agency (USEPA) [2] as "a dose (or concentration) that produces a predetermined change in response rate of an adverse effect (called the benchmark response or BMR) compared to background." This method was developed by Crump [3] and Dourson et al. [4] as an improvement over the NOAEL/LOAEL method for developing noncancer risk values. Due to significant advancement in computing capabilities and continuous efforts in the development of BMD modeling software by the USEPA in recent years, this method has been adopted widely by the risk assessment community. The BMD software is freely available from the USEPA's website: http://www.epa.gov/ncea/bmds/.

The BMD/BMC is calculated by first fitting one or more flexible mathematical model(s) to the observed dose–response data, as shown in Fig. 2.2. To avoid extrapolating well below the observed range, the BMR is usually chosen at the lower end of the experimentally detected response range. The adequately fitted model is used to identify the dose (i.e., BMD) corresponding to the BMR. Often, a statistical confidence lower bound (usually the 95% lower bound on the dose, as depicted by the blue line in Fig. 2.2) is used instead of the central estimate dose response at that point to account for statistical uncertainty and to ensure a health-protective result. This lower bound is referred to as the BMDL or lower-bound benchmark concentration (BMCL) and is used in place of a NOAEL or LOAEL as the POD in the derivation of risk values.

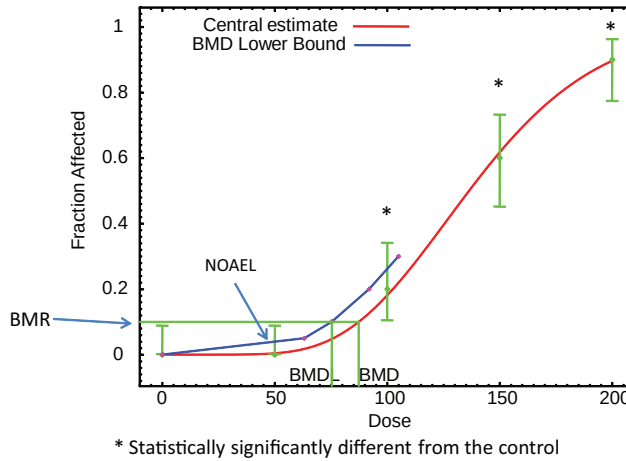


Figure 2.2 An example of estimating a NOAEL and BMDL from a dose–response curve with a sample size of 50 animals per dose group.

BMD modeling has a number of advantages over the NOAEL/LOAEL approach for developing risk values [2–8]. First, the BMD is not limited to being one of the experimental doses. BMD modeling uses all the information provided by the dataset and the BMD is much less dependent on dose spacing than is the NOAEL/LOAEL approach; thus the BMD can give a much better estimate of the toxicological response at the low-dose range when the dose spacing is large. A second advantage of the BMD approach is that it uses more of the dose–response data, unlike the NOAEL/NOAEL, which is based on a

single dose. This means that the BMD can incorporate information about the slope of the dose–response curve. In addition, a BMD can be calculated even if a study did not identify a NOAEL, removing the need for extrapolation from a LOAEL.

Another advantage of BMD modeling is that it reflects uncertainty and variability, particularly uncertainty associated with sample size. All other things being equal, smaller sample size results in reduced statistical power and therefore wider confidence limits. Because NOAELs are often identified on the basis of statistical significance, a smaller (less powerful) study would tend to result in higher, less protective NOAELs (since it would be less likely that smaller changes are statistically significant) as the POD than experiments with a larger sample size (see Figs. 2.2 and 2.3) [9]. Theoretically, a lower POD should be used when there is more uncertainty in the toxicity data. Using the BMD approach, a smaller sample size would tend to result in wider confidence limits and a lower (more conservative) BMDL, corresponding to the greater uncertainty with the smaller study.

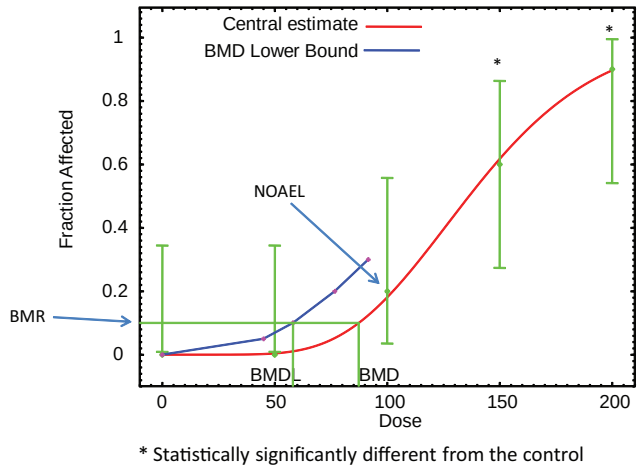


Figure 2.3 An example of estimating a NOAEL and BMDL from a dose–response curve with a sample size of 10 animals per dose group.

Another limitation of the NOAEL approach is that it is based on a combination of scientific judgment and statistical analysis, and the role of scientific judgment is often a source of controversy. In contrast,

the BMD is a function of a defined response level, which can lead to increased consistency in response comparison across endpoints. However, the use of the BMD does not remove all controversy or the need for scientific judgment, since such judgment plays an important role in the definition of the BMR, and a given BMR (e.g., 10% change in organ weight) may have very different biological implications, depending on the target organ/tissue (e.g., liver vs. brain weight).

Though there are many advantages to using BMD to calculate reference values (i.e., reference doses [RfDs] for oral exposure and reference concentrations [RfCs] for inhalation exposure), there are also a number of limitations, some of which are shared with the NOAEL/LOAEL approach. First, the results are limited by the quality of data available, and some studies do not report data in a form appropriate for modeling (see the following section). The data also need to exhibit a dose-related trend, ideally containing data point(s) at the lower end of dose–response range. Toxicological judgment still plays a critical role, and proper identification of the studies and endpoints to be used for modeling must be done to ensure a useful BMD/BMC is derived [10]. Without good quantitative data or quality data reporting, there may be substantial uncertainty in the resulting BMD/BMC (and therefore the estimate of risk values), despite the apparent precision resulting from modeling. Finally, conducting BMD modeling increases the amount of time and resources needed for an analysis.

2.5 Identifying the Critical Effect

Upon exposure to a toxic agent, individuals may develop more than one adverse response. As the dose increases, the first adverse effect (or its precursor changes in structure or function) that occurs is identified as the critical effect. Protecting exposed individuals from the critical effect would imply that other adverse responses are also prevented. Therefore, for risk assessment purposes, the POD for deriving a risk value is usually based on the dose–response data for the critical effect.

In practice, the critical effect is identified by a comparison of subthreshold dose estimates (e.g., the highest NOAEL, the lowest LOAEL, or the BMDL) for observed adverse effects from each

toxicity study in order to identify the most sensitive effect that would have the lowest subthreshold dose. To do so, the results of the hazard identification are reviewed to identify the adverse effects relevant to humans, the doses at which these effects occur and the corresponding NOAEL, LOAEL, or BMDL. The adverse effect with the lowest NOAEL/BMDL or LOAEL is considered the most sensitive adverse response (i.e., critical effect), and the corresponding NOAEL, LOAEL, or BMDL will serve as the POD for deriving a risk value.

During the comparison of the different adverse effects, it is critical to consider the limitations inherent in each subthreshold estimate rather than indiscriminantly selecting the smallest threshold estimate among all of the adverse effects. For example, BMD modeling for a dose–response with smaller sample size (e.g., a sample size of 10 in a subchronic study) would result in a lower BMDL estimate than a similar dose–response with a larger sample size (a sample size of 50 in a chronic study) (see Figs. 2.2 and 2.3). In this case, the chronic study with a longer exposure duration and large sample size results in greater statistical power and provides more reliable dose–response information; therefore, it would form a better basis to identify the dose–response threshold.

2.6 Low-Dose Extrapolation

Noncancer toxicity is defined as a health effect other than cancer and gene mutations that is due to the effects of environmental agents on the structure or function of various organ systems. By definition, a database for derivation of a dose–response estimate for noncancer toxicity should ensure that both appropriate and adequate numbers of endpoints have been evaluated. As stated in the USEPA risk assessment methods [11], "the minimum laboratory animal toxicological database requirement for the derivation of an RfD/RfC with low confidence is a well-conducted subchronic bioassay that evaluated a comprehensive array of endpoints, including an adequate evaluation of portal of entry (e.g., respiratory tract) effects, and established an unequivocal NOAEL and LOAEL. For a higher confidence RfD/RfC, chronic bioassay data, two-generation reproductive studies, and developmental studies in two different mammalian species are usually required."

For nonlinear dose–response assessment, derivation of a reference value from a POD for the critical effect is accomplished by using UFs or data-derived adjustment if data allow accounting for uncertainty and variability in the derivation. These extrapolations need to account for interindividual extrapolation from average healthy humans to sensitive humans when the available POD for the critical effect is identified directly from average human population. Adjustments for interspecies extrapolation from laboratory animal data to humans are also needed when the critical effect is identified from an animal study. Furthermore, additional UFs are used when the overall toxicity database is incomplete, when a LOAEL is used as the POD, and/or when data from a subchronic study are extrapolated to estimate chronic effects.

While there are modest differences in specific applications between different regulatory agencies and organizations, all of them have factors to account for differences between average humans and sensitive humans and differences between experimental animals and humans. The other factors address lack of knowledge (uncertainty) and may be addressed separately or holistically. For example, the USEPA has separate factors for (1) the lack of a NOAEL, (2) extrapolation to lifetime exposure, and (3) gaps in the database, while the International Programme on Chemical Safety (IPCS) and Health Canada consider these three areas holistically as data gaps (see Table 2.1). For each of these areas, the preference is to base the factor on the available understanding of the chemical's mode of action and toxicity (e.g., whether there is information suggesting an increased toxicity when exposure duration increases or gaps in the database could be supplemented by the database for a similar compound); default values are used in the absence of such data. The composite UF is determined as the product of the individual UFs.

2.7 Interspecies and Human Interindividual Variability

Historically, UFs have been used to account for animal to human variability (interspecies) and human interindividual variability (also known as intraspecies) in derivation of acceptable level of exposure [12, 13]. Human data are always the preferred data to derive human

risk values. When such data are neither available nor adequate, toxicity data from experimental animals may be used. In this case, it is assumed that the results observed in experimental animals are relevant to humans, and humans are more sensitive than animals at a given dose or concentration. If a POD is based on human data, then a UF value of less than 10 or even 1 is appropriate for interspecies variability. When the POD is identified on the basis of animal data, and there are no adequate data to further characterize the difference between animals and humans, then an interspecies uncertainty factor (UF_A) will be applied to account for the extrapolation from animal to human. A reduced default UF_A (e.g., 3) will be applied when dosimetric adjustments (as discussed in detail in following sections) are used to account for some of the toxicokinetic differences between animals and humans [14, 15]. A full default UF_A of 10 is used when dosimetric adjustments are not employed [12, 13].

Table 2.1 Default uncertainty factors used by regulatory agencies

UFs	EPA	ATSDR	RIVM	Health Canada	IPCS
Human interindividual	10 (3.16 × 3.16)	10	10	10 (3.16 × 3.16)	10 (3.16 × 3.16)
Interspecies	≤10* (3.16 × 3.16)	10	10	10 (2.5 × 4)	10 (2.5 × 4)
Subchronic to chronic	≤10	NA	10	1–100	1–100
LOAEL to NOAEL	≤10	10	10		
Database	≤10	NA	NA		
Modifying factor	Discontinued	NA	NA	1–10	1–10

*Currently the USEPA uses 3 with a dosimetric adjustment to calculate the human equivalent dose (HED) for oral exposure or the human equivalent concentration (HEC) for inhalation exposure for a POD.

The factor for human interindividual variability accounts for the natural differences (e.g., metabolism) that occur between human subpopulations and for the fact that some subpopulations may be more sensitive than the average population. If an RfD is based on

human data gathered in a known sensitive subpopulation, a value of less than 10, perhaps even 1, may be chosen for this factor (e.g., an human interindividual UF of 1 was used in the USEPA Integrated Risk Information System [IRIS] fluorine assessment) [16]. When the available data do not adequately characterize the response of sensitive individuals, a human interindividual UF (UF_H) with a default value of 10 is used to account for a lack of information on potential sensitive subpopulations.

Recently, there has been a significant advancement in using toxicokinetic data to inform quantitative relationships between animals and humans as well as between sensitive humans and average humans. These advancements include (a) the application of categorical dosimetric adjustments in the estimation of a human equivalent dose (HED) or the human equivalent concentration (HEC) for a particular effect [11], (b) data-derived chemical-specific adjustment factors (CSAFs) [17], and (c) the use of fully data-dependent physiologically based pharmacokinetic (PBPK) models to directly calculate HEDs or HECs. The latter provides the most data-rich and reliable dose–response estimate along with decreases in uncertainties in the reference value derivation [18] (see Fig. 2.4). In the following sections, we will focus our discussion on the current application of these new methods in the risk assessment arena.

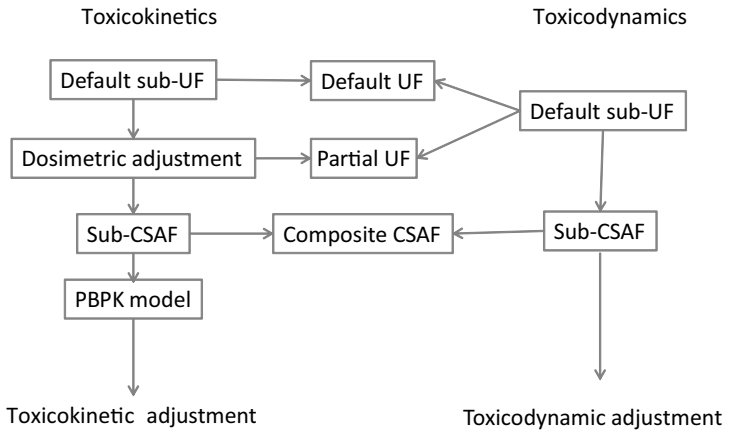


Figure 2.4 Continuum of using toxicokinetic and toxicodynamic data to inform quantitative relationships between animals and humans as well as between sensitive humans and average humans.

2.8 Dosimetric Adjustment

When human data are not available or are inadequate, it is necessary to use animal data to define the POD. An interspecies UF is commonly used to extrapolate animal data to average healthy humans. This UF is considered to be composed of subfactors for toxicokinetics (how the body absorbes, distributes, metabolizes, and excretes the chemical) and toxicodynamics (how the body responds to the chemical). If no information is available on the quantitative differences between animals and humans in either these two subcomponents, then a default value of 10 is used. If information is available on any of these two subcomponents, then this information is used along with a default value for the remaining subfactor. For example, if a dosimetric adjustment is conducted to calculate an HEC for the POD, it will account for a toxicokinetic difference between animals and humans. As the result, only a default subfactor (e.g., a factor of 3 used by the USEPA) is used to account for the remaining uncertainty in the toxicodynamic difference from animals to humans [11].

2.8.1 Inhalation Exposure to Particles

The concept of using data to replace the toxicokinetic component of the interspecies UF has been introduced by the USEPA in its inhalation RfC methods [11]. A key element of this adjustment is estimation of the "dose" (i.e., agent mass deposited per unit surface area or tissue volume) delivered to specific target sites in the respiratory tract or made available to uptake and metabolic processes for systemic distribution. In this adjustment, it is assumed that an effective (target tissue) dose in a particular species is expected to be equally toxic when achieved in some other species (e.g., humans). Adjustment factors are used to adjust the observed exposure levels (i.e., NOAELs, LOAELs, etc.) in laboratory animals to estimate a concentration that would be an equivalent exposure to humans (i.e., $NOAEL_{HEC}$, $LOAEL_{HEC}$, etc.). The calculation of HECs takes into account whether the material is a particle (aerosol) or a gas (or vapor), the anatomic location of the target for the endpoints(s) of interest (systemic, or regions of the respiratory tract), and differences in breathing rates and respiratory tract regional surface areas in experimental animal species and humans. These HECs then form the basis for comparison and choice of the critical effect and study.

Many inhalation toxicity studies using laboratory animals use discontinuous exposure regimens, which are often for six to eight hours per day and five days per week. Inhalation RfCs are usually constructed to reflect a continuous exposure. By extension, the inhalation risk value also is assumed to be protective for discontinuous exposures at the same air concentration. Normalization to some given exposure (e.g., 24 hours/day continuously for a lifetime) is needed to adjust for the wide variety of experimental exposures to permit comparisons between studies [11]. Therefore, the first step is to calculate a duration-adjusted exposure level.

The duration-adjusted exposure levels in milligrams per cubic meter for experimental animals can be calculated as

$$\text{NOAEL}_{\text{ADJ}}\ (\text{mg/m}^3) = E\ (\text{mg/m}^3) \times D\ (\text{hours/day})/24\ (\text{hours}) \times W\ (\text{days/week})/7\ (\text{days})$$

where $\text{NOAEL}_{\text{ADJ}}$ = duration-adjusted NOAEL exposure levels (mg/m^3), E = experimental exposure level (mg/m^3), D = number of hours exposed per day, and W = number of days of exposure per week.

The derivation of the $\text{NOAEL}_{\text{HEC}}$ for insoluble and approximately spherical particles is calculated from the $\text{NOAEL}_{\text{ADJ}}$ as

$$\text{NOAEL}_{\text{HEC}}\ (\text{mg/m}^3) = \text{NOAEL}_{\text{ADJ}}\ (\text{mg/m}^3) \times \text{RDDR}_{\text{r}}$$

where $\text{NOAEL}_{\text{HEC}}$ = the NOAEL exposure levels dosimetrically adjusted to an HEC and RDDR_{r} = regional deposited dose ratio for the respiratory tract region (r) of interest for the toxic effect.

The RDDR_{r} is most easily calculated using the software (RDDR software) available as a supplement to the USEPA RfC methods document [11] or the multiple-path particle deposition (MPPD) model developed jointly by the Chemical Industry Institute of Toxicology (CIIT, currently the Hamner Institutes for Health Sciences) and the Dutch National Institute for Public Health and the Environment (RIVM) [19]. The respiratory tract can be divided into extrathoracic, tracheobronchial, and pulmonary regions; thus, a region-specific RDDR for these regions of the respiratory tract as well as extrarespiratory (systemic) effect can be directly calculated by the RDDR software [11]. To run these programs, the software requires input on the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (σ_{g}) of the particle size distribution, in addition to the animal species and body weight information (for

estimation of region-specific surface area in the airway) for which calculation of an $RDDR_r$ is desired.

It is important to calculate the HEC for each observed adverse effect in a particular region before the final selection of POD because it is difficult to identify which NOAEL/BMCL is lower compared to other NOAELs/BMCLs. This could be illustrated in the following hypothetic example. In subchronic inhalation rodent studies, epithelium damage was observed in the tracheobronchial region of rats and mice after treatment with particles (MMAD of 2.3 μm and σ_g of 1.8). The duration-adjusted $NOAEL_{ADJ}$ is 45 mg/m^3 (in rats) and 30 mg/m^3 (in mice), respectively. Without a calculation of the HECs, the $NOAEL_{ADJ}$ from the mouse study appears to be a more sensitive (lower) effective dose (see Table 2.2). However, the RDDR for the tracheobronchial region in rats is 0.863 and in mice is 1.738. After dosimetric adjustment, as shown in the Table 2.2, the final $NOAEL_{HEC}$ from the rat study is 39 mg/m^3 versus 52 mg/m^3 in mice; the rat $NOAEL_{HEC}$ appears to be a more sensitive (lower) effective concentration. Therefore, it might be more appropriate to use the rat $NOAEL_{HEC}$ as the POD to derive an inhalation risk value. This comparison clearly demonstrates that it is critical to conduct dosimetric adjustment before the final selection of the effective concentration for the critical effect.

Table 2.2 Comparison of dosimetric adjustment for particle inhalation exposure

	Rat study	**Mouse study**
$NOAEL_{ADJ}$ (mg/m^3)	45	30
MMAD (μm)	2.3	2.3
σ_g (μm)	1.8	1.8
BW (g)	180	31.6
$RDDR_{TB}$	0.863	1.738
$NOAEL_{HEC}$ (mg/m^3)	39	52

2.8.2 Inhalation Exposure to Gas/Vapor

The derivation of the $NOAEL_{HEC}$ for exposure to gas or vapor is different from that for particles, which is based on an adjustment factor of regional gas dose ratio (RGDR).

$$NOAEL_{HEC}\ (mg/m^3) = NOAEL_{ADJ}\ (mg/m^3) \times RGDR_r$$

where $RGDR_r$ = the regional gas dose ratio for the respiratory tract region (r) of interest for the toxic effect.

Similar to the calculation of the RDDR, the method for $RGDR_r$ calculation is also dependent on the location of the effects observed. The two categories of gases with the greater potential for respiratory tract effects are gases in category 1 and category 2. Category 1 gases are defined as gases that are highly water soluble and/or rapidly irreversibly reactive (i.e., the propensity for dissociation as well as the ability to serve as substrate for metabolism) in the respiratory tract. Gases in category 2 are defined as gases that are moderately water soluble that may be rapidly reversibly reactive or moderately to slowly irreversibly reactive in respiratory tract tissue. For the effects in the respiratory airway due to exposure to category 1 or 2 gases, the $RGDR_r$ can be calculated on the basis of the ratios of respiratory minute volume (V_E) and surface area (SA_r) of the respiratory tract region of interest for animals and humans [20, 21].

$$RGDR_r = (V_E/SA_r)_A/(V_E/SA_r)_H$$

where V_E = minute volume (can be calculated on the basis of the equation shown below) (L/min) and SA_r = surface area for region r (default values can be found in Table 4.4 in the USEPA's RfC methods [11]).

$$\ln(V_E) = b_0 + b_1 \ln(BW)$$

where b_0 and b_1= species-specific parameters that can be found in Table 4.6 in the USEPA's RfC methods [11] and BW = body weight (kg).

Gases or vapors in category 3 are relatively water insoluble and unreactive in the respiratory tract regions. Thus, the relatively limited dose reaching these respiratory tract regions does not appear to result in any significant toxicity, although some respiratory tract toxicity may be related to recirculation. The uptake of these gases occurs predominantly in the pulmonary region and is perfusion limited. The site of toxicity is generally remote to the principal site of absorption in the pulmonary region. In addition to category 3 gases, category 2 gases have the potential for significant accumulation in the blood and, therefore, have the potential for both respiratory and remote toxicity. For the remote toxicity effects due to exposure to category 2 or 3 gases, the $RGDR_r$ is calculated as a ratio of the

blood:gas (air) partition coefficient ($H_{b/g}$) of the chemical for the laboratory animal species and the humans. A default value of 1.0 is used when one or both of the blood:gas (air) partition coefficients are not available for the chemical of interest.

$$RGDR_{ER} = (H_{b/g})_A/(H_{b/g})_H$$

where ER = extrarespiratory (systemic) and $H_{b/g}$= blood:gas(air) partition coefficient for human or animal (this chemical specific information can be found from publications such as Gargas et al. [22]).

For example, for a chemical with an $H_{b/g}$ (mouse) of 5.79 and an $H_{b/g}$ (human) of 8.94, an $RGDR_{ER}$ of 0.648 can be calculated (e.g., 0.648 = 5.79/8.94). Please note that some regulatory agency may use a value of 1.0 when the ratio of the blood:gas partition coefficient for the laboratory animal species and the humans is larger than one.

2.8.3 Oral Exposure

For an oral exposure dose level, a dosimetric adjustment has been routinely done for cancer risk assessment by the USEPA [23] but not for noncancer risk assessment. However, this practice has been changed recently to using a similar approach in both cancer and noncancer risk assessment [24]. As stated in USEPA's *Guidelines for Carcinogen Risk Assessment* [23], "As a default for oral exposure, a human equivalent dose for adults is estimated from data on another species by an adjustment of animal applied oral dose by a scaling factor based on body weight to the 3/4 power." This adjustment is calculated on the basis of the following equation:

$$Dose_H\,(mg/day) = Dose_A\,(mg/day) \times (BW_H/BW_A)^{3/4}$$

where $Dose_H$ = daily human dose (mg/day), $Dose_A$ = daily animal dose (mg/day), BW_H = human body weight (kg), and BW_A = animal body weight (kg).

It is important to note that the dose unit in this equation refers to an applied daily dose (e.g., mg/day) instead of a common experiment dose expressed as a body weight normalized unit (mg/kg body weight-day). When the common experiment dose unit of mg/kg-day is used, the adjustment needs to be rearranged, as shown in the following equation, to reflect the introduction of body weight data in the aforementioned equation.

$$\text{Dose (mg/day)} = \text{Dose (mg/kg-day)} \times \text{BW (kg)}$$

$$\text{Dose}_H\,(\text{mg/kg-day}) = \text{Dose}_A\,(\text{mg/kg-day}) \times (BW_A/BW_H) \times (BW_H/BW_A)^{3/4}$$

$$= \text{Dose}_A(\text{mg/kg-day}) \times (BW_A/BW_H)^{1/4}$$

For example, a daily average NOAEL of 50 mg/kg-day is reported from a chronic rat study. If the rat body weight is 0.25 kg and the human body weight is 70 kg, the HED can be calculated as follows:

$$\text{Dose}_{HED}\,(\text{mg/kg-day}) = \text{Dose}_A\,(\text{mg/kg-day}) \times (BW_A/BW_H)^{1/4}$$

$$= 50 \times (0.25/70)^{1/4}$$

$$= 50 \times 0.2445$$

$$= 12\ \text{mg/kg-day}$$

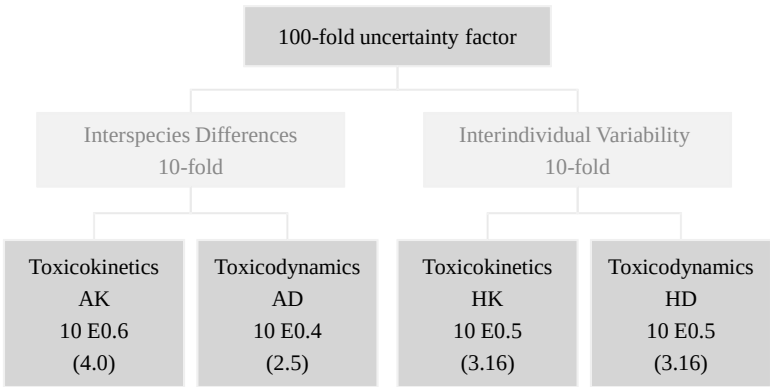
When the HEC $NOAEL_{HEC}$ from an inhalation study or HED $NOAEL_{HED}$ from an oral study is used as a POD in noncancer risk assessment, the toxicokinetic difference from animals to humans has been accounted for; therefore, only a subfactor (UF_A) of 3 is needed to account for the remaining interspecies uncertainty from animals to humans.

2.9 Data-Derived Chemical-Specific Adjustment Factors

Toxicity is a response due to exposure to a particular agent. The dose–response analysis captures a quantitative relationship between the amount of exposure through the route of exposure and the response that occurred in a target organ/tissue. Any difference in terms of response due to exposure to a particular agent could be considered due to the differences in two areas: the difference in the amount of chemical agent or its metabolite that reaches the target organ/tissue after exposure to the same external dose of the chemical agent (i.e., toxicokinetics) and the difference in response to the same target organ/tissue dose of the agent or its metabolite (i.e., toxicodynamics).

Recently, a number of regulatory agencies such as IPCS [17], Health Canada [25], and the USEPA [26] started to introduce a data-derived adjustment factor approach proposed by Renwick [27] in their risk assessment process. In this approach, each UF

(interspecies and interindividual variability) is divided into subfactors to allow for separate evaluations of differences in toxicokinetics and toxicodynamics as shown in Fig. 2.5. The IPCS [28, 29] has developed a comprehensive framework for the incorporation of quantitative data in the adjustment factors to account for interspecies differences or human interindividual variability in either toxicokinetics or toxicodynamics in the risk assessment process. As shown in Fig. 2.5, incorporation of toxicokinetic or toxicodynamic data becomes possible if each factor of 10 is divided into appropriately weighted subfactors. When multiplied, the default values for these subfactors will give the original default values of 10 for each corresponding UF before the application of data-derived subfactors.



The default values for each subfactors are based on IPCS (2005).

Figure 2.5 A relationship between chemical-specific adjustment factors and uncertainty factors. The default values for each subfactors are based on IPCS (2005).

For the application of this CSAF, the continuum of processes leading to chemical toxicity was split at the level of delivery of the active chemical species (either parent compound or a circulating active metabolite, responsible for the adverse effect) to the target tissue/organ. The events leading up to the delivery of the active chemical species were considered toxicokinetics, and events within the target tissue/organ were considered toxicodynamic. Therefore, the toxicokinetic CSAF subfactors include absorption, distribution, metabolism, and excretion of the active chemical species, while toxicodynamic CSAF subfactors account for the tissue/organ response to

internal dose of the active chemical species. When the comparison data are available for deriving any of these CSAF subfactors, the default subfactors can be replaced with the corresponding CSAF subfactors, and the final composite UF will consist of CSAF subfactors as well as remaining default uncertainty subfactors. PBPK models can also be used to develop CSAFs. However, if the PBPK model incorporates bioactivation and/or detoxification processes within the target tissue/organ, it is necessary to reconsider the subdivision between kinetics and dynamics because the calculation of tissue dose in a PBPK model may include both toxicokinetics and some aspects of toxicodynamics.

Development of the toxicokinetic part of CSAF for interspecies or human interindividual differences will be based on an appropriate dose metric, such as the area under the curve (AUC) or the clearance rate or peak concentration (C_{max}) for the active chemical species. A critical step in this approach is to identify the active chemical species (e.g., parent or a metabolite) that are responsible for the observed toxicity effect of interest. Without knowledge of the active chemical species, one cannot appropriately address the implications of kinetic differences as well as dynamic differences. Development of the toxicodynamics part of CSAF for interspecies or human interindividual differences can be based on a quantitative comparison of the concentrations that cause an effect of defined magnitude (e.g., EC_{10}, the effective concentration resulting in a 10% response for the endpoint of concern). Such data could be obtained from in vitro studies with the appropriate tissue or cell types.

The interspecies CSAF can be estimated on the basis of a comparison of differences in the mean parameter estimates (e.g., central tendency of AUC for toxicokinetics or EC_{10} for toxicodynamics) between the test animal species at the exposure level close to the NOAEL or LOAEL and adult humans at the potential environmental exposure level. Human interindividual CSAF can be based on data that define the variability in the relevant parameter estimates in healthy human adults as well as potentially susceptible subgroups. The human variability could be presented as unimodal, with the sensitive subpopulation comprising either the lower "tail" of the distribution for the healthy population of the dose causing a specified effect, or bimodal, with the sensitive subpopulation having its own independent distribution apart from the main population. To protect the sensitive population, the CSAF could be calculated as the

parameter estimate at the percentile of interest (e.g., 95%) for the sensitive population (in either unimodal distribution or the sensitive subpopulation distribution) divided by the parameter estimate at the mean in the main population distribution.

One example of using the toxicokinetic data to derive an interspecies toxicokinetic CSAF subfactor is the USEPA boron IRIS assessment [26]. On the basis of the mode of action analysis, the critical toxic outcome (i.e., decreased fetal birth weight) of boron exposure is most likely related to a continuous exposure over an extended period during fetal development, and the most appropriate estimator for fetal internal dose is the average steady-state circulating boron concentration in the mother because boron is freely diffusible across biological membranes and will rapidly and evenly equilibrate in all body water compartments. Boron is not metabolized and almost entirely eliminated in the urine; thus clearance of boron by the kidney can be used as the key toxicokinetic parameter. Assuming steady-state conditions, clearance, expressed in units of mL/min (volume of plasma cleared of the substance per unit time), is inversely related to plasma concentration. Therefore, an interspecies adjustment factor for toxicokinetics (AF_{AK}) is calculated on the basis of the ratio of pregnant rat clearance (Cl_A) and pregnant human clearance (Cl_H) after adjustment for oral absorption (fa) and body weight (BW).

$$AF_{AK} = (Cl_A \times fa_A/BW_A)/(Cl_H \times fa_H/BW_H)$$

where Cl = the clearance rate (mL/min), fa = the fraction of ingested boron absorbed into the body from the gut, and BW = body weight (kg).

On the basis of the kinetic studies of U. S. Borax [30], Vaziri et al. [31], and Pahl et al. [32], the mean boron clearance for pregnant rats was 1.0 mL/min and that for pregnant women was 66.1 mL/min. The mean body weights for pregnant rats and pregnant women from those studies are 0.303 and 67.6 kg, respectively. The absorption fraction in rat was 0.92 (Schou et al. [33]) and in humans was 0.95 (Vanderpool et al. [34]). The resulting CSAF subfactor (AF_{AK}) is 3.3.

$$\begin{aligned} AF_{AK} &= (Cl_A \times fa_A/BW_r)/(Cl_H \times fa_H/BW_H) \\ &= (1.0 \times 0.92/0.303)/(66.1 \times 0.95/67.6) \\ &= 3.3 \end{aligned}$$

The human interindividual variability in elimination could also be estimated on the basis of variations in physiological parameters. Using the glomerular filtration rate (GFR) to estimate the human interindividual variation in boron elimination is a good example of developing a CSAF subfactor by using available physiological data and scientific judgment [26]. Human interindividual variation in boron absorption and distribution is very limited. Because no information on boron clearance in pregnant women is available and boron is excreted entirely through the urinary route, the variation of the boron clearance rate in pregnant women was estimated on the basis of the available information on GFR during human pregnancy. By evaluating the GFR variation, the variability of elimination in the pregnant women could be estimated; therefore, a CSAF subfactor could be established. The data on GFR in pregnant women from three studies (Dunlop et al. [35], Krutzen et al. [36], and Sturgiss et al. [37]) were used to estimate the mean GFR and its standard deviation. The human interindividual variation in boron elimination was estimated on the basis of the ratios between the mean GFRs and the lower percentile estimate (the difference between the mean and three standard deviations), which resulted in a ratio of 2 [26].

$$AF_{HK} = GFR_{AVG}/(GFR_{AVG} - 3SD_{GFR})$$
$$= 2$$

For the boron assessment [26], there are no toxicodynamic data sufficient to warrant the replacement of the toxicodynamic part of default values for either UF_A or UF_H for boron; therefore, AF_{AD} and AF_{HD} are each assigned a default value of 3.16. The overall composite UF was calculated as follows:

$$\text{Composite UF} = \text{Data-derived } AF_{AK} \times \text{Default } UF_{AD} \times \text{Data-derived } AF_{HK} \times \text{Default } UF_{HD}$$
$$= 3.3 \times 3.16 \times 2 \times 3.16$$
$$= 66$$

In this example, the data-derived AF_{AK} was 3.3, which was not much different from the default subfactor of 3.16. Nevertheless, using this data-derived CSAF to replace default interspecies toxicokinetic subfactor captured the available data on interspecies difference in toxicokinetics and, thus, increased confidence in the estimated risk value (i.e., RfD).

Although the framework proposed by the IPCS provides guidance on the use of available toxicokinetic and toxicodynamic data to develop CSAFs, the application of this approach may vary among different regulatory agencies depending on the chemicals of interest, and available data (see Table 2.1). For example, Health Canada uses evenly divided default subfactors (3.16-fold for both toxicokinetics and toxicodynamics) for human interindividual extrapolation; however, it uses unequally divided default subfactors (fourfold for toxicokinetics and 2.5-fold for toxicodynamics) for interspecies extrapolation (also see Fig. 2.5). In contrast, the USEPA applies evenly divided subfactors (3.16-fold for both toxicokinetics and toxicodynamics) for both interspecies and human interindividual extrapolations. As a result, the risk values proposed by various agencies on the same chemical might vary to some extent due to the difference in the selection of default subfactors (e.g., boron assessments by the IPCS, and the USEPA).

2.10 PBPK Model

PBPK modeling is a mathematical modeling technique for predicting the absorption, distribution, metabolism, and excretion (ADME) of administered parent or active metabolites in humans and other animal species. Due to the recent advances in computational power, more and more PBPK models have become available for risk assessment purposes. As such, PBPK modeling has become the tool of choice to develop estimates of target tissue concentration in both animals and humans (i.e., directly calculating HED/HEC or to conduct route to route extrapolation), eliminating the need for interspecies dosimetric adjustment.

PBPK modeling incorporates multiple anatomically, physiologically, and biochemically described compartments that represent the tissues and organs with interconnections to blood; therefore, it is possible to meaningfully extrapolate from one species to another by simply taking into account physiological differences (different organ volumes, blood flows, etc.) [38, 39]. PBPK modeling can be used to predict the internal tissue/organ dose, which in turn can serve as the starting point to estimate the external exposure dose/concentration from other routes of exposure (route-to-route

extrapolation) and to estimate the external exposure on the basis of the PBPK model developed for another species (e.g., human PBPK model), as depicted in Fig. 2.6.

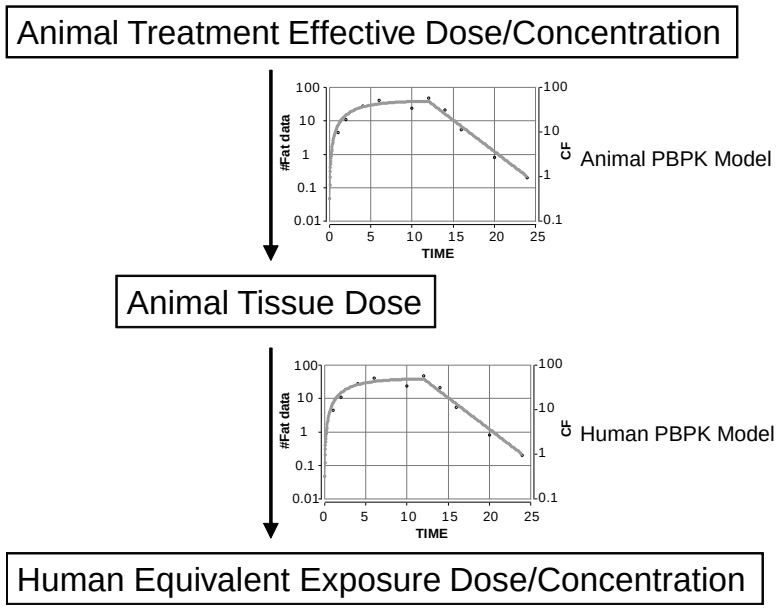


Figure 2.6 An example of using PBPK models in interspecies dose extrapolation.

One example of using PBPK models to derive inhalation RfCs is a recent hazard assessment of trichloroethylene (TCE) by the USEPA [40]. On the basis of the toxicity database, fetal heart malformations in Sprague–Dawley rats exposed on GDs 1–22 are considered one of the most sensitive toxicity effects after inhalation exposure to TCE. Due to a lack of comprehensive developmental toxic studies after inhalation exposure during gestation, an oral exposure developmental study was used to estimate an internal dose point of departure (idPOD) for the observed developmental toxicity effect. The oral treatment doses were first converted to internal dose metrics (TCE metabolized by oxidation/$kg^{¾}$/day), and then a BMD modeling exercise was conducted on the basis of the incidence of fetal heart malformations at the corresponding internal dose metrics. The estimated $BMDL_{01}$ (BMR was set to 1% due to severity of defects, some of which could have been fatal) of 0.0142 mg TCE

metabolized by oxidation/$kg^{¾}$/day is selected as the idPOD to estimate an RfC. Separately, the human PBPK model was applied to obtain the human equivalent exposure (HEC or HED) corresponding to the idPOD. Considering the human population model characterizes individual-to-individual variation, in addition to its uncertainty, the overall 99th percentile of the combined uncertainty and variability distribution was used for deriving the HEC (HEC_{99} of 0.0037 ppm) from the idPOD. Because the PBPK-estimated HEC_{99} replaces the application AF_{AK} (interspecies toxicokinetic adjustment) and AF_{HK} (human interindividual toxicokinetic adjustment) in the calculation of the RfC, the composite UF of 10 consisted of the remaining UF_{AD} (interspecies uncertainty subfactor for toxicodynamics) and UF_{HD} (human interindividual uncertainty subfactor for toxicodynamics), and the RfC is calculated as follows:

$$\begin{aligned} RfC &= BMDL_{01\ HEC99}/UF \\ &= 0.0037/10 \\ &= 0.00037 \text{ ppm } (2\ \mu g/m^3) \end{aligned}$$

In this example, an animal PBPK model was used to calculate the animal internal dose from an oral external dose for the critical effect. After the estimation of the idPOD, a human PBPK model was used to calculate the human equivalent inhalation concentration corresponding to the animal idPOD. This process covered not only a route-to-route extrapolation (i.e., oral dose to inhalation concentration) but also animal to human toxicokinetic extrapolation, therefore eliminating the need for a toxicokinetic subfactor within interspecies UF_A (i.e., UF_{AK}). By taking advantage of a human PBPK population model, the HEC at the overall 99th percentile of the combined uncertainty and variability distribution was calculated and used as the POD to derive the RfC. Therefore, it is unnecessary to apply a toxicokinetic subfactor within the human interindividual UF_H (i.e., UF_{HK}). The same approach was also used in an IRIS assessment of vinyl chloride [41].

As shown in the TCE risk assessment, PBPK models can be a very powerful tool in dose–response assessment. However, to use PBPK models in risk assessment, there are several requirements for a PBPK model to be considered adequate for risk assessment purposes [42–44]:

1. The model should be able to simulate the dose metrics in the test species and/or humans for the exposure route and exposure scenario of relevance.
2. The model should be calibrated for the species and life stages of relevance to the risk assessment.
3. The model should consist of parameters essential for simulating uptake via routes associated with human exposures as well as the critical study chosen for the assessment.
4 The model should be able to provide predictions of the time course of concentration of the toxic moiety or appropriate surrogate (parent chemical or metabolite) in the target organ or tissue.
5. The model should be peer-reviewed and evaluated for its quality and predictive capability.

Thus, it is very important to carefully evaluate the available PBPK models to determine whether these models provide required functions, as mentioned, and could be used in dose–response assessment.

2.11 Linear Dose–Response

The linear dose–response analysis approach is used when the mode of action information indicates that the dose–response curve at low dose is or is expected to be linear. This approach has been frequently used for cancer dose response as a default approach by regulatory agencies. Cancer dose response is generally considered to be linear in the low-dose region when the agents are DNA reactive and have direct mutagenic activity or there are high human exposures or body burdens near doses associated with key precursor events. Linear extrapolation is considered to be health protective and is used as a default approach when the weight of evidence evaluation of all available data is insufficient to establish the mode of action for a tumor site and when it is determined to be scientifically plausible on the basis of the available data [23].

Similar to the nonlinear dose–response analysis, the linear dose–response approach also includes two steps, identifying a POD and extrapolating from the POD to lower doses (e.g., environmental exposure levels). To estimate a POD, the BMD software (e.g.,

multistage model) is often used to model the tumor incidence data and to estimate a POD near the lower end of the observed range, without significant extrapolation to lower doses (see Fig. 2.7). Whenever possible, dosimetric conversion will be conducted preferably with toxicokinetic modeling such as a PBPK model. When PBPK models are not available or cannot provide appropriate dose metric estimates, default cross-species dosimetric scaling can be employed to estimate HED for oral exposure or HEC for inhalation exposure as discussed in previous sections. The resulting POD is expressed as a HED or HEC, respectively.

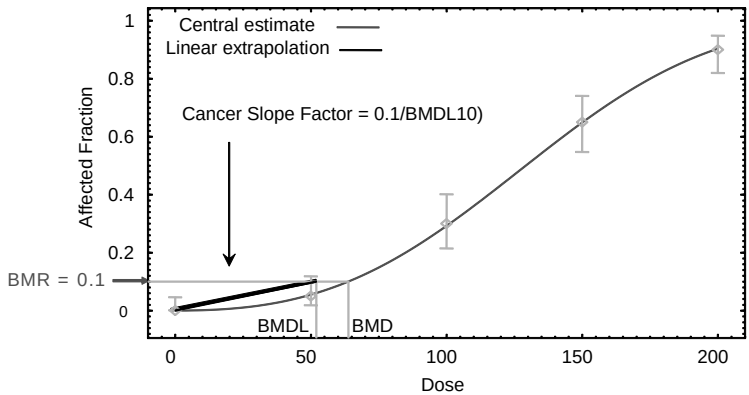


Figure 2.7 An illustration of identifying POD from a dose–response curve and a linear low dose extrapolation from the POD.

For linear extrapolation to a low dose, a straight line will be drawn from the POD to the origin, corrected for background (see Fig. 2.7). This implies a proportional (linear) relationship between risk and dose/concentration at low doses/concentrations. The slope of this line, known as the slope factor, is an upper-bound estimate of risk per increment of dose/concentration that can be used to estimate risk probabilities at different exposure levels. The slope factor can be calculated as BMR/$BMDL_{10}$ if the $BMDL_{10}$ (with BMR of 10% extra risk) is used as the POD.

For example, a set of cancer incidence data was modeled against the treatment doses after allometric scaling adjustment to HEDs, and the estimated $BMDL_{10}$ is 1.5 mg/kg-day (HED) at a BMR of 10% extra risk.

$$\text{Slope factor} = \text{BMR}/\text{BMDL}_{10}$$
$$= 0.10 / 1.5 \text{ (mg/kg-day)}$$
$$= 0.067 \text{ (mg/kg-day)}^{-1}$$

Therefore, the oral cancer slope factor is 0.067 $(\text{mg/kg-day})^{-1}$. Exposure associated with a specific risk (e.g., 1 in 1 million humans as many regulatory agencies would examine) can be estimated from this cancer slope factor on the basis of following equation:

$$\text{Dose (1/million)} = \text{Risk}/\text{Cancer slope factor}$$
$$= 1 \times 10^{-6} / 0.067 \text{ (mg/kg-day)}^{-1}$$
$$= 1.5 \times 10^{-5} \text{ mg/kg-day}$$

On the basis of this oral cancer slope factor, oral exposure to this chemical at 1.5×10^{-5} mg/kg-day for life time would result in an additional risk of 1 in 1 million humans.

Although the cancer slope factor can be calculated for each particular tumor response, one chemical compound might produce multiple types of tumors in the treated animals. In this case, basing the cancer slope factor on only one tumor type may underestimate the carcinogenic potential of a chemical that is observed to induce neoplasia at multiple sites in a bioassay [45]. Thus, it might be necessary to estimate the risk of developing any combination of tumors at all sites. A newly developed MS-Combo model incorporated into the USEPA's BMD software package (after version 2.2) allows users to calculate the BMD and BMDL for any combination of tumors observed in a single bioassay.

A major assumption of the MS-Combo model is that different tumor types are independent of one another (i.e., tumors in one organ are not metastasized from another organ). Individual tumor types can first be modeled with the regular multistage dichotomous model to determine which model setting (degrees of multistage model) best fits the data. This allows individual tumors to be fit with models that best characterize the specific shapes of their dose responses. The MS-Combo model then incorporates the parameter estimates from the best fitting models for each individual tumor types into a final combined model. On the basis of this combined tumor model, the POD (i.e., BMDL) can be estimated.

A good example of using the MS-Combo model to calculate combined tumor POD for cancer risk assessment can be found in a

recent USEPA hazard assessment of technical grade dinitrotoluene (tg-DNT [46]). Chronic oral treatment with tg-DNT produced multiple types of tumors (hepatocellular carcinoma and/or neoplastic nodules (OSF = 2.3×10^{-1} $(mg/kg\text{-}day)^{-1}$), mammary fibroadenomas (OSF = 1.0×10^{-1} $(mg/kg\text{-}day)^{-1}$), and subcutaneous fibromas (OSF = 2.8×10^{-1} $(mg/kg\text{-}day)^{-1}$) in male rats [47]. For each tumor type, tumor incidence data were first fit with regular multistage model to decide the best-fitting model. Then, the MS-Combo multiple tumor model (BMDS version 2.2.2) was used to generate the final combined tumor model by maintaining the best-fitting individual models for each tumor types. The estimated $BMDL_{10}$ for combined tumors was 0.224 mg/kg-day (HED), and on the basis of this POD, the cancer oral slope factor is 4.5×10^{-1} $(mg/kg\text{-}day)^{-1}$. This cancer slope factor captures the overall tumor response in relation to exposure doses at low-dose range.

2.12 Summary

Dose–response assessment plays a central role in chemical risk assessment. Along with hazard identification, dose–response assessment provides a foundation to estimate acceptable levels of exposure that forms a basis for risk management action. In the past two decades, there has been significant improvement in risk assessment, especially in the area of dose–response assessment. For example, the POD determination has also been evolving from the traditional NOAEL/LOAEL approach to mathematical modeling (e.g., BMD modeling) with much more emphasis on using all the dose–response information from the available data, whenever it is possible. Similarly, the advancements in understanding of toxicokinetics have facilitated the shift in dose extrapolation from default UFs, through categorical dosimetric adjustment, to data-derived chemical specific adjustments, and sophisticated toxicokinetic modeling (e.g., PBPK model). Vast improvements in computational power have contributed significantly to the implementation of dose–response modeling, and data-driven toxicokinetic modeling for better identifying PODs and interspecies and human interindividual dose extrapolation. Additionally, recent emphasis on consideration of the weight of evidence for mode of action for both cancer and noncancer effects also significantly contributed to delineation and consideration of

appropriate key events for subsequent dose–response analysis [48, 49]. These latest data-based approaches have further refined the accuracy of the assessment, therefore providing a stronger scientific basis for regulatory action.

It can be predicted that in the near future, more and more data-based methodologies will be implemented in the area of dose–response assessment. A significant initiative has been made in the use of toxicogenomic data to assist dose–response assessment [50, 51]. Other new computational approaches such as the quantitative structure–activity relationship (QSAR) could also play a more significant role in dose–response assessment [52]. The current point estimate approaches used in the POD selection, dosimetric adjustment, or dose extrapolation could be further improved with an incorporation of distribution information from all the data involved in the assessment by using a probabilistic approach so that the final outcome of dose–response assessment would be a distribution of potential risk at specified level of exposure. Therefore, it will not only allow a quantitatively estimate risk at a level of exposure but also assist in the risk management decision-making process when the exposure level exceeds the acceptable level of exposure for the majority of the population.

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Chapter 3

Recent Advances in Cancer Risk Estimation

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3.1 Introduction

Cancer risk assessment as a systematic and quantitative endeavor has by now a long history. The underlying approach has been to identify a cancer potency, or "slope factor," describing the relationship between risk and dose at low dose levels, which can then be combined with exposure data to calculate a risk estimate for a particular scenario or measured situation. The source data for potency estimation have most frequently been animal tumor incidence data in long-term bioassays, and this will be the focus of this chapter. However, epidemiological data have also been used when available and have often been important either as the primary basis of the estimate or at least as a check on the plausibility of estimates obtained from animal study data.

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A variety of possible approaches to the analysis of bioassay data for risk estimation were considered in the early stages of development of this methodology. But its widespread use is usually considered to have started with the development of the linearized multistage model and software to fit this to cancer incidence data by Crump et al. [1, 2] in the late 1970s and 1980s. These methodological developments were supported by recommendations for their use, initially by Anderson and the Carcinogen Assessment Group of the United States Environmental Protection Agency (USEPA) [3]. This was followed by formal risk assessment guidelines for the USEPA [4]. The state of California also developed risk assessment guidelines at this time [5]. The basic methodology was also extended to include fitting of time-to-tumor data to a time-dependent version of the underlying multistage cancer model, which can be valuable in the analysis of datasets with substantial intercurrent mortality or variable dosing schedules [6]. The methods thus established continued in use well into the twenty-first century, although more user-friendly versions of the software were developed in parallel with the exponential increase in the power of personal computers [7].

An alternative approach to cancer dose–response analysis was proposed by Moolgavkar and Knudson [8]. Their model was designed to include a quantitative accounting for cell division, resulting in expansion of a clone of mutated cells, and cell death or terminal differentiation, which removed cells from the pool of those capable of further proliferation. The model in principle allowed for several successive stages of mutation on the way to the final appearance of a fully malignant clone of tumor cells, as has been observed in actual human tumors [9]. However, the mathematical complexity of such cell proliferation models has generally limited their implementation to no more than two stages of successive mutation. This type of model has stimulated a lot of research and discussion of possible mechanisms but has not in practice been widely used in risk assessments for regulatory purposes, because of the large number of parameters required to be determined for the model and the necessity of using independent measures or estimates for some of these, especially cell proliferation rates. Extensive analyses using this type of methodology were developed, for example, for formaldehyde

[10], but this approach has not, at least to date, appeared in a final USEPA toxicological review for that compound.

In parallel with the development of these methods and tools, which mainly modeled the underlying toxicodynamic features of cancer dose response, there has been extensive development of toxicokinetic modeling, especially the more or less realistic physiologically based pharmacokinetic (PBPK) models. It is often observed that the uptake, metabolism, and elimination of the carcinogenic substance (and/or a procarcinogen and metabolites) is nonlinear, especially at the higher doses employed in experimental animal studies [11, 12]. This nonlinearity, often appearing as a leveling off or "saturation" of the dose response at higher applied doses, presents difficulties in fitting the data with the typical multistage model. Starting with initial studies of a number of volatile toxicants such as styrene [13], methylene chloride [14], and perchloroethylene [15], PBPK models were used to determine internal dose metrics at the target site(s) for tumorigenesis. This often resulted in better fit to the multistage model than could be obtained with an applied dose metric. PBPK modeling was also used to inform the extrapolation from animal test species to humans, although this sometimes involved large uncertainties because of the scarcity of reliable toxicokinetic data to parameterize the human models. However, extensive use of these techniques subsequent to these early examples has improved the methods and increased confidence in them to the point where it is now more or less standard practice to at least evaluate whether use of PBPK modeling and appropriate internal dose metrics is informative when deriving a cancer potency estimate. PBPK modeling has also been used in many risk analyses to address the question of interindividual variability in the target species (humans) as well as in the test species [16].

In addition to these developments in the quantitative methodology for risk assessment it is important to recognize the continuing expansion of the database of studies that provide the input data for these calculations. The development of the National Toxicology Program's (NTP's) cancer bioassays (now with 611 printed long-term study reports according to the current *Management Status Report*) has provided a key resource for cancer incidence data on compounds of interest, for identification of potentially carcinogenic chemicals, for characterization of both cancer and noncancer

pathology associated with exposure to these chemicals, and for the quantitative data necessary to calculate potency values. This program [17] has also developed an important quality standard for the design, implementation and reporting of long-term bioassays. It is also important to recognize the contribution of the International Agency for Research on Cancer (IARC). Although the monograph series only addresses hazard identification rather than quantitative risk assessment, this obviously is an essential first step in identifying substances for evaluation from the dose–response perspective. The IARC has also, via its successively updated prologues to the monograph series [18], made important contributions to the debate on study evaluation criteria, and the inclusion of supporting data such as genetic toxicity, studies of mechanism and chemical structure–activity comparisons.

This evolutionary approach and relatively established position of the linearized multistage method has been considerably revised in the last 10 years. The immediate stimulus to many of these changes was the publication of the USEPA's revised guidelines for carcinogen risk assessment in 2005 [19]. This document was the final product of a lengthy effort to update the original 1986 guidelines [4], which had previously resulted in "proposed" [20] and "interim final" [21] draft guidelines. Several of the changes in carcinogen risk assessment methodology that have been introduced recently were prefigured in those earlier draft guidelines proposals and have become more or less standard practice since the availability of the final guidelines. Another component of the discussion on methodology was the risk assessment guidelines published by the state of California's Air Toxics Hot Spots program [22]. Also, various inputs from the National Science Foundation, while not necessarily endorsing specific methodologies, encouraged the updating of guidelines for carcinogen risk assessment methodology [23] and provided comment on specific risk assessments. This affected the form of hazard assessment documents by encouraging the provision of greater detail on systematic literature review [24] and analysis of methodological data.

Among the various recent changes and emerging concepts, several are presented here as being of particular interest:

- Replacement of the longstanding linearized multistage model for cancer with the benchmark dose (BMD) method as the

standard tool for dose–response analysis of both cancer and noncancer toxicity data
- Interest in allowing for greater sensitivity to early-in-life exposures to carcinogens
- Development and increasing acceptance of methods for generating an overall potency estimate for cancer incidence after exposure to multisite carcinogens
- Incorporation of mechanistic data into risk assessments
- Potential for use of data from high-throughput screening methods and other novel experimental methods in risk assessment

3.2 Benchmark Dose Method

Dissatisfaction with the statistical inadequacies of the traditional lowest-observed-adverse-effect level (LOAEL)/no-observed-adverse-effect level (NOAEL) method of analyzing noncancer health effect data led to the proposal of an alternative approach that was described by Crump [25]. This method, referred to as BMD analysis, used mathematical models to fit the response data across all dose levels examined in the study and by means of this mathematical fit identified a BMD (and, specifically, the 95% lower confidence limit on this estimate, referred to as the BMDL) corresponding to a standardized response rate, usually 5% or 10% for dichotomous data. Health-protective levels were then selected by application of uncertainty factors to this BMDL in a similar way to their application to LOAELs and NOAELs. This approach was widely tested for a range of noncancer data types, especially in the early stages with developmental toxicity data that have particular statistical problems that are hard to accommodate in the LOAEL/NOAEL methodology. Eventually guidelines [26] for the use of this methodology were developed and applied generally for noncancer risk assessment.

Concurrently with this development of BMD methodology for noncancer effects, consideration was given to its use for cancer risk assessment [17]. This was partly prompted by an interest in reconciling the previously very different dose–response analysis methods for cancer and noncancer effects. There was also a concern that although the multistage model as originally proposed

by Armitage and Doll [27] had been fairly successful in describing cancer dose–response curves quantitatively, it was increasingly clear that its assumed correspondence with actual biological mechanisms [9] was very limited. Even somewhat more realistic models in reality fell some way short of fully describing the true biological mechanism of action, and these had not been much used for risk assessment because of their mathematical complexity and uncertainties in the values of the many key parameters. The BMD approach was therefore attractive since it is applicable, with appropriate extrapolation strategies, to both cancer and noncancer incidence data, and the justification of the model used to fit the data is based purely on the quality of fit to those data rather than any assumption a priori that the model corresponds to actual chemical or biological events.

The adoption of this methodology as the default approach for quantitative cancer risk assessment has resulted from the adoption of final guidelines [16, 19] recommending its use, and also from the development by the USEPA of software (BMDS) and supporting documentation [28] to implement the method, starting development in 1995 with release of an initial version in 1999 and with many revisions and extensions since then. Parallel to the initial areas of application of this methodology, the initial versions of this software were primarily designed around the needs of noncancer data analysis, although a dichotomous multistage model was included from the start, and in fact Crump had pointed out in his original publication [21] that the linear, quadratic, and polynomial models that he evaluated were similar to those used for cancer analysis, although with fewer constraints on possible parameter values. In 2007, a version of BMDS was released with a multistage cancer model that incorporates the constraints (in particular, extra risk calculation and non-negative values for β coefficients) detailed by the USEPA 2005 guidelines and provides a unit risk calculation. This represents a recent consensus that the multistage polynomial model is in general the best mathematical fit for cancer incidence data. Departures from this state of affairs most commonly can be accommodated best by using toxicokinetic models, as noted previously, and/or by mortality corrections such as the poly-3 correction favored in recent NTP bioassay reports. It is perhaps worth noting that the model referred to here is specifically the "multistage" model, in contrast to the "linearized multistage" model previously used in cancer risk assessment.

The process of linearization, which consists of ignoring the β coefficients for higher polynomial terms and considering only the 95% upper confidence limit (95% UCL) of the linear term, is not used in BMD analysis: the BMD and its 95% lower confidence limit (BMDL) are estimated from the full polynomial fitted, not just the linear term. Other models, and in particular those typical noncancer models with implied thresholds, are not considered appropriate for most cancer risk estimates, in line with the low-dose linearity assumption (in the absence of compound-specific evidence to the contrary) generally recommended [16, 19].

The use of the multistage polynomial as the usual model for fitting tumor incidence data, and the similar use of likelihood maximization procedures to optimize and evaluate the fit obtained, ensures that in most cases the results obtained by the BMD procedure are very similar to those obtained with the earlier linearized multistage procedure. The essential difference between the two is in the method of estimating the slope in the low-dose region. Having determined a BMDL the usually recommended procedure is linear extrapolation from this dose and response point to zero response above background with zero exposure: the slope of this line is the cancer potency. Compared to the previous linearized multistage method, this approach explicitly avoids the assumption that the actual fitted model has any particular significance outside the range of the observed data, instead relying strictly on the low-dose linearity assumption.

The low-dose linearity assumption is recommended both for carcinogens with a known mechanism supportive of this assumption, and for those of unknown mechanism or where such mechanistic details as are known do not have a clear implication for the form of the dose–response curve. For those few carcinogens where there is sufficiently convincing evidence for a mechanism that results in a threshold, a health-protective level may be determined by application of uncertainty factors to the BMDL, similarly to the approach used for a noncancer health effect. This default assumption of low-dose linearity has remained constant through the various generations of adopted and proposed cancer risk assessment guidelines form the USEPA and other authorities.

The extrapolation from a BMDL does sometimes produce a potency estimate that differs from that obtained by the linearized

multistage approach. This is seen in some cases where the fitted polynomial is highly curved (i.e., the quadratic and higher terms in the polynomial dominate the best fit equation). In these cases the BMDL-derived potency may be somewhat higher, depending on the confidence limits around the linear term. Usually such differences are reduced if the 5% benchmark response rate (BMR) is used to determine the BMDL rather than the initially recommended 10%. A 5% increase in response rate above background is commonly within the range of observed data in bioassay results, and where this is the case it may be preferred as the BMR, although in practice this choice seldom makes a difference in the observed BMD and BMDL, since most datasets show a dose response very close to linear at incidences of 10% or less.

There are numerous examples of current practice in the use of the BMD model in risk assessment. As a fairly straightforward example, the USEPA's toxicological review of biphenyl [29] recently published on the Integrated Risk Information System (IRIS) shows the use of BMD methodology in the analysis of both cancer and noncancer endpoints, including a "typical" linear extrapolation potency analysis for liver tumors in female mice. This also demonstrates the ability to use the BMD analysis to deal with a cancer endpoint that is, unlike most, shown to involve a mechanism that probably has a threshold: the bladder tumors induced by biphenyl in male rats were found to be associated with and caused by the formation of bladder calculi, a high-dose phenomenon. In this case a reference dose (RfD) for this effect was estimated by application of an uncertainty factor to the BMDL. Another somewhat more complex example of the application of the BMD methodology appears in the recently finalized toxicity review of 1,4-dioxane [30], also developed by the USEPA for the IRIS program. This illustrates typical current methods and reporting standards for BMD analysis of both cancer and noncancer data. The attached figure, recalculated with the data from Kano et al. (2009) [31] used by the USEPA in its report, shows a typical fit using the multistage cancer model in BMDS. The analysts in this report also illustrate a number of interesting alternative ways to achieve a reasonable potency estimate, including the use of nonstandard models where the multistage model could not be fit to the data sufficiently well. However, even in these cases they adhere to the default linear low-dose extrapolation procedure.

Although some toxicokinetic models have been proposed, the analysts determined that the applied dose metric was more appropriate than a calculated internal dose in this case ("dose" on the *x*-axis in Fig. 3.1 is in mg/kg-day) because of uncertainties in the mechanism. It is conceivable that they would have had fewer troubles in fitting the multistage model to some of the datasets if they had elected to use an internal dose metric instead.

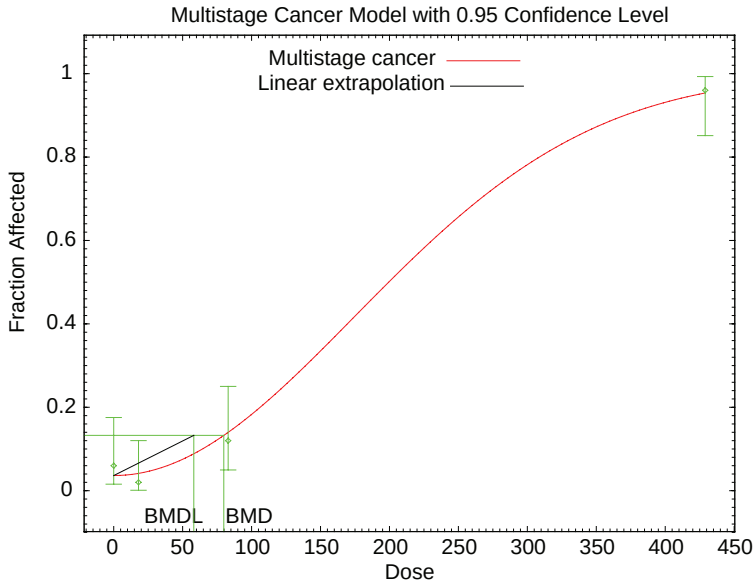

Figure 3.1 Multistage cancer model for combined incidence of hepatic adenomas and carcinomas in female F344 rats exposed orally to 1,4-dioxane.

An interesting footnote to this general move to replace the linearized multistage approach by the BMD method is that although there have been occasional efforts to develop a "model free" approach to the analysis of time-to-tumor data [32], these have not been widely accepted. Instead, analysts needing to analyze time-to-tumor data have tended to return to the earlier formulation of the time-dependent version of the multistage model [6]. Rather than relying on the linearization procedure and ignoring the higher terms of the polynomial, it is possible to use the full polynomial to estimate a 95% lower confidence limit on the risk-specific dose for an end-of-

study tumor incidence of 5% or 10% and use this as a BMDL. This clearly assumes more reliability of the enhanced multistage model, in particular for time extrapolation within the study period, than is assumed by the simple use of the non-time-dependent version as a curve-fitting tool to describe the whole-life incidence data. This BMD-oriented approach was possible with the TOXRISK software [7], but this software is unfortunately no longer available or supported. The USEPA has recently developed a new program to implement the time-dependent version of the multistage model and offers this as an adjunct to the BMDS software package: it will be interesting to see how widely this will be used.

3.3 Risk of Carcinogen Exposures Early in Life

Concerns that exposure to toxic chemicals including carcinogens early in life (including exposures in utero, as well as during infancy, childhood, and adolescence) may result in greater risk than corresponding exposures to adults have been expressed [33]. These were noted by the National Research Council (NRC) [20] and have led to various legislative initiatives including the state of California's Children's Environmental Health Protection Act. These concerns have stimulated several developments in cancer risk estimation methodology, notably in the USEPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* [34] and the state of California's cancer risk estimation guidance for the Air Toxics Hot Spots program [19]. Both these guidance documents recommend the application of age-specific sensitivity factors of 10 for infants (birth to age 2 and during the third trimester of pregnancy in the California guidelines) and 3 for children (ages between 2 and 16) as a policy default to allow for the expected increased risk of exposure to carcinogens during these life stages. These factors are applied to exposures during these periods during the risk calculation for a particular exposure scenario, rather than by simply adjusting the potency factor, in order to make proper allowance for exposures that are discontinuous or shorter than the entire life span. This also allows for the consideration of age-specific intake rates, such as breathing rates and consumption of food and drinking water [35], rather than relying on adult values or whole-life averages as was done in earlier exposure assessment procedures.

The technical background to these proposals is described more fully in Chapter 4 on children sensitivity and so will not be repeated here. It should be noted that application of these adjustments can have substantial impacts on the risk estimates calculated for the general population, where children would be expected to be present among those exposed. The California guidelines recommend use of these adjustments for all carcinogens except those for which a different degree of adjustment (or none) is indicated by compound-specific data. Since relatively few compounds have actually been systematically examined for life-stage-specific cancer potency, and those examined are consistent with (and provide justification for) the recommended adjustment factors, the number of cases where these adjustment factors would not be used is expected to remain small. The USEPA, on the other hand, recommended that the adjustment factors only be applied for compounds where a "mutagenic mode of action" was found to apply, which potentially excludes more compounds depending on how this restriction is interpreted. However, that agency has so far been unable to finalize any guidance on what is meant by this phrase, and indeed there are some significant logical contradictions in the concept, not least of which is that several compounds whose age-dependent carcinogenicity was examined by the USEPA and the California Office of Environmental Health Hazard Assessment (OEHHA) have been argued by some as examples of either a nonmutagenic or an undetermined mode of action. These nevertheless showed similar age dependence of potency to other compounds whose mutagenicity is established and is consistent with the proposed adjustments. In practice, a number of recent carcinogenicity assessments by the USEPA have actually taken a pragmatic approach to the proposed definition of mode of action, which is probably most reasonable under their circumstances.

3.4 Potency of Multisite Carcinogens

In many carcinogenesis bioassays the findings in any particular species and sex of test animals are of tumors occurring at one particular site and of one presumed cellular origin [36]. If additional tumor sites are observed, they often show considerably lower sensitivity. In view of these generalities, it has been the usual

practice for many years to use the potency at the most sensitive site in the most sensitive strain, sex, and species as an indicator of human cancer risk [3]. The inherent conservatism of this approach has been presumed to provide sufficient coverage for any additional risk to humans associated with minor sites of tumor incidence. However, there are a number of carcinogens of public health importance where tumors occur at multiple sites in one experiment (i.e., for a single test species, strain, and sex) [37]. In some cases the overall risk of cancer in a bioassay is substantially greater than the extra risk attributable to any single site. In view of this, and noting the fundamental principle that concordance of site and tumor type between animal test species is not assumed [20, 38], it is necessary to examine the overall risk of cancer at all sites in the bioassay in estimating the human cancer potency.

Several different approaches to this problem have been used. The simplest is to base the potency estimate on the total number of affected animals rather than the incidence at any one site [4]. This can be undertaken fairly simply if the study report includes this statistic or provides individual animal data from which it may be extracted. This is generally the case for NTP bioassay reports, although frequently not possible with studies published in scientific journals, unless the original authors are available and willing to share their raw data. However, there are several possible problems with this type of analysis. Where incidence rates at several sites are substantial the cancer risks from all sites (which are assumed to be independent) will be underestimated by this method since some animals will bear multiple unrelated tumors [39]. Also, differences in background rates and shapes of the dose–response curves may be obscured in the combined incidence data, which could also result in an underestimate of the combined risk [40].

A second approach, which was recommended by the NRC [20] in 1994, is to simply add the calculated potencies at the various independent sites. This is analogous to the approach taken for cancer risks from exposures to multiple chemicals, which are assumed to be additive. This is likely to produce a reasonable combined estimate when overall tumor incidences are low. However, since cancer potencies are reported as 95% UCLs on the low-dose slope, when these are added there is some overestimation of the 95% UCL of the combined potency value. Such overestimation may be relatively

minor for small potencies and low multiplicity of tumor types, but these conditions do not necessarily apply in the analysis of animal bioassay data. To provide a more statistically robust estimate of the combined potency, methods have been developed that aim to take into account the actual likelihood distributions for the potency estimates. Initially these focused on the traditional linearized multistage model, where the potencies at different sites are estimated as the 95% UCL (q_1^*) on the linear term (q_1) of the fitted polynomial. The USEPA's analysis of 1,3-butadiene carcinogenicity [41] using data from the NTP's second inhalation bioassay in the mouse [42] attempted this by assuming that the likelihood density functions for q_1 for the various tumor types were normal. In this case the combined 95% UCL can be estimated by adding the maximum likelihood estimates for each of the individual tumor site slope estimates (which for normal distributions are the same as the means) and calculating a combined standard deviation from the individual site standard deviations (which for normal distributions is simply related to the 95% UCL: $UCL_{95} = 1.645\sigma$).

The problem with this relatively straightforward approach is that the assumption of a normal shape to the likelihood density function for q_1 is not valid, although for simple polynomial fits it may be a workable approximation. In reality the likelihood density distribution for q_1 is not symmetrical, since it is limited by the definition of the model to zero as a lower-bound but at least notionally extends to infinity at the upper-bound. Also, it is not necessarily a smooth single-peaked curve. In cases where higher terms of the polynomial contribute to likely (although not necessarily optimal) fits the function for q_1 may show multiple peaks of shoulders representing different plausible model forms. Because of this a preferred method has been to determine the actual shape of the likelihood density distributions of q_1 for each contributing tumor site and to add these systematically to develop a combined distribution. The 95% UCL of this combined distribution is then used as the combined potency estimate. Some initial efforts using this approach have been reported by analysts with the state of California [43–45], where the shapes of the q_1 likelihood density functions for each site were determined using a modified version [46] of the MSTAGE program [47] to fit the linearized multistage model to tumor incidence data. These functions were then combined using the Monte Carlo procedure implemented

by the Crystal Ball add-in to the Microsoft Excel spreadsheet. More recently, this method was used to examine the multisite cancer potency of 1,3-butadiene and to compare the results obtained by this method to the results of the USEPA's simpler method [48] and by doing a single potency analysis for all tumor-bearing animals. It was found that, as shown in Table 3.1, the potency estimate based on all tumor-bearing animals was a significant underestimate compared to the estimate obtained by either of the distribution-based methods, while straightforward addition of q_1^* values resulted in a significant overestimate. The method assuming normality for the likelihood density functions ("Sum of $q_1 + 1.645\sigma$" in the table) underestimates the combined potency by a small amount (12% – 14%) relative to the presumably most accurate Monte Carlo addition method.

Table 3.1 Overall potency estimates for cancer risk of exposure to 1,3-butadiene, based on tumors observed in the NTP (1993) bioassay in mice [36]

	Total q_{animal} estimate $(mg/kg\text{-}day)^{-1}$	
Basis	**Males**	**Females**
All animals bearing selected tumors	n.d.	0.19
Multisite potency calculations (whole-life incidences, poly-3-corrected)		
Sum of q_1 (maximum likelihood estimate)	0.25	0.31
Sum of q_1^* (95% UCL)	0.41	0.47
Sum of $q_1 + 1.645\sigma$	0.31	0.37
q_1^* combined distribution	0.36	0.42

*Calculated values from the Refs. [35, 42].

The multisite tumor potency method based on Monte Carlo addition of the likelihood density distributions for individual tumor sites has been used in some recent regulatory risk assessments, including the *no significant risk level* calculations for glycidol [49] and *tris*(1,3-dichloro-2-propyl) phosphate (TDCPP) [50] under the state of California's Proposition 65 program.

In its assessment of carcinogenic potency for 1,4-dioxane [26], the USEPA used a different Monte Carlo procedure, using a Bayesian

approach to determine the combined distribution for the multisite BMD: a BMDL was estimated as the 5% point on the posterior distribution.

More recently, the USEPA group responsible for the BMDS software has added an "MS-Combo" module [51] that allows calculation of the BMDs and BMDLs for a combination of tumor sites. Individual sites are modeled with the multistage cancer model, and the likelihood profile, BMD, and BMDL for a combined distribution are estimated by addition of parameters from the individual fits. The combined risk estimate has a polynomial form similar to the individual site estimates:

$$P(d) = 1 - \exp[-(\beta_0 + \beta_1 d + \beta_2 d_2 + \ldots)]$$

where $\beta_0 = \Sigma\beta_{0i}$, $\beta_1 = \Sigma\beta_{1i}$, $\beta_2 = \Sigma\beta_{2i}$, . . . for all tumor sites ($1 < i < j$).

Exact forms of the functions describing the likelihood for each of the individual fits may also be added to determine the overall likelihood profile, and differentials of this combined function inform a stepwise optimization routine to determine the value of the 95% lower confidence limit of the BMD for the combined distribution. This approach is similar to the Monte Carlo method described previously, except that, consistent with the BMD method, the parameter of interest for low-dose extrapolation is the BMDL for a chosen benchmark response (typically 5% or 10%), instead of the upper bound on β_1. To the extent that higher polynomial terms appear in the fits for individual tumor sites their contribution is reflected in both the BMD and BMDL (as opposed to the linearized multistage approach, where they affect the 95% UCL but not the MLE of the low-dose slope factor). On the other hand, it is recommended to determine the degree of the optimum fit polynomial for each tumor site individually before doing the multisite analysis, and to use this information as a fixed input to the MS-Combo analysis. In this case uncertainty related to the degree of the fitted polynomial is not considered, whereas this is captured by at least some versions of the linearized multistage software.

In practice the MS-Combo feature of the BMDS software generally produces potency estimates that are substantially identical to those obtained using the linearized multistage/Monte Carlo method. It is also computationally more efficient than the Monte Carlo approach, which is a time- and resource-intensive "brute force" method for

solving distribution function problems, and is methodologically consistent with the currently preferred BMD approach to carcinogenic potency estimation.

3.5 Risk Assessments Incorporating Mechanistic Information

It has been a common thread throughout the development of cancer risk assessment that the dose–response and variability models used should reflect the underlying biological events, insofar as these are understood. Indeed, the development of risk assessment models described in the introduction to this chapter reflects this objective, although more recent developments have brought further recognition of the limitations of our knowledge. We typically lack sufficient detailed information about the rates and interrelationships of the many processes involved in carcinogenesis to construct a fully representative and quantitatively reliable model of the actual events. This is especially difficult since some of the underlying molecular events are essentially unobservable in real time and may only be deduced from their observed consequences. However, there have been some notable successes in identifying key events and processes, starting with the presumption of somatic mutational events as a result of covalent DNA modifications for many carcinogens. Not only is there a large literature demonstrating formation of DNA adducts for many carcinogens [17], but these have in some cases been shown to follow the assumed linear dose–response characteristics at low doses [52]. Analysis of genetic modifications observed in cells transformed in vitro and tumor cells has revealed further details of the process of chemical carcinogenesis. These mechanistic revelations have been generally supportive of the paradigms used in quantitative risk assessment for DNA-reactive carcinogens.

More specific investigations have been undertaken with carcinogens where a different mechanism of action is suspected. Some notable examples of such investigations have involved carcinogens such as phthalates and other peroxisome proliferation inducers, chemicals causing thyroid follicular cell tumors [53], and the various chemicals causing deposition of α-2u globulin in the male rat kidney. These investigations have indicated mechanisms that are

unique to a particular test species and in some cases to a specific strain and/or sex. It is believed that these mechanisms are unlikely to apply to humans, so the carcinogenicity finding in that test animal is considered not relevant to human risk. These findings are well established in the specific cases noted, but often the true picture is a good deal more complicated than the simplified arguments advanced initially. Thus the male rat kidney tumors associated with α-2u globulin deposition are a well-established phenomenon with certain chemicals, but this does not mean that all tumors observed in male rat kidneys are caused by this mechanism. The USEPA developed detailed guidance to assist in identifying examples of this particular phenomenon [54]. However, these guidelines are not always respected in some publications that propose identification of no human relevance for a particular carcinogenicity observation.

Similarly, there is a large literature on peroxisome proliferation inducers, some of which are liver carcinogens in rodents, and the finding that this particular response in rodents is associated with these chemicals functioning as agonists for the peroxisome proliferator-activated receptorα (PPARα) nuclear receptor, an important regulator of lipid metabolism and cell growth. It was thought that this mechanism did not operate in humans, since the peroxisome proliferation response is absent or minimal in humans exposed to prototype PPARα agonists. Also the peroxisome proliferation and tumor formation are not seen in PPARα-null mice exposed for 11 months to the prototype agonist Wy-14,643 [55]. However, subsequent work showed that the picture is a lot more complicated both with regard to other related PPARs [56] and with regard to different toxicological responses in the liver to different PPAR agonists [57]. This does not necessarily prove that there is a human liver cancer risk from any of these PPAR agonists, but it certainly clouds the picture compared to the original hypothesis that all such risk could be clearly discounted. Coincidentally, it has been shown that many phthalates (which were among the original "stars" of the PPARα saga) have other serious toxicological effects, namely, reproductive and developmental toxicity, which have an entirely different mechanism from the rodent liver carcinogenesis or other PPAR-related responses [58–61]. These other responses may in fact be more important from a public health point of view than any possible cancer risk.

While not wishing to detract from the importance of mechanistic studies in developing an understanding of the implications of carcinogenesis findings for human risk, these examples should serve as a warning not to accept proposed mechanisms uncritically. This is especially true for the risk assessor working with a mandate to protect public health. The acceptance of a mechanistic proposal for publication in a scientific journal certainly implies its plausibility, but if acceptance of this proposal implies the discounting of what would otherwise be a substantial risk to human health, this requires a substantially higher degree of confidence. Similarly, it is often seen that a chemical produces tumor responses at several different sites, all of which have been proposed to act by different mechanisms that are argued to indicate that the risk to humans is minimal (MTBE is a typical example [62]). This situation in effect runs into the multiple comparisons problem. If one were to suppose that the probability that any one of three such hypotheses was correct was 60% (an arbitrary, if perhaps overly charitable, Bayesian prior estimate), the expected probability that all of the hypotheses was correct and that there was therefore no risk to humans would be 22%. This is quite a long way short of the standard required for confidence in public health decisions, usually quoted as 95% for quantitative limits. Such multiple explanations are also sometimes seen sequentially, where after an explanatory hypothesis for a single observation of carcinogenicity is shown to be incorrect or incomplete, another hypothesis for lack of human relevance is developed. It is preferable that research programs be driven by dispassionate inquiry rather than the desired outcome.

The examples discussed so far have been primarily directed to answering the question of human relevance, but mechanistic studies have also made important contributions to the quantitative aspects of human cancer risk estimation. Probably the best-known and longest established of these is the development of the toxicity equivalence factors (TEFs) used to estimate both cancer risks and other dioxin-like toxicities of chlorinated dioxins, dibenzofurans, and coplanar PCBs. These rely on the structural similarities observed among congeners of these chemical types and on the observation that many of their toxic effects, including carcinogenicity, are believed to have an underlying common mechanism of binding to the *AhR* nuclear receptor. This receptor is well known as the

regulator for the expression of polycyclic aromatic hydrocarbon (PAH)-metabolizing cytochromes (e.g., the CYP-1A series in the liver) [63]. A panel of experts convened by the World Health Organization has developed the TEF values based on a variety of endpoints, many of which are short-term or in vitro measurements that can be readily applied to the full range of dioxin-like congeners, unlike the expensive and time-consuming carcinogenicity bioassay protocol. These TEFs are used as scaling factors relative to the carcinogenic potency (or toxicity measures for other dioxin-like effect endpoints) for 2,3,7,8-tetrachlorodibenzodixin, one of the most potent and widely studied members of the series, in estimating the risk from any of the listed dioxin-like compounds. The TEF values have gone through a number of iterations, the most recent being published in 2005 [64].

This approach, using a combination of structure–activity comparisons and mechanistically based endpoint assessments has also been used in other series of related compounds, notably the PAHs [65–67]. The USEPA is in the process of developing a considerably revised and extended version of its earlier guidance, which promises to take into account a wide range of data on many more compounds within the overall PAH class and to take account of new methods and mechanistic information. While currently still in a review draft form [68], this promises to be an important contribution to the topic once finalized.

Other examples where mechanistic information has contributed to the quantitative assessment of cancer risk are numerous, especially in regard to the use of toxicokinetic modeling, which has by now a long history of contributing to risk assessment of carcinogens, as noted earlier. Current methods include efforts to identify and quantitate the actual reactive species responsible for damaging DNA (and/or interacting with other receptors, depending on the chemical), to extrapolate both from higher doses to lower doses and to estimate the effects of interindividual variability, especially in humans. Current developments in the area are reviewed elsewhere in this book by Yang et al.

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Chapter 4

Consideration of Infants and Children in Risk Assessment

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4.1 Introduction

There are critical developmental periods (windows of susceptibility) during which developing organ systems are uniquely vulnerable to injury from exposure to xenobiotic chemicals [1–3]. Toxicity of a chemical at different life stages depends on many factors, some related to disposition, such as the relative activation and detoxification, and others related to the innate sensitivity to the toxic effects, including the presence of windows of susceptibility. Different toxicological targets emerge over time as an organ or tissue develops, making prediction of toxicity to a young organism on the basis of studies in mature animals or adult humans uncertain. Further, toxicokinetic processes change over time from fetal life through childhood, resulting in differences in toxicity of xenobiotics by age.

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Age-related physiological and behavioral differences can also affect the degree of exposure to environmental chemicals. The following sections discuss age-related toxicodynamic and toxicokinetic differences and approaches by the United States Environmental Protection Agency (USEPA) and California EPA's (Cal/EPA) Office of Environmental Health Hazard Assessment (OEHHA) to address infants and children in risk assessment.

4.1.1 Toxicodynamics: Changing Targets of Toxicity

Development involves a complex series of events that change the structure and function of developing organs. For example, Rice and Barone [4] describe critical stages of development in the central nervous system (CNS) during fetal development and childhood involving rapid cell proliferation, migration, and differentiation. These developmental processes must be exquisitely coordinated both in time and space for complete brain development. Chemical-induced disruption to any of these processes may be irreversible and result in adverse structural or functional neurological deficits in the developing child. Coordinated developmental processes occur in every organ and organ system. Exposure to chemicals that disrupt developmental processes can potentially disrupt the structure and function of any organ system. Since development continues into young adulthood, exposures during childhood should be viewed as having potential to cause developmental toxicity. For example, structural maturation of the CNS continues through adolescence, involving both cell proliferation and synaptic pruning (loss of selected neurons) [5]. Disruption of these processes could lead to functional alterations in higher level functions of the brain. Similarly, the peripubertal period presents windows of susceptibility for the reproductive organs and mammary glands, which are experiencing rapid growth and concomitant rapid cell proliferation and differentiation.

4.1.2 Children Can Experience Different Exposures Than Adults

Because infants and children are growing and more physically active than adults, they have higher energy and oxygen requirements per pound resulting in greater consumption rates of food, air, and water

[6]. In the same environmental setting, children may receive greater doses of environmental toxicants on a body weight basis than adults through common exposure pathways such as inhalation and ingestion.

Children can experience unique exposures through the foods (and nonfood items) they eat. For a formula-fed infant, formula may be the sole source of fluids and nutrients for the first several months of life. When reconstituted with tap water, formula can be a source of water-borne contaminants, and infants may receive larger doses (mg/kg body weight) of these contaminants than older children and adults. Children tend to eat more of some food items than other, such as apples, and can receive higher doses of environmental contaminants in those products.

Breast milk is an exposure pathway unique to infants. Physicochemical characteristics such as lipophilicity and the ability to bind to protein are important to the transfer of a contaminant from the mother's plasma to the milk [7, 8]. An infant's daily intake of lipophilic contaminants, such as brominated flame retardants or halogenated dioxins and furans (PCDD/Fs), from breast milk may be substantially greater (up to 2 orders of magnitude) than an adult's intake [9, 10]. Some chemicals may be actively transported into milk, such as perchlorate, which is transported via the sodium iodide symporter [11].

Mouthing behavior in toddlers and outdoor play can result in higher exposure to dust- and soil-borne contaminants in children relative to adults. An oft-cited example is children poisoned by lead in paint because of mouthing and hand-to-mouth activity.

Data from the US Centers for Disease Control (CDC) and Prevention's National Health and Nutrition Examination Survey (NHANES) indicate, on the basis of urine measurements, that children experience higher internal doses of some environmental chemicals than adults in the general population, such as organophosphate pesticides and polybrominated diphenyl ethers [12].

4.1.3 Disposition of Toxicants Changes with Age

Pediatric pharmacology researchers have in the last decade taken advantage of the explosion in molecular biology tools to evaluate the ontogeny of drug metabolism and elimination processes. Risk

assessors can use this knowledge in evaluating toxicokinetics of environmental contaminants. While an adult has mature processes driving absorption, metabolism, distribution and excretion, in the neonate maturational processes in cells and tissues impact the disposition of drugs, and by extension environmental contaminants, and contribute to variability in the response to toxicants [13].

A number of studies have demonstrated age-related differences in drug disposition [14–16]. The major differences relative to adults occur in preterm and full-term neonates and young infants [15, 17]. In addition, interindividual variability is greatest in the youngest children, apparently due to variability in maturation of critical metabolism and elimination pathways [15, 16, 18]. Genotypic variation determines rates of metabolism of toxicants and sometimes pathways of metabolism. These genetic polymorphisms are superimposed on variability in maturation of metabolic enzymes and elimination processes in the liver and kidney. Prediction of the metabolism and elimination of xenobiotics on the basis of known effects of genotypic variation on kinetics is limited to periods of development where the genotype is fully expressed (e.g., there is phenotypic and genotypic concordance) [19, 20]. Finally, nutritional and disease status will affect the kinetics of xenobiotics. It is obviously difficult to account for all these variables quantitatively, given the large data gaps in our knowledge of the toxicity of environmental toxicants. Nonetheless, it is important to consider these factors when assessing risk to infants and children from exposure to environmental toxicants, for example, to inform the choice of uncertainty factor (UF) in deriving safe exposure levels.

4.1.3.1 Absorption

A number of physiological and morphological factors that influence the absorption of xenobiotics differ by age. These include factors influencing both passive and active absorption across the gut, across the skin, and through the lung. Passive diffusion across the gut epithelium is the mechanism by which most toxicants are absorbed into the systemic circulation [21]. Infants have a higher stomach pH than adults, which alters the dose of certain ionizable compounds for infants relative to adults. Gastric emptying time, gastrointestinal motility, and bile secretion are all lower in infants than in adults, which slows absorption of chemicals [13, 22]. Slower appearance of

the xenobiotics in the blood of infants following oral administration and a longer clearance results in a longer time to peak concentration, a flatter peak, and slower elimination [16].

Active transport processes also differ by age as maturation of transport processes occurs in the gut epithelium. For example, *P*-glycoprotein transports many substrates into the gut epithelium and across the blood–brain barrier [16]. Maturation of this and other transporters would affect the rate of absorption of many compounds, including some xenobiotics. The intestinal microbiome also influences absorption and other kinetic processes including metabolism [13]. Further, nutritional status influences absorption across the gut. For example, absorption of lead across the gut is increased in children with iron deficiency, a condition occurring most often in children under five years of age and in children from lower socioeconomic groups. These children also have higher lead exposures from lead-based paint in older housing [23].

Dermal exposure to toxic chemicals may result in higher dose to an infant or small child due to the ratio of surface area to body weight of infants, which is more than two times greater than in an adult. Further permeability of the stratum corneum is higher in infants. There are several examples where dermally applied chemicals (e.g., hexachlorophene, isopropanol, aniline) were more toxic to infants than adults [24, 25].

Absorption across the lung can also differ by age, in part due to differences in morphology of the respiratory tract. For example, modeling of fine-particle deposition indicates that infants likely have a larger percentage of fine-particle deposition in the alveolar portion of the lung than older children and adults [26]. Chemicals associated with fine airborne particulate matter may be deposited and absorbed to a greater extent in the bronchiolar-alveolar region in infants. Infants have increased alveolar ventilation and a smaller functional residual capacity than adults, resulting in faster pulmonary absorption in neonates [16], as observed following administration of anesthetics prior to surgery.

4.1.3.2 Distribution

Once absorbed into the systemic circulation, environmental contaminants distribute to various tissues, depending on a number of factors such as physicochemical properties of the chemical,