A Framework for Assessing Risks to Children from Exposure to Environmental Agents

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In recent years there has been an increasing focus in environmental risk assessment on children as a potentially susceptible population. There also has been growing recognition of the need for a systematic approach for organizing, evaluating, and incorporating the available data on children's susceptibilities in risk assessments. In this article we present a conceptual framework for assessing risks to children from environmental exposures. The proposed framework builds on the problem formulation → analysis → risk characterization paradigm, identifying at each phase the questions and issues of particular importance for characterizing risks to the developing organism (from conception through organ maturation). The framework is presented and discussed from the complementary perspectives of toxicokinetics and toxicodynamics. *Key words:* children's health, developmental toxicity, framework, life stage, risk assessment, toxicodynamics, toxicokinetics. *Environ Health Perspect* 112:238–256 (2004). doi:10.1289/ehp.6182 available via *http://dx.doi.org/* [Online 25 November 2003]

A Proposed Framework for Assessing Risks to Children

Over the past decade a dramatic increase has occurred in the recognition and concern for children as a potentially susceptible population for exposure to toxic environmental agents. The U.S. federal government has developed and implemented many new policies and programs to assess and reduce environmental risks to children. As the body of knowledge on children's health and risk factors expands rapidly, there is an increasing need for the systematic application of this knowledge on children in the risk assessment process. The evaluation of children's health risks from environmental exposures should be structured, informed, and guided by the best available information on the many factors influencing children's exposures (e.g., activity patterns, diet, physiology) and sensitivities (e.g., toxicokinetics and toxicodynamics). This kind of information needs to be organized and presented in a format that focuses on its application to risk assessment.

In July–August 2001 the International Life Sciences Institute (ILSI) Risk Science Institute (RSI) held a workshop to begin to address these needs [see Olin and Sonawane (2003) for a complete list of workshop participants]. The objective of the ILSI RSI workshop was to develop a framework for assessing children's health risks from exposure to environmental agents, focusing principally on hazard characterization (i.e., hazard identification and dose–response assessment) in the traditional risk assessment paradigm. Issues related to children's

exposures have been addressed elsewhere [e.g., U.S. EPA (U.S. Environmental Protection Agency) Risk Assessment Forum (2000)]. In the workshop and in this article, the term "children" was defined to include humans from conception through organ maturation (in adolescence) (see "Risk Characterization and the Framework" for further discussion of this definition). It was recognized that the effects of childhood exposures may present during childhood or later in life and that the framework should incorporate this understanding. Use of a framework can reveal what already is known as well as what is not yet known, identifying critical data gaps and research needs.

The framework for assessing risks to children from exposure to environmental agents incorporates many of the principles and elements of other frameworks and risk assessment guidance developed by the U.S. EPA over the past decade (e.g., U.S. EPA 1997; U.S. EPA Risk Assessment Forum 1992). It is responsive to, and consistent with, the directives articulated in the U.S. EPA Administrator's policy guidance (U.S. EPA 1995a) and Executive Order 13045 (Clinton 1997).

The proposed framework, presented in Figure 1, is broadly analogous to frameworks previously established by the U.S. EPA for use in its risk assessment/risk management process. It must be emphasized repeatedly that risk assessment is an iterative, not a linear, process. This concept is rigorously reinforced by the graphic inclusion of many arrows coursing back and forth, up and down, and around the framework.

The proposed framework retains the three major steps envisioned in the risk assessment phase of the risk assessment/risk management process—problem formulation, analysis, and risk characterization—refining each to capture the areas of special emphasis for the life stages constituting "childhood" (i.e., conception through adolescence). As with these other frameworks, the proposed framework for assessing risks to children from exposure to environmental agents visualizes its role within the larger context of an integrated process. This integrated process is illustrated in Figure 2. The integrated process presumes that before any significant effort is made to conduct a risk assessment, a planning and scoping exercise has been conducted to assure an understanding of the purpose(s) for which the assessment is being done and what its scope should and/or can be, given available information. As the 1997 Cumulative Risk Assessment Guidance (U.S. EPA 1997) states:

> The risk manager must explain clearly why the assessment is being performed and what questions need to be addressed. The manager must also advise the assessors, economists, engineers, and

other contributing experts on the planning team of any interested party, affected party, or policy interests to be considered in the context of the risk issue. These factors may influence the risk management options, management goals, key participants, data sources, selection of assessment end points, or the schedule for developing the assessment. The manager and assessment planning team must discuss any regulatory basis for the risk assessment and what kind of information is required to satisfy such requirements.

The Guidance goes on to say:

Initially, the risk assessor and manager (and the planning team) need to evaluate and select the kind of risk information, exposure scenarios and assessment issues that need to be covered. At this point, most EPA assessments focus on technical information related to the sources, effects, populations and the routes of exposure. Reasons to limit the technical scope of the assessment must be stated explicitly and must include details on limitations on resources, data, the impact of risk elements on the risk estimate, and methods available. In cases where an element of risk is likely to be important, but no valid data are available, the assessor must highlight this deficiency or use judgment or assumed values to approximate the missing data. Such judgments and approximations

must be noted clearly and explained to the manager in the risk characterization.

Problem Formulation

The stage is now set to proceed to the first phase of risk assessment: problem formulation. Problem formulation continues and expands the characterization of exposure and effects as well as the examination of the adequacy of scientific data and data needs, policy and public health issues, and specific factors to define the feasibility, scope, and objectives for the risk assessment. Problem formulation provides an early identification of key factors to be considered to develop a scientifically sound risk assessment. It should include a statement of the key questions the risk assessment is seeking to answer, with a rationale for focusing the assessment on particular ages or toxic effects.

The proposed framework identifies three dimensions in problem formulation—exposure, host factors, and biological effects—and emphasizes, by inclusion of arrows pointing in both directions between the three, the

reciprocal dependence of each dimension upon the others. Problem formulation is grounded in a clear articulation and understanding of several key elements:

- Objective—Defining the purpose of the risk assessment. Why is it being done? How will it be used? What is the public health need? What is (are) the risk question(s) being asked?
- Overall scope—Determining the scope of the risk assessment, general or specific. Is the assessment to consider, for example, all developmental phases from *in utero* through adolescence in the general population and all possible sources and routes of exposure (aggregate and cumulative), or is it confined to specific scenarios such as children living near a specific Superfund site potentially exposed via air, soil, and groundwater?
- Exposure considerations: Preliminary identification of life stages potentially affected—Identifying the life stages likely to be affected, given the properties of the environmental agent(s) and the defined scope of the assessment. Qualitatively characterize the sources, duration, and pattern of exposures to women of childbearing age and/or children, as appropriate, including potential for dietary, drinking water, soil, and air exposures, pharmaceutical use, and other sources. Will all ages be at risk for exposure (e.g., from air toxicants, water contaminants), or are we only concerned with prenatal exposures, newborns (e.g., from nursing exposures), or older children (agents in diet or soil, or pediatric drugs)? This decision may be site specific (e.g., only children of a certain age are exposed, if it is a day care center that 3- to 5-year-olds attend) or it may be less specific and thus dependent upon the exposure characteristics of several different life stages/age groups.
- Biological effects considerations: Preliminary identification of toxic effects and kinetic and dynamic profiles—What do we know about the chemical being evaluated that may be important for considering age-specific risk? Does the chemical cause known organ-specific toxicity? What organs are affected, and how are these organs potentially differentially susceptible during development? What should be the specific time periods of concern? Do we know of kinetic or dynamic considerations that might make the chemical differentially toxic during development?
- Result of problem formulation—The outcome of this phase of risk assessment should be the accumulation of the information needed to develop a conceptual model, shown in the proposed framework as a task linking the problem formulation phase to the analysis phase. The conceptual model can be either a diagram/flow chart or a written description of the predicted key
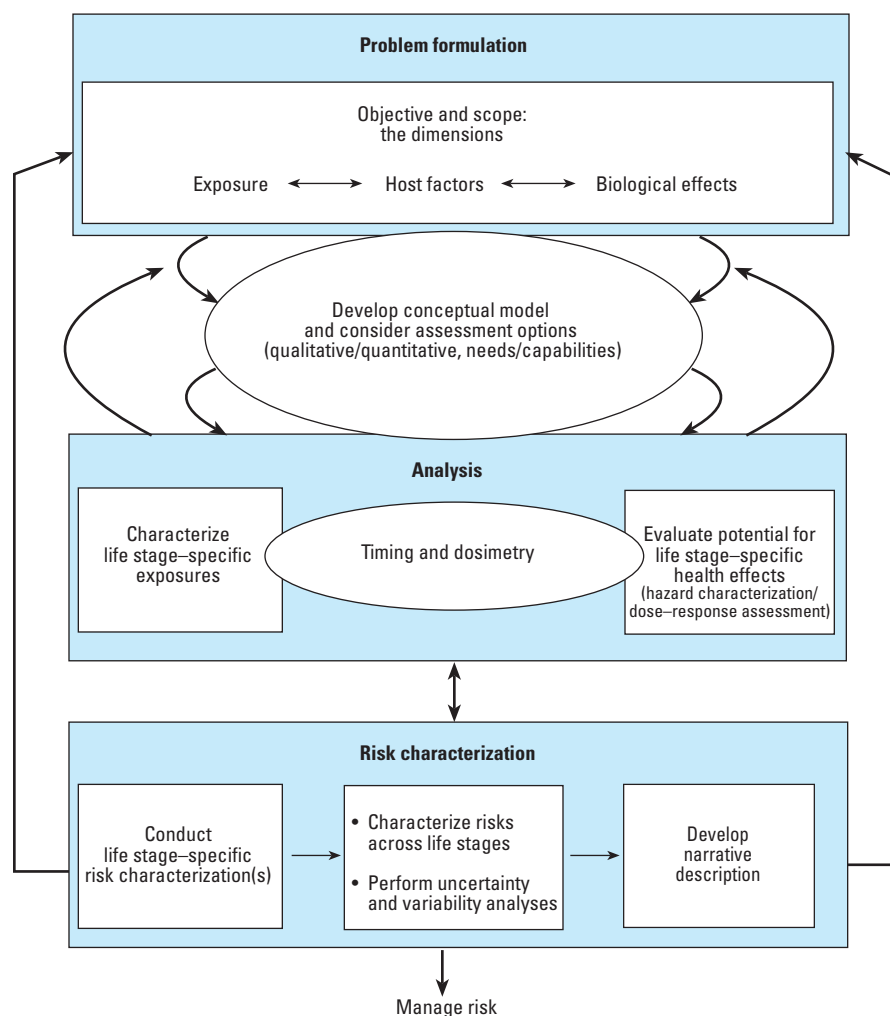

Figure 1. Proposed framework for assessing risks to children from exposure to environmental agents.

relationships between the host factors and the biological effects, informed by the initial identification of exposure scenarios, exposed life-stage groups, and the identified characteristics and toxicological end points of the chemical(s) that may contribute to children's risk.

Analysis

The analysis phase of risk assessment consists of an in-depth characterization of exposures and evaluation of the potential health effects (hazard characterization) on a life stage–specific basis. The hazard characterization should include both hazard identification and dose–response assessment. The life stages for which the analysis is to be conducted will have been identified, at least tentatively, in the conceptual model. It is important to note that the proposed framework incorporates the concepts of timing and dosimetry as unifying factors for both the exposure assessment and hazard assessment components of the analysis.

- Characterization of age-specific exposures— Characterize exposures for all life stages of interest. Are quantitative exposure data available? Can exposures be estimated? Can life stages/age groups be ranked by exposure? Which life stages/age groups are most likely to be exposed more than adults?
- Evaluation of potential for life stage–specific health effects—Consider data available for hazard identification and dose–response assessments for specific life stages. (Access

information on the capabilities of humans and animal models in the selected life stages to absorb, metabolize, and excrete xenobiotics and on the timing of developmental vulnerabilities in terms of organ and systems growth/maturation.) Evaluate the toxicokinetic profile of the chemical to understand major clearance pathways and mechanisms for activation and detoxification. Evaluate the toxicodynamic effects of the chemical ranging from cellular/molecular mechanisms of action to identifying the critical target organs and types of toxic effects. Consider how each potentially exposed age group might handle the chemical in terms of kinetic factors [Which developmental life stages are likely to have greater internal dose (per unit of exposure) than adults based upon absorption, clearance, activation/detoxification?] and toxicodynamic/vulnerability factors (What are the critical periods of organ or systems development that can be affected by the chemical based upon its mechanism of action? What are the target organs and toxic effects of concern?).

- Consider need for further assessment— Determine the need for continuing with the assessment based upon the following three issues: *a*) unique effects (Are there any life stage/toxic effect combinations that represent novel toxicities that would not be seen in adult-only exposure scenarios?); *b*) quantitative differences in effect (Are there any developmental life stages in which a greater

effective exposure dose and/or greater adverse reaction is likely compared with adults? If so, prioritize for further analysis.); *c*) lack of adult risk assessment: if there is no preexisting or relevant adult risk assessment, then continue with the children's analysis. This may indicate that children's exposure issues are unique in this scenario such that an adult assessment is unnecessary. In making this decision, one must remain mindful of legislative or other mandates that may direct what is or is not to be done.

- Consider assessment options and their feasibility and appropriateness for prioritized life stages—Based upon the public health needs, data available, and level of quantitative and qualitative assessments possible, an appropriate level of analysis for the risk characterization will be determined. Methodological options include the following: *a*) Qualitative approaches in which the risk for one or more developmental life stage is described as an additional uncertainty or source of intersubject variability in the adult-based assessment. Although such an approach may not drive the risk assessment, it could add weight to its overall conclusions. *b*) Semiquantitative approaches in which uncertainty factors are modified as needed, or a specific children's uncertainty/safety factor is considered. *c*) Quantitative analysis of exposure differences in which standard exposure equations are modified to capture children's behaviors and life-stage exposure variables. *d*) Quantitative analysis of kinetic differences, using life stage–specific physiologically based toxicokinetic (PBTK) approaches, if feasible and appropriate. *e*) Quantitative analysis of toxicodynamic differences based upon dose–response assessment of effects in developmental life stages relative to adults. *f*) Quantitative approaches to describe interchild variability within a given life-stage group. This analysis phase can determine that a single approach suffices, or that a combination of two or more of these options is needed, or that other approaches are required to address the children's risk questions raised in problem formulation.

Risk Characterization

The final phase of the risk assessment process is risk characterization. Risk characterization is the final integrative step of risk assessment for both ecological and human health assessment for any life stage. The U.S. EPA Risk Characterization Policy states that "risk characterization integrates information from the preceding components of the risk assessment and synthesizes an overall conclusion about risk that is complete, informative, and useful for decision makers" (U.S. EPA 1995b).
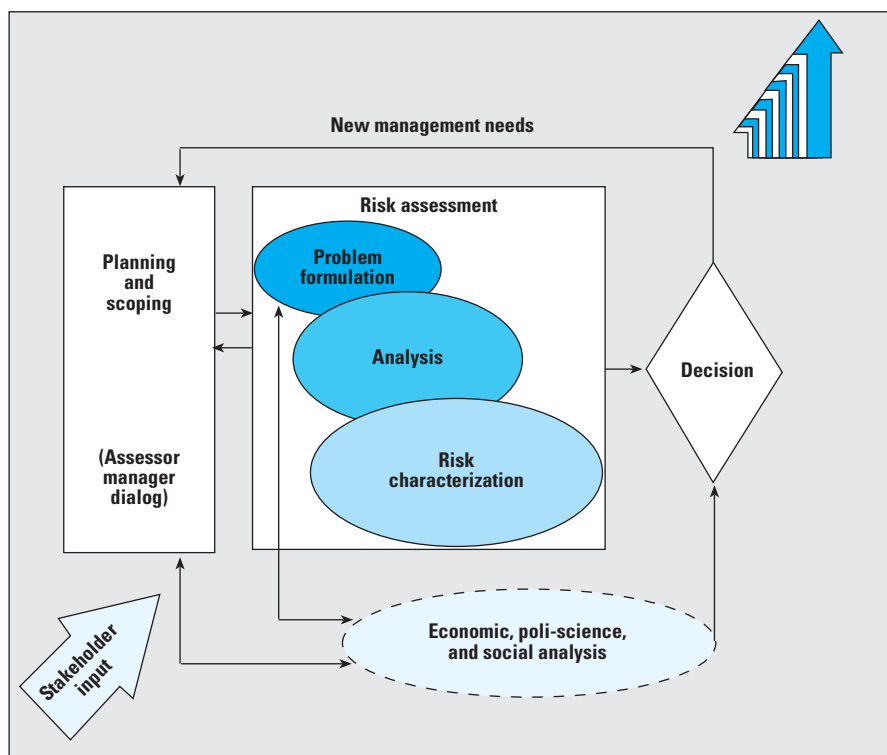

Figure 2. Stages in the integrated risk assessment process (U.S. EPA 1997).

Risk characterization employs the methods selected in the earlier phases to calculate or otherwise assess risks to life-stage groups prioritized for detailed analysis. It results in a statement of the likelihood that children's risks for specific effects will be higher or lower than adult risks, to what degree these groups may differ, and how this impacts overall risk conclusions regarding the scenarios analyzed. High-quality risk characterizations also include analyses of uncertainty and variability and describe the impact(s) of these two factors on the integrity and accuracy of the assessment.

• Conduct life stage–specific risk assessment(s)—This step is the natural culmination of the preceding phases and could range in level of complexity from a straightforward justification for use of a particular uncertainty factor to a highly refined quantitative analysis incorporating mode of action, dose–response analysis, and child-specific toxicokinetic and toxicodynamic data.

• Characterize risks for children—Develop narrative description of the overall process of consideration of potential risks for children and the conclusions from the risk assessment, including characterization of variabilities and uncertainties and identification of critical assumptions, confidence in the database, data gaps, and research needs. This would also include a discussion of comparative risks for children versus adults.

Toxicokinetic Considerations in Understanding Children's Health Risks from Exposure to Environmental Agents

We now consider how an analysis of toxicokinetics could be conducted within the proposed framework, including the types of data and considerations that would be needed at each step in the process. The goal is not to provide detailed instructions or guidelines for how the toxicokinetic analysis should be conducted. Instead, the focus is on creating a broad perspective that ensures that the relevant questions related to absorption, distribution, metabolism, and excretion (ADME) of xenobiotics across the various developmental stages (*in utero* through adolescence) are addressed.

The approach taken in a risk assessment is influenced by the type of data available. In some cases, assessment of children's risks by extrapolation from animal data for *in utero* or juvenile life stages may be possible. Alternatively (or perhaps additionally), the database may permit an extrapolation from human adults to early life stages. Although the latter type of extrapolation is the primary focus of this toxicokinetic discussion, much of what is discussed and recommended is also relevant to direct extrapolation from animal toxicity studies.

Our overall approach is to *a*) identify the key toxicokinetic determinants that tend to govern internal dose in general; *b*) summarize what is known regarding these determinants for the *in utero* period and for children; *c*) describe how this information can be used to better refine internal dose estimates for these early life stages; *d*) discuss how the toxicokinetic approaches and methods fit into the overall children's risk assessment framework; and *e*) identify critical data needs. Toxicokinetics is addressed primarily in the problem formulation and analysis phases, providing input to risk characterization.

Problem Formulation for the Toxicokinetic Analysis

Problem formulation begins with a statement of the purpose of the assessment, followed by definition of the problem/issue being addressed and identification of potential methods and data sets that may be applied.

First, the broad goal of toxicokinetic assessment is to improve the characterization of risks by developing more accurate internal dose estimates for specific life stages and between genders, species, dose routes, and exposure patterns. Toxicokinetic assessment can remove some of the uncertainty in risk assessment by replacing interspecies scaling defaults with more precise estimates of internal dose. This allows the internal dose associated with toxicity in experimental animals to be related to the internal dose humans may experience via environmental exposures under various conditions of exposure. Further, toxicokinetic assessments can take into account the range of interindividual variability (where such distributions have been described) to show both the central tendency and upper-bound estimates of internal dose. Toxicokinetic assessments also can illuminate the mechanism of toxicity by providing various estimates of internal dose whose relationships to adverse effect can be tested with regression or other correlational analyses. Those dose metrics (e.g., metabolites vs. parent compound) best correlated to toxicity are also most likely to be related to the toxic mechanism. These functions are equally relevant to risk assessments involving the *in utero* and postnatal periods.

Each toxicokinetic assessment needs to consider the specific objectives for the risk scenario being analyzed. This involves an understanding of scenario-specific factors that affect exposure and chemical-specific toxicodynamic factors that affect target organ and key internal dose metric(s) (e.g., parent compound vs. metabolite). Problem formulation also needs to take stock of the key toxicokinetic factors that generally tend to govern internal dose,

identify the types of chemical-specific and developmental data needed for a children's toxicokinetic analysis, then develop a set of analytic options for conducting the analysis. These aspects of problem formulation are summarized briefly below.

Exposure inputs to toxicokinetic analysis. Exposure assessment is a particularly critical input to children's toxicokinetic analysis, given that per body weight, children's exposure patterns and rates often differ considerably from those of adults. Problem formulation identifies the most likely route(s) of uptake (gastrointestinal absorption, dermal penetration, respiratory tract absorption), assesses whether first-pass effects will occur (e.g., hepatic extraction before systemic circulation) after oral exposure, and considers which contact sites will receive the largest applied dose. It also considers how dose rate will be affected by exposure scenario, whether sporadic (e.g., soil ingestion) or continuous (e.g., inhalation), and whether occurring as a single bolus (e.g., pica ingestion) or more evenly spread out (e.g., contaminants in drinking water or diet). The behaviors and physiologic factors that lead to greater exposures during childhood were recently summarized (U.S. EPA Risk Assessment Forum 2000).

The following factors need to be extracted from exposure assessment for input to toxicokinetic analysis for children: *a*) ages at which exposure occurs and behaviors that lead to exposure; *b*) route(s) of exposure; *c*) chemical form of contaminant in exposure medium and estimates of bioavailability; *d*) pattern of exposure (intensity—how much inhaled, ingested, contacted per event; frequency—how often; duration—over how many days, weeks, or years); *e*) estimate of daily dose (external exposure dose), which also considers body weight, breathing rate during rest and play activities, etc. Information should be sought on distributions of exposure across children's age groups to prioritize age groups for further analysis and to prepare for a toxicokinetic analysis that can represent the range of exposures and internal doses considered possible.

Toxicodynamic factors to consider in toxicokinetic analysis. Toxicodynamics and mechanism of toxicant action have a direct bearing on how the toxicokinetic analysis will be framed. Problem formulation should evaluate target organ specificity to determine the compartments for which estimates of internal dose will be needed. This is also affected by mechanism of action considerations, which may show that metabolic activation at one site leads to toxicity in another. The mechanism of action also can determine which dose metric(s) need to be evaluated, both in terms of parent compound versus metabolites and in terms of peak versus area under the curve

(AUC) doses, and provides critical input for PBTK model development. During the analysis phase, this information needs to be combined with knowledge about children's functional capacity in these critical pathways to derive internal dose estimates specific to children.

Generally important toxicokinetic determinants. Regardless of whether animals or humans (adults or children) are being modeled, certain toxicokinetic inputs are likely to be more influential than others in determining internal dose. Recognition of this early in the process ensures that the analyst will prioritize these inputs for special attention and thus decrease uncertainties (to the extent possible) in the areas most likely to drive the assessment. However, the goal of problem formulation is not to eliminate any toxicokinetic factors from consideration; for certain chemicals or age groups, additional factors may take on a more prominent role. The importance of such additional factors may only come to light after an initial analysis and may be part of an iterative process.

Key factors for which data should be sought include absorption rate for the relevant exposure pathways, distributional factors (chemical residence at contact site vs. systemic distribution), blood flow to liver and other metabolizing or target organs (Kedderis 1997), size of storage compartments such as fat or muscle, availability of plasma protein binding sites and maternal, placental, and embryo/fetal factors for the analysis of *in utero* exposure, metabolism rates for both activation and detoxification pathways at the various life stages in which exposure occurs, and ability to eliminate xenobiotics or their metabolites via renal or biliary clearance or via pulmonary gas exchange. In each of these areas, chemical-specific data are needed to identify the main pathways of chemical activation, detoxification, and clearance as seen in adults or animal models, and then age-specific data are needed to adjust these factors for the *in utero* or childhood period.

Listing of analytic options. The toxicokinetic assessment could proceed along different tracks depending upon the level of quantitative detail necessary and feasible. These options range from a completely qualitative description of the issues and uncertainties in children's toxicokinetics to a fully quantitative analysis involving PBTK modeling and Monte Carlo analysis. Problem formulation should evaluate whether the types of data for these analyses are available for the particular chemical and life stages being analyzed.

Analysis of Toxicokinetic Data

This phase of the toxicokinetic assessment reviews the chemical-specific toxicodynamic and toxicokinetic information described above, together with the child/age group-specific toxicokinetic information. The major questions raised in this phase are *a)* How does toxicokinetics affect the toxic mechanism of the chemical via activation or detoxification pathways? *b)* What are the key dose metrics and target organs for the chemical being analyzed? *c)* What are the major *in utero* or child-specific toxicokinetic factors that may alter chemical fate? *d)* Based upon internal dose considerations, which age groups should be prioritized for more detailed analysis? *e)* Which toxicokinetic analytical methods are best suited to evaluating children's internal dose? *f)* Once the analysis is conducted, what do the results tell us about how internal exposure can vary (per unit external dose) across developmental stages and between children and adults? The stepwise process outlined below is intended to address these questions.

Analysis of chemical-specific data. The first task is to combine toxicodynamic and toxicokinetic information from animal models and, to the extent available, from adult humans; this type of data will usually be lacking in children or juvenile animals. The combination of toxicodynamic and toxicokinetic data is used to understand the toxicokinetic determinants of toxicity— whether metabolism represents detoxification or activation or both. This involves identification of the various enzymes that may be important in chemical activation and removal (e.g., phase I enzymes—specific cytochrome P-450s (CYPs), peroxidases, dehydrogenases; phase II systems—glucuronidation, sulfation, glutathione conjugation; other enzymes—epoxide hydrolase, serum esterases). Because an enzyme can be part of an activation pathway for one chemical and yet be detoxifying for another [e.g., glutathione *S*-transferases (GSTs), epoxide hydrolase, various CYPs], determining the role of a given enzyme is highly chemical specific. Therefore, the toxicological significance of the presence or absence of an enzyme at a particular developmental stage is also chemical specific.

Known or anticipated target organs for the chemical should be identified, and the key activation and detoxification steps in each target organ should be evaluated. Internal dose metrics for the active form of the chemical can be selected for each target organ. Because target sites can be different in the fetus or child from those in adults, toxicity information for early life stages is particularly important, when available.

Other toxicokinetic determinants of chemical fate should be evaluated so the mechanisms and factors involved in chemical absorption (from scenario-relevant portals of entry), distribution (e.g., serum binding, partitioning), and excretion (e.g., renal, biliary, exhalation) are understood.

This segment of the analysis phase should provide the following chemical-specific information: identification of key metabolic pathways for activation and detoxification; target organs for which dosimetry information would be needed; key dose metrics that should be modeled or evaluated in other ways; toxicokinetic factors that control chemical distribution and excretion.

Analysis of age group–specific toxicokinetic factors. Numerous toxicokinetic factors differ across life stages, particularly because of rapidly changing physiology and the immaturity of various systems *in utero* and in early life. However, the importance of any single factor in altering internal dosimetry depends upon the toxicokinetic mechanisms involved in the ADME of the chemical, the life stage where exposure occurs, and the interplay of other factors that may tend to accentuate or offset the dosimetry change. For example, immature metabolism in early life via phase I enzymes may have less influence on internal dose and long-term retention of highly lipid-soluble organochlorines whose toxicokinetics is most influenced by partitioning into lipids. An example of offsetting factors would be where immaturity of metabolism causes both the activation and detoxification steps to be slow relative to those of older age groups. In this case, formation of toxic metabolites may be low, but their removal may be sufficiently impeded (perhaps also by slow renal and biliary clearance) to create similar or higher levels of ultimate toxicant at key target sites.

These considerations illustrate the need for this phase of the analysis to take into account the various ADME differences possible in early life. The following general discussion provides a framework into which chemical-specific information can be added to focus the toxicokinetic analysis for children.

Absorption factors. The exposure scenario (age groups involved, contaminated media, behaviors leading to exposure) will determine the route of uptake: oral, dermal, inhalation, or a combination of several routes. Also, as stated above, the scenario will dictate the frequency and intensity of exposure, which can differ substantially across age groups. Aside from differences in exposure, the amount of uptake can differ because the percent absorption from the gastrointestinal (GI) tract, respiratory tract, and skin may be different in newborns and infants relative to older children and adults.

Oral absorption. Greater oral absorption of lead (Pb) has been documented in infants relative to adults and attributed to greater pinocytic activity of intestinal epithelium prior to closure. This nonselective uptake mechanism may also increase the absorption of other metals and organic compounds, as suggested by data in juvenile rats [Kostial et al. 1978;

NRC (National Research Council) 1993]. In rodents, closure occurs around the time of weaning.

A variety of other factors may influence oral absorption in different age groups, including nutrient deficiencies (e.g., low iron or calcium intake increases the absorption of lead; low stomach pH up to 2 years of age; blood flow and surface area of GI tract absorptive regions; presence of milk in stomach) (NRC 1993).

The possibility of higher GI uptake of ingested chemicals early in life should be evaluated within the context of behavior of the chemical in the gut. If it is generally well absorbed in rodents and adult humans by the oral route (e.g., small organic molecules), then any increase in absorption during early life stages may not create a large difference in uptake. However, for poorly absorbed chemicals (e.g., a variety of metals), increased uptake in children may be an important factor in the exposure and risk assessment. For such chemicals, the mode of absorption should be investigated to determine whether these mechanisms may be enhanced in early life. Further, GI uptake data for this and analogous chemicals in children or juvenile animals should be sought. These efforts may allow age group-specific adjustment factors to be applied for GI absorption.

Respiratory dosimetry and absorption. PARTICLES AND AEROSOLS. Inhalation exposure during early life may lead to a different degree of exposure than at older ages because of the greater respiratory volume per surface area in young children. On average this can lead to an approximately 2 fold increase in respiratory tract exposure (per unit surface area) of young children compared with adults (NRC 1993; U.S. EPA 1999, 2000). Preliminary modeling efforts for young children suggest that this differential can be larger when considering local deposition (Martonen et al. 2000). This exposure dose differential for particles and aerosols may be of particular consequence to young children who are sensitive to respiratory irritants and allergens because of asthma or other conditions. Further, in asthma the changes in breathing pattern and respiratory volume/resistance may create local exposure patterns different from those in healthy children or adults. Therefore, it is important to analyze respiratory deposition of particles and aerosols in children, both healthy and asthmatic. This can be aided by the development of regional deposited dose ratio (RDDR) models that take into account respiratory physiology at different life stages as well as a distribution of particle sizes. These models would be similar to those currently used in extrapolating from animal-to-human data for inhalation reference concentration (RfC) development (U.S. EPA 1994). Additional consideration should be

given to whether there are life stages where mucociliary clearance and macrophage clearance of particles are substantially different from those of adults. Until these models are available, the greater inhalation volume per respiratory surface area in young children should be considered for input into the analysis.

REACTIVE GASES. RfC methodology uses the regional gas dose ratio (RGDR) to extrapolate from extrathoracic dose in animals (where the toxicity data are obtained) to adult humans. This adjustment factor is based upon the difference in respiratory volume per surface area in the upper respiratory tract across species. This methodology can be extended to children of various ages by use of upper respiratory tract surface area and inhalation volumes for these ages. Lacking RGDR data, the overall approximately 2-fold differential in inhalation volume per respiratory surface area described above may be assumed as a first approximation for reactive gases.

NONREACTIVE GASES. Uptake of this class of inhaled chemicals is currently modeled by estimating the difference in pulmonary absorption (net systemic uptake) between test animals and adult humans. Uptake across alveoli is driven by the blood:air partition coefficient, respiratory rate, cardiac output, and systemic extraction (e.g., partitioning into lipid, removal from circulation via metabolism or excretion). However, for chemicals that rapidly reach steady state (e.g., perchloroethylene), the major (but not only) determinant of net uptake is the partition coefficient. In this case, increased respiration and cardiac output also lead to increased exhalation. This may be especially relevant for neonates and infants whose metabolic and renal capacities are immature and quite limited. The blood:air coefficient is affected by the presence of carriers (e.g., hemoglobin) or lipid in blood, which can vary across species and age groups. It may be possible to develop a database of children's partition coefficients (across age and chemicals) based on partition coefficients determined *in vitro* and to model uptake of these gases in children based upon data sets in adult animals and humans.

Dermal absorption. For toxicokinetic assessment of dermal absorption, a major consideration is whether dermal contact with the contaminated medium is greater in children of certain ages than adults because of behavioral factors (e.g., crawling; play activities leading to high percentage of the body becoming covered with soil) or physiological factors (higher skin surface area per body weight in young children) (NRC 1993). A second consideration is whether children's skin is more permeable to chemicals than the skin used to derive uptake factors, typically

adult animal or human skin. As full-term newborns have a well-developed stratum corneum, it is generally believed that the age of the child has little bearing on dermal permeability (U.S. EPA 1992). This has been shown in limited *in vitro* testing using skin from neonates and adults (U.S. EPA 1992; Wester et al. 1985). However, the skin of premature neonates can be substantially more permeable than that of full-term neonates because of immaturity of the stratum corneum (Barker et al. 1987; U.S. EPA 1992). This potential for increased dermal uptake in premature neonates may be an important factor in scenarios where these neonates are dermally exposed to contaminants present in bath water or to chemicals in hygienic or diaper-rash products. Dermal penetrability may also be enhanced when skin is damaged or highly hydrated (U.S. EPA 1992). These conditions are more prevalent in infants whose skin under a diaper is more likely to be excessively hydrated and possibly compromised by irritation and rash.

A final consideration is whether chemical sorption on the exposure matrix (e.g., soil) significantly impedes dermal penetration. Although this factor affects adults as well as children, in certain cases binding to the exposure matrix may substantially decrease dermal uptake and thus reduce the importance of any child/adult differences in this route of exposure.

Distribution factors. Distribution into systemic compartments depends upon a number of chemical-specific factors: lipid and water solubility, as these determine partition coefficients; chemical size; ability to be carried by transporters across membranes, and affinity for plasma or tissue proteins. Age group–specific factors that affect chemical disposition include lipid and water content of the body (generally more water and less lipid in neonates), quantity of plasma protein binding sites (fewer in neonates; those that do exist may be less available for xenobiotic binding than at older ages), and more permeable blood–brain barrier in neonates. These factors tend to increase the volume of distribution for many chemicals in early life (Ginsberg et al. 2002, 2004). Higher volume of distribution can lead to lower blood concentrations and longer chemical half-lives, as the chemical is less available to the central compartment for transfer to sites of metabolism (e.g., liver) and elimination (kidney, lung, bile). However, the interplay of distributional, metabolic, and elimination factors can be complex, thus defying use of a simple adjustment factor to compensate for differences in chemical distribution in children. PBTK modeling for children holds the greatest potential to combine the absorption factors described above with distribution, metabolism, and elimination information to enable predictions of

blood and tissue concentration estimates over time. Short of this approach, there are some simplified generalizations regarding distribution and age groups that may be useful in the assessment: greater permeability of the blood–brain barrier in early life can produce higher chemical concentrations in the central nervous system (CNS) of children; lower lipid content in early life would cause less storage and retention of lipophilic chemicals; less plasma protein binding in early life might accentuate chemical toxicity because of a greater percentage of free chemical in the circulation (as suggested for lidocaine, cisplatin and other drugs; Kakiuchi et al. 1999; Zemlickis et al. 1994). The importance of such distributional differences across age groups may be described qualitatively for the chemical under assessment. However, PBTK modeling would be needed to quantitatively incorporate such factors.

Two additional distributional phenomena critical to early life exposures are placental transport of chemicals from mother to fetus, and partitioning of chemicals from maternal blood into breast milk. The existing database suggests that most chemicals can cross the placenta, although the rates can vary depending upon molecular size, lipophilicity, and serum protein binding (Ginsberg et al. 2002, 2004). This suggests that toxicant exposure in the mother will generally lead to toxicant exposure of the fetus, although maternal metabolism/clearance factors may lead to lower concentrations in the fetus compared with the mother. Thus, fetal exposure needs to be considered where maternal exposure occurs. Fortunately, there are PBTK models that describe pregnancy and fetal exposure (Clewell et al. 1999; Krishnan and Andersen 1998). Similarly, the partitioning of chemicals into breast milk has been evaluated for various types of chemicals and can be described via modeling approaches in the absence of empirical data (Byczkowski et al. 1994).

Metabolism factors. A companion article (Ginsberg et al. 2004) in this mini-monograph summarizes a variety of *in vitro* data (enzyme levels and function) and *in vivo* data (therapeutic drug pharmacokinetic studies) that show that young children, particularly in the first 2 months of life, are immature with respect to metabolic and renal clearance. This appears to be a consistent finding across a number of metabolic pathways including a variety of CYPs (including CYP1A2 and CYP2E1, two that are particularly important in toxicant activation), glucuronidation, serum esterases, epoxide hydrolase, and perhaps also GSTs. There are fetal forms of some enzymes (e.g., CYP3A7, GST-pi), but these appear to have a different range of specificities from the adult forms. Renal function is also immature

in the first weeks to months of life, leading to prolonged half-life of a variety of renally cleared drugs. This condition changes by 6 months of age, and for a time, some enzymatic functions (most notably CYP1A2) appear to become somewhat more active than in adults (Dorne et al. 2001; Ginsberg et al. 2002, 2004; Renwick et al. 2000). This may be a function of the higher liver mass per body weight (and assumed higher hepatic blood flow per body weight) that exists in children compared with adults. This becomes normalized when scaling across ages on a surface area rather than body weight basis (Gibbs et al. 1997), which suggests that beyond 6 months of age a surface area correction may be a good first approximation of how metabolism changes with age once a system has reached functional maturity (~6 months–1 year for many systems).

The significance of these changes in metabolism with postnatal development depends upon whether chemical metabolism leads to activation or detoxification, which pathways are involved in activation and detoxification, and whether blood flow limitations to the metabolizing tissue (e.g., the liver) prevent the full expression of changes in enzymatic function (Kedderis 1997). The importance of changes in metabolic function also depends upon whether other clearance pathways (e.g., renal, biliary, exhalation) can compensate for slow metabolism in early life.

The interplay of distributional, metabolic, and renal factors is best understood and quantitatively evaluated via PBTK models. However, if these models do not exist and cannot be developed within the scope of the children's risk assessment being performed, then some simplifying first approximations of chemical clearance and metabolic activation may be possible from the existing literature. *In vitro* and *in vivo* data sets that are available provide quantitative data on metabolic processing in children relative to adults for a wide variety of pathways and drugs (Ginsberg et al. 2002, 2004; Hines and McCarver 2002; McCarver and Hines 2002; Renwick et al. 2000). At a minimum, the slower clearance of many chemicals very early in life should be qualitatively discussed in terms of internal dosimetry and risk implications for children (e.g., more of parent compound but less of metabolites present in tissues; also possibly slower removal of metabolites). It should be noted that neonate/adult differences in half-life can be large relative to the default assumptions for interindividual pharmacokinetic variability (3.16 factor), especially when considering the full range of results from individual neonates (Ginsberg et al. 2002). This may warrant semiquantitative approaches such as adjusting uncertainty factors to incorporate pharmacokinetics into risk assessments for

newborns. However, this will take careful consideration of chemical mechanism of action (activation and detoxification pathways) and potential blood flow limitations. Ultimately, a PBTK model would provide the best assurance that all relevant pharmacokinetic factors have been accounted for when estimating internal doses.

In utero, placental, fetal, and maternal factors can all play a role in chemical metabolism. A variety of placental enzymes exist and can be induced by maternal exposure to cigarette smoke and other types of drugs and toxicants (Juchau 1980). Metabolism by the fetus itself can in some cases outweigh the importance of maternal metabolism in terms of fetal toxicant exposure. This has been seen with fetal mice, whose risk of lung tumors from maternal exposure to 3-methylcholanthrene was greatest when the fetal mice had induced levels of CYP1A1 and the mother was noninducible (Anderson et al. 1989; Miller et al. 1990). Lower tumor incidence was seen in offspring when the mothers were inducible, demonstrating the protective role maternal metabolism can have, even when that metabolism leads to more toxic moieties. Another issue is that fetal metabolism may create metabolites less able than parent compound to cross the placenta back to the maternal circulation [e.g., zidovudine (Garland et al. 1998); hormonal agents (Slikker et al. 1982)]. This could lead to an accumulation of metabolites in fetal tissues. However, the fetus may have lower exposure to reactive metabolites because of the lack of activating metabolic pathways, as is recognized for CYP2E1 in the fetus (Cresteil 1998).

Understanding the time course for the development of *in utero* metabolic capabilities can identify gestational periods important for toxicokinetic assessment of placental or fetal activation. These placental and fetal metabolism factors are best incorporated into a modeling framework to be useful in risk calculations.

Elimination factors. As mentioned above, renal elimination of drugs is generally reduced in newborns, which is consistent with developmental studies on the maturation of renal glomerular filtration and tubular secretory functions. Biliary excretion can also be diminished in newborns because glucuronidation capability and other hepatic functions are immature in the first months of life. However, enterohepatic circulation is functional in early life, which can lead to substantial reabsorption of chemicals excreted in bile (Suchy et al. 1987). Exhalation of volatile chemicals may be enhanced in the young because of high ventilation rates per body weight and the fact that other clearance pathways are immature. Although PBTK modeling of these elimination pathways in conjunction with other toxicokinetic inputs

specific to children is the ideal, one can assume that elimination via renal and biliary systems will be slower in newborns than in adults. This can lead to potentially greater levels of parent compound or metabolites in newborns, depending upon their primary route of clearance. The children's risk assessment should consider the implications of deficits in chemical elimination during this early life stage.

Selection of age groups for special focus. Review of chemical-specific and age group–specific data may reveal a specific age group of particular concern from a toxicokinetic perspective. These would be age groups where the toxicokinetic mechanisms central to absorption, distribution, activation, detoxification, and elimination of the chemical are expected to be most different from those of adults. In general, as outlined in the preceding sections, neonates (both premature and full term) through the first several months of life are most different from older age groups and adults. Therefore, this age period should be carefully considered for the possibility of substantive changes in internal dose relative to adults. Somewhat older age groups (6 months to 2 years) are also important from a toxicokinetic perspective in that these groups generally have greater hepatic extraction and shorter chemical half-lives because of larger liver size per body weight (Gibbs et al. 1997; Ginsberg et al. 2002, 2004). *In utero* may also be a critical life stage from a toxicokinetic perspective, as most chemicals cross the placenta and placental or *in utero* enzymes may be sites of chemical metabolic activation. The other portions of the risk assessment (exposure assessment; toxicity assessment) may identify key life stages that need to be fully analyzed, regardless of whether unique toxicokinetic considerations exist in those stages.

Changes in toxicokinetic function may be defined within specific age groupings as a way to compile and organize the data. It appears that rapid maturational changes that occur within the first weeks and months of life warrant subdividing that period into several age groups. Beyond that age, broader age groupings are possible, given that changes in metabolism and other factors may be possible to scale allometrically based upon body surface area. Alternatively, for these age groups a continuous physiological model based upon a set of equations that describe physiological development of organ systems and blood flows may be suitable (Pelekis et al. 2001). The risk assessment may dictate that certain age groups be the prime focus on the basis of exposure issues or toxicodynamic issues. The toxicokinetic portion of the analysis can accommodate this focus, providing some idea of how internal dose may be affected by the

stage of development during these critical exposure or susceptibility periods. However, the risk assessment should also take on as a focus those age groups that appear to have the unique toxicokinetic features relative to adults (particularly the first weeks and months of life as described above).

Selection of analytical approach. The possible approaches for assessing toxicokinetic factors as part of a children's risk assessment are PBTK modeling, semiquantitative assessment of children's internal exposure relative to adults, and qualitative description of the issues and uncertainties. Each approach has advantages and disadvantages, as described below.

Quantitative approaches: PBTK models. PBTK models have had great utility in refining risk assessments involving extrapolation of exposure and toxicity across species. The same will likely be true of PBTK modeling for children. However, models do not currently exist that take into account the numerous factors that can create toxicokinetic differences between children and adults or that are calibrated against actual toxicokinetic data in children. Several initial efforts (Gentry et al. 2002; Haddad et al. 1999; Pelekis et al. 2001) form useful building blocks, and we can expect children's PBTK models to evolve over the next few years. Therefore, although this analytical tool holds great promise for providing refined estimates of internal dose in children, it has the drawback of requiring a period of intensive model development. Other drawbacks include the fact that PBTK modeling requires a large amount of empirical data for model calibration and validation, and these types of data are not available for environmental toxicants in children. Therefore, such modeling will be difficult to validate and will often depend upon developing confidence in the model structure by simulating pharmacokinetic data in children who have been exposed to therapeutic drugs. While this will introduce uncertainties, it does not invalidate this approach.

The combination of PBTK models with Monte Carlo approaches is potentially quite useful, incorporating the distribution of children's capacities in the various ADME areas. In this way, variability within a given age group of children can be explicitly examined, and predetermined percentiles of the distribution of internal dose (e.g., 50th or 90th percentile) can be selected for inclusion in risk calculations. Alternatively, the full distribution can be used in combination with distributions for other risk inputs (exposure, dose response) for a complete probabilistic description of risk. In this way, the toxicokinetic contribution to variability and uncertainty in the assessment can be explicit and readily expressed.

Although PBTK efforts are recommended for children's risk assessments, not all types of assessments may warrant this level of effort,

even when working children's models are available. For example, risk assessments in which the exposure or dose–response inputs are associated with a high degree of uncertainty may not warrant extensive effort to refine the toxicokinetic component. In such cases, screening-level analyses may be the only realistic option. Additionally, in cases where toxicokinetic properties of the chemical have not been well studied in rodents and adult humans, children's models become more uncertain and less worthwhile. Therefore, the choice of whether to utilize PBTK approaches for children depends upon whether refined estimates of internal dose are feasible for children and worthwhile relative to the level of analysis being conducted in other portions of the risk assessment.

Semiquantitative approaches. When there is no need to conduct a detailed quantitative analysis or when such an analysis is not feasible, the analyst can consider a semiquantitative approach. In these cases, review of the underlying chemical-specific and age group–specific databases may suggest a potentially greater internal dose at certain early life stages than in adult humans or in the laboratory animals from which toxicity data are extrapolated. For example, if the chemical could be metabolically activated to toxic metabolites by CYP3A7 [e.g., aflatoxin B_1; 2-amino-3-imidazoquinoline (Hashimoto et al. 1995; Kitada et al. 1989)], a form of CYP prevalent *in utero* and just after birth, this may constitute a sufficient rationale to develop at least a semiquantitative or screening-level estimate of relative internal dose in this age group. This would be the case especially if the active metabolite formed from CYP3A7 metabolism is expected to be poorly detoxified and excreted in this age group. This screening-level approach can be seen as supplementing the existing set of uncertainty factors that already exist in non-cancer risk assessment, specifically the half-log (3.16-fold) uncertainty factor for interindividual variability in toxicokinetics (Renwick 1998). Although that uncertainty factor is designed to address a large array of general interindividual differences that might affect toxicokinetic handling of xenobiotics (e.g., genetics, gender, disease states, other concomitant exposures, age), it may not always be adequate to address specific differences between subgroups of the population (e.g., children of certain age groups). The semiquantitative assessment could evaluate whether a sufficient difference between children and adults might exist in the direction of increased toxicant dose to warrant an age group–specific adjustment factor for toxicokinetics.

Because this would not be a comprehensive PBTK approach, the semiquantitative assessment would focus upon one or several key toxicokinetic factors/pathways. An assessment

that does not incorporate all factors that may influence the estimate of internal dose has the disadvantage of greater analytic uncertainty. However, by taking stock of key factors with obvious implications for internal dose differences in children, the analysis can point out what types of concerns exist, how large the across-age differences may generally be, and whether more detailed PBTK assessment is ultimately needed to refine the dose estimate.

The semiquantitative assessment can evaluate how known differences in key toxicokinetic pathways (Ginsberg et al. 2002, 2004) may affect the absorption, metabolism, and elimination of the chemical under analysis. The analysis would be semiquantitative and comparative in that the size of functional differences between children and adults (e.g., child/adult ratio) would be used as an initial estimate of the change in internal dose in a particular age group. Other toxicokinetic factors may increase or decrease the influence of changes in, for example, a specific CYP pathway (e.g., differences in blood flow, protein binding, distribution to CNS, renal elimination changes, phase II conjugation activity). These factors would need to be considered for their possibility to alter or negate the importance of the key child/adult difference upon which the analysis is focused. It is essential that this screening level approach be described as providing only a crude estimate of the differences in internal dose that may be possible, and that the various uncertainties be made explicit.

Qualitative approaches. When the review of chemical-specific and age group–specific toxicokinetic data suggests that child/adult differences may not cause substantially higher internal dose in children, or where this review indicates large areas of uncertainty, a purely qualitative approach may be adequate. This approach can summarize what is known about the toxicokinetic properties of the chemical vis-à-vis the development of toxicokinetic functions *in utero* and in children. This can lead to a discussion of how these various factors may interact to alter internal dosimetry relative to adults and if there are age groups where such alterations are more likely. If this is a considerable source of uncertainty, it may affect how much confidence is placed on the overall risk assessment regarding *in utero* and children's exposures.

Engaging the Framework: Addressing Modeling and Data Needs

The toxicokinetic assessment framework described above involves a large array of parameters that need to be informed by empirical data for a variety of age groups. Given that there are very few toxicokinetic

data for environmental chemicals in children, a large number of data gaps will need to be filled for individual chemicals, either through new data acquisition (e.g., studies in juvenile animals) or by reliance on surrogate chemicals (e.g., drugs that have similar metabolism/clearance pathways and have been tested in children).

To engage the framework there is an urgent need for the development of well-calibrated, and to the extent possible, validated PBTK models for children. These models can be extensions of adult models with appropriate adjustments for the physiologic and metabolism/elimination differences that exist for children at specific developmental periods. This modeling can progress in stages from initial descriptions of children's growth and maturation (changes in body weight and water/lipid composition, body compartment sizes, tissue blood flows, ventilation rates, and serum protein binding capacity) to more complete, chemical-specific PBTK models with age-specific activity data for the major metabolism and elimination pathways of the chemical. Model development would likely involve a number of case studies for specific chemicals, which would then lead to a flexible modeling framework that would describe the underlying physiology of children's development and be adaptable to a variety of chemicals and age groups. A similar approach can be used for the *in utero* period, in which established models for specific chemicals can be adapted to new chemicals for which there is exposure during pregnancy.

There are several general toxicokinetic data and modeling needs to make this framework fully feasible:

- Develop an accessible database of evaluated age-specific physiological parameters in humans and animals
- Expand data available on juvenile animal toxicology and toxicokinetics
- Develop, calibrate, and validate PBTK model(s) for early life stages
  - Submodels for respiratory deposition and uptake (particles, gases)
  - Case studies
- Chart the development of lung clearance mechanisms
- Characterize *in utero* dosimetry
- Compile data and create model(s) for lactational transfer of chemicals
- Continue building database on ontogeny of metabolic enzyme systems

In addition to these general needs, there may be a variety of chemical-specific data needs for any individual analysis, depending upon the extent of toxicokinetic evaluation the chemical has already undergone and the degree to which it has been modeled in test animals and humans.

Toxicodynamic Considerations in Understanding Children's Health Risks from Exposure to Environmental Agents

Complementing the consideration of toxicokinetics are the toxicodynamic issues; i.e., how to use known dynamic differences in development to better understand children's susceptibility to environmental agents, and how and when such information could be used in an overall risk assessment framework for evaluating children's health risks.

The term "developmental dynamics" is used here to describe the biochemical, molecular, cellular, organ, and organism processes that change throughout development and that define and characterize the developing organism at each life stage. Toxicodynamics is the response of these normal developmental processes to toxicant exposure. Such alterations need to be considered in both a temporal and a dose-related context to understand the immediate and long-term consequences of such changes.

In this section we *a*) identify the role of toxicodynamics in applying the proposed framework for assessing children's risks, *b*) evaluate life-stage considerations by developmental organ system, illustrating their significance for the respiratory, immune, and nervous systems, *c*) discuss developmental processes and their implications for toxicodynamics in children's risk assessment, and *d*) identify several critical data needs.

Toxicodynamics in the Risk Assessment Framework

Toxicodynamics is an integral component of the proposed framework (Figure 1). Table 1 describes for each phase of the risk assessment process (problem formulation, analysis, and risk characterization) how developmental dynamic information could impact risk assessments for children. We consider the major contribution of toxicodynamics to be within the analysis and risk characterization stages of the overall children's risk assessment framework.

The problem formulation stage of the children's risk assessment framework provides an opportunity for the assessor to understand the purpose and focus of the risk assessment and can help to define the breadth of the toxicodynamic assessment. In addition, by identifying chemicals or chemical classes to be evaluated, the problem formulation stage provides critical input regarding potential biological systems for consideration (i.e., do we already know that this class of chemicals is neurotoxic?). If yes, then specific dynamic factors relevant for the developing nervous system should be considered in the analysis. Likewise, if this class of chemicals is known to

affect particular cellular or molecular processes, namely, chemicals known to affect apoptotic processes, then organ systems that use these dynamic processes should be considered in the analysis. In addition, because such apoptotic processes occur at specific times in normal development, critical windows of vulnerability could be identified. Thus, both critical time periods and target organ systems could be identified for further evaluation using dynamic information. During problem formulation, the risk assessor needs to take into consideration the life stage of the population(s) of concern and the specificity of the agent of concern.

The proposed children's risk assessment framework emphasizes the need to connect toxicodynamic considerations with concurrent toxicokinetic considerations. For example, to assess developmental toxicodynamics it is essential to understand if the parent compound or a metabolite would be expected to reach developing tissues. In addition, it is important to know what dose levels and at what times such exposures would be expected; hence, the importance of timing and dosimetry considerations within the analysis phase of the framework. The assessment of toxicodynamics also informs quantitative considerations in dose–response and risk characterization.

Early Life Stages and Susceptibility

Data on life stages and critical windows of susceptibility in development have been summarized and discussed in a number of recent workshop/workgroup reports and reviews (Adams et al. 2000; Adkins 2000; Dietert et al. 2000; Lemasters et al. 2000; Pinkerton and Joad 2000; Pryor et al. 2000; Rice and Barone 2000; Selevan et al. 2000; Weiss 2000; Zoetis and Walls 2003). Figure 3 is a summary of developmental life stages, including those early life stages considered in this article (i.e., preconception through adolescence). Identification of distinct life stages facilitates identification and characterization of potential windows of susceptibility. It is also important to be able to compare life stages and particular end points and patterns of dynamic processes across species. In this discussion we use a series of general, simplified life-stage events (summarized in Figure 3) that include conception, embryonic, fetal, newborn, neonatal, preweaning, weaning, juvenile, puberty, adolescence, adulthood, and old age.

Of course, there are a number of different ways of viewing the period called childhood. In considering the toxicodynamic processes involved with the adverse effects of xenobiotic materials, it is helpful to be aware of the way different organizations and disciplines have viewed and subdivided the developmental periods.

Categorization of life stages by dietary age divisions. As an example, the Institute of Medicine (IOM) of the National Academies of Science (NAS) is establishing updated dietary recommendations for nutrients. As has been the situation with prior guidelines, the nutrient recommendations are targeted toward a series of age ranges that correlate with the physiological demands of normal growth and development. The age ranges that are used for the present set of dietary reference intakes (IOM 1997, 2001; Murphy et al. 2002), including the newly established tolerable upper intake levels, divide the childhood period into three major life-stage categories that are then subdivided into narrower age ranges. The three major developmental categories are infancy, childhood, and adolescence. Intakes during pregnancy and lactation are also considered separate categories.

The infancy period is subdivided into the first and second 6 months after birth. Lactation demands and the age of introduction of solid foods were considered in establishing this division. The childhood period is subdivided into two periods (1–3 years and 4–8 years). The adolescent period is divided into two periods (9–13 years and 14–18 years) based on the beginnings of puberty and growth demands. Males and females are treated differently during the adolescent period.

The IOM dietary age divisions are important considerations in risk assessment because nutrition and food exposure pathways can have a strong impact on the evaluation of all environmental effects. Accordingly, it may become possible to link nutritional status in a particular age group with toxicant exposure and manifestation of effects.

Categorization of life stages by behavior and exposure windows. Likewise, exposure assessors have identified a different series of age-related exposure windows based on age-specific behaviors and physiological considerations. For example, differences between toddlers and neonates in exposure to carpet and floor, in hand-to-mouth exposure pathways, and in dietary patterns and exposures are defined by differences in activities as well as physiological development. Exposure considerations in developing life-stage categories are reviewed in the U.S. EPA children's exposure factors handbook (U.S. EPA 2000) and the U.S. EPA risk assessment forum document on issues associated with considering developmental changes in behavior and anatomy when assessing exposure to children (U.S. EPA Risk Assessment Forum 2000).

Many other examples of discipline-based differences in childhood categorizations could be cited. For instance, child psychologists or childhood developmental specialists define life stages using behavioral landmarks. Although it was not considered important for the children's risk assessment framework to fully integrate all the various categorical views of childhood and major developmental periods, it is important to be aware that these differences exist among disciplines.

Recognizing categorical overlap between disciplines. Although general developmental life stages do not always match the discipline-specific exposure windows, the differences should not prevent the examination of dose–response relationships for critical developmental end points. They do, however, highlight the need for the framework to be robust enough to allow for iterative interactions between exposures and effects analysis in both the problem formulation and analysis stages. The overlap of categories also identifies a need for risk assessment methods that would allow risk assessors to relate outputs from the exposure assessments to those from discipline-specific assessments using multiple exposure times during various life stages.

Table 1. How does developmental dynamic information impact risk assessments for children?

Problem formulation
  Determination of risk assessment context and scope
  Definition of scope provides context for risk assessment and leads to the identification of relevant life stages, systems, or processes of interest for the risk assessment
  Determination of relevant exposure pathways/scenarios will provide context for identifying relevant developmental life stages
  Determination of chemical-specific factors will also provide context for the identification of potential life stages for evaluation, as it will identify potential toxicological processes of interest and hence identify developmental systems for potential evaluation
  Identification of cross-species relevancy of potential responses
Analysis
  Identification of uniquely susceptible dynamic processes
  Identification of developmental milestones and/or end points for testing/assessment
  Identification of functional consequences of processes if altered
  Illustrate the interrelatedness of dynamic developmental processes and thus identify impacts that could occur at later life stages and within other organ systems
  Identification of immediate or delayed responses
Risk characterization
  Define dose–response relationships, especially dose, time, and response relationships
  Characterize potential magnitude of effect, reversibility, repair, functional reserve, etc., of dynamic developmental processes

Thus, the timing and dosimetry relationships shown in Figure 3 become very important for the hazard/risk assessment equation and hence linkage with developmental hazard identification and dose–response assessments.

Examples of the Importance of Life-Stage Considerations

We selected three specific organs or biological systems to evaluate and illustrate distinct life-stage considerations: the immune, respiratory, and nervous systems. These were chosen to illustrate the importance of toxicodynamic considerations for children's risk and were not intended to be comprehensive. Many other organ systems in development would also be important in this context, including but not limited to cardiovascular and endocrine development (Barr et al. 2000).

Figure 4 shows the initial appearance of organ systems during gestation in humans and in rodents. Note that the relative temporal initial development of each organ system, defined by the first appearance of cellular structure of each system, can vary greatly across species.

Respiratory system. Figure 5 shows the temporal development of the respiratory system in humans and in rodents (Pinkerton and Joad 2000). The human respiratory system involves the formation of a highly ordered airway branching system with 25,000 distinct terminations giving rise to more than 300 million alveoli as well as the differentiation and proliferation of over 40 different cell types. The transition of the lungs from a simple protruding bud of tissue from the foregut into a highly organized, integrated, complex structure that is innervated, ventilated, and vascularized is a multi-step process. Obvious from Figure 5 is the fact that although remarkable structural changes occur during the embryonic development such as pseudoglandular, canalicular, and saccular stages of prenatal development, changes to the lungs continue into the postnatal developmental period. Approximately 80% of the alveoli present in the adult lung are formed after birth. Numerous metabolic and biochemical functions of the lungs undergo development and maturation during the postnatal time frame, which includes the proliferative period of the alveolar phase of postnatal lung growth.

Physiologic development of the lungs continues to increase in large measure during the period of alveolar expansion in the postnatal period. The alveolar period of growth also encompasses further development of the airways. Although branching morphogenesis of the bronchial tree is essentially complete at birth, the airways continue to undergo maturation, growth, and expansion through early adulthood.

A number of studies suggest that the processes of cellular differentiation, branching morphogenesis, and overall lung growth can be affected by exposure to chemicals and particles. Both embryogenesis and fetal gestation represent critical periods of cellular differentiation and branching morphogenesis. The effects of exposure, however, are likely to be different for each period of development. For example, during embryogenesis and fetal development, cell number, type, and function of the airways and alveoli may be significantly affected by exposure to a diverse number of substances and/or conditions. However, because cells continue to differentiate and divide during the postnatal period, chemical exposure during the postnatal period is also likely to affect the respiratory system, but in a different manner based on changes in the process of differentiation and morphogenesis (Smiley-Jewell et al. 1998). As growth is essentially complete by the end of adolescence, exposure to chemicals and other factors at this time are likely to have completely different consequences in the adult compared with those found in children (Fanucchi et al. 1997; Plopper et al. 1994; Smiley-Jewell et al. 1998).
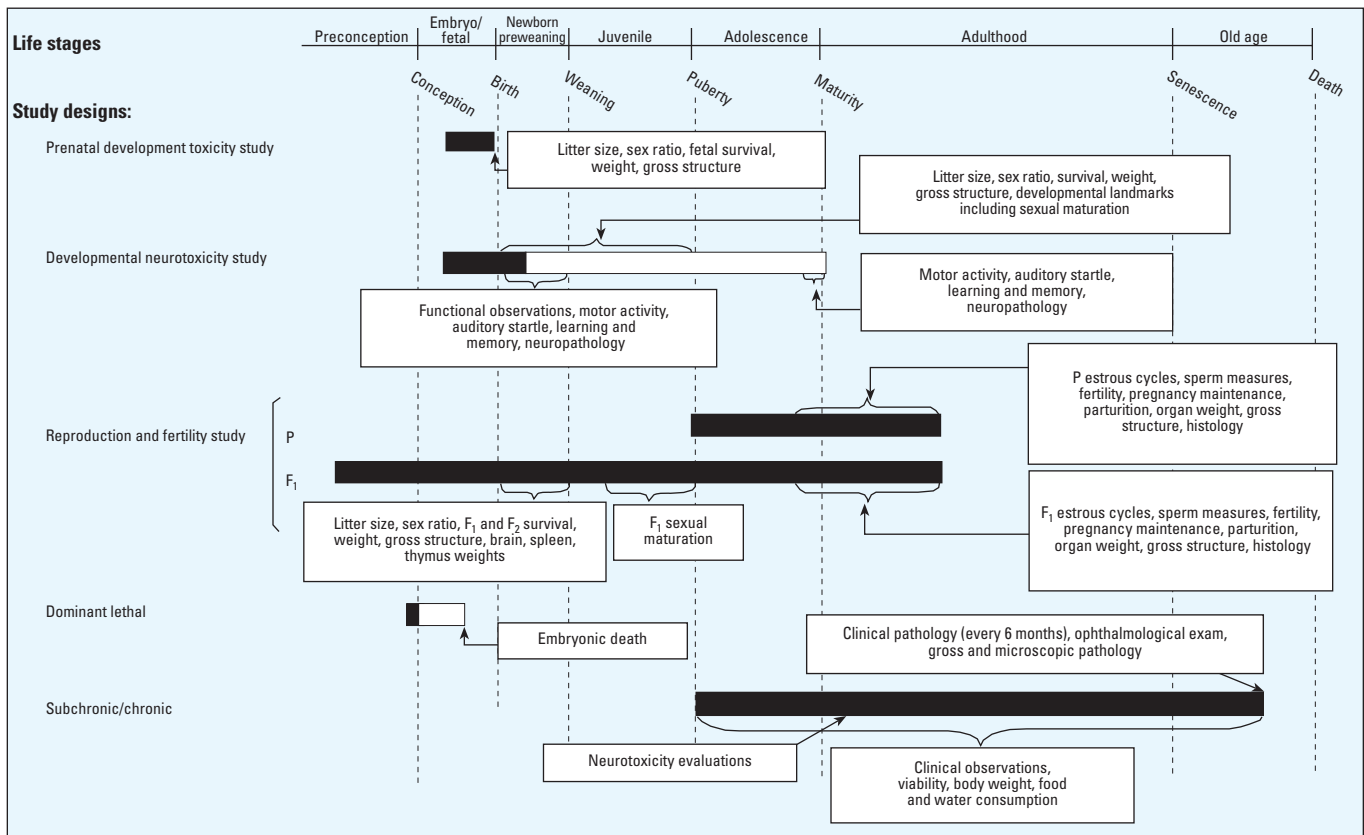

Figure 3. Exposures and end points related to developmental toxicity evaluations. Figure adapted from Kimmel (2001).

Inhalation exposure to substances during critical windows of development may have profound effects that would not be seen if the same exposure were to occur in the adult. Because lung development occurs over the entire prenatal period, exposure effects can have significantly different consequences depending upon whether they occur during the pre- or postnatal period of life. Although our understanding of these changes at this time is extremely limited, one would expect that abnormal developmental changes that occur in the prenatal period because of exposure to a variety of chemicals may have long-term effects persisting into adult life. Examples of altered lung growth or functional deficits in respiration have been shown to result from exposure early in organogenesis to neonatal and adolescent developmental time periods (Pinkerton and Joad 2000). In contrast, structural abnormalities in the lung that result from exposure to environmental toxicants are believed to be manifested only while lung morphogenesis is still occurring, i.e., including the neonatal but not adolescent developmental periods. Very recent studies have suggested that the development of asthma and immune disorders of the respiratory system may result from exposures during organogenesis as well as throughout neonatal and adolescent development (Pinkerton and Joad 2000).

Immune system. The immune system undergoes a number of key changes throughout embryonic, fetal, neonatal, and juvenile development that would be expected to alter the potential risk from environmental exposure to toxicants. Figure 6 shows a comparison of some of these critical changes for immune system development in humans and in rodents. Five specific stages in the development of a mature immune system are illustrated and include initiation of hematopoiesis, migration of stem cells and expansion of progenitor cells, colonization of bone marrow and thymus, maturation to immunocompetence, and establishment of immune memory. These specific stages were chosen as they represent discrete steps in the formation of the mature immune system and also represent periods in which differential vulnerabilities to immunotoxicants would be anticipated (Holladay and Smialowicz 2000). Early embryonic issues concern the location and source of stem cells to seed the primary immune organs such as the thymus. The initiation of hematopoiesis is a benchmark that signals the appearance of cells necessary to sustain immune development. Obviously, exposures occurring before versus after the beginning of hematopoiesis, the migration of stem cells and expansion of progenitor cells, and the emergence of the bone marrow as an important progenitor cell source might lead
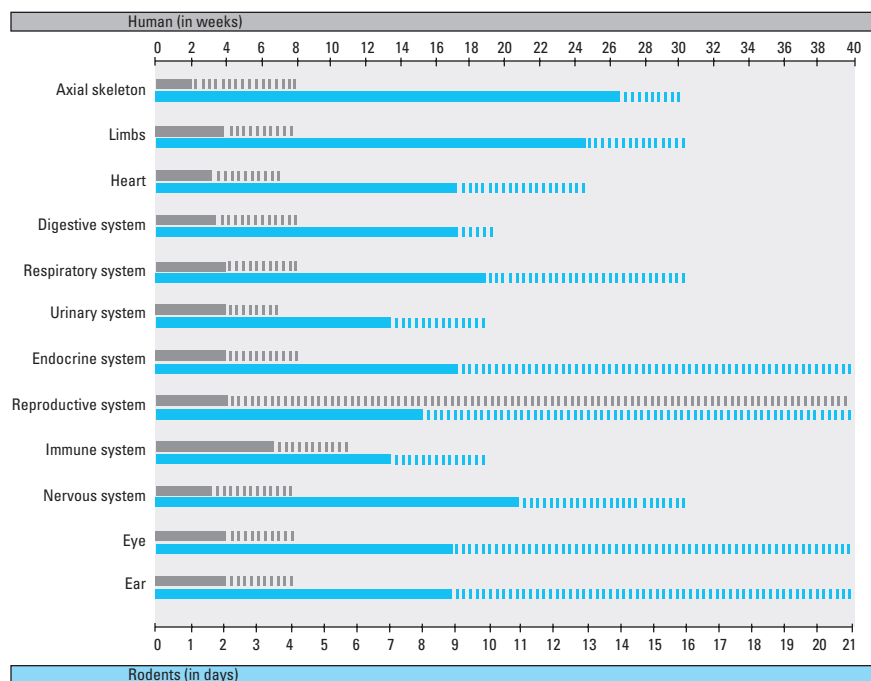

Figure 4. Initial appearance of organ systems during gestation in humans and rodents. A comparative timeline for the initial appearance of cellular structure of the various components of each organ system during gestation is given for humans and rodents. The solid bars represent time to initial appearance, and the hashed bars represent the time window of appearance of these initial organ systems. Note that for some complex organ systems, the appearance of many initial structures within that organ may be over an extended period of development. The connections and maturation for most of these systems continues until after birth for both species. Figure reproduced from Faustman et al. (2003) and reprinted with permission of the Institute for Risk Analyses and Risk Communication at the University of Washington.
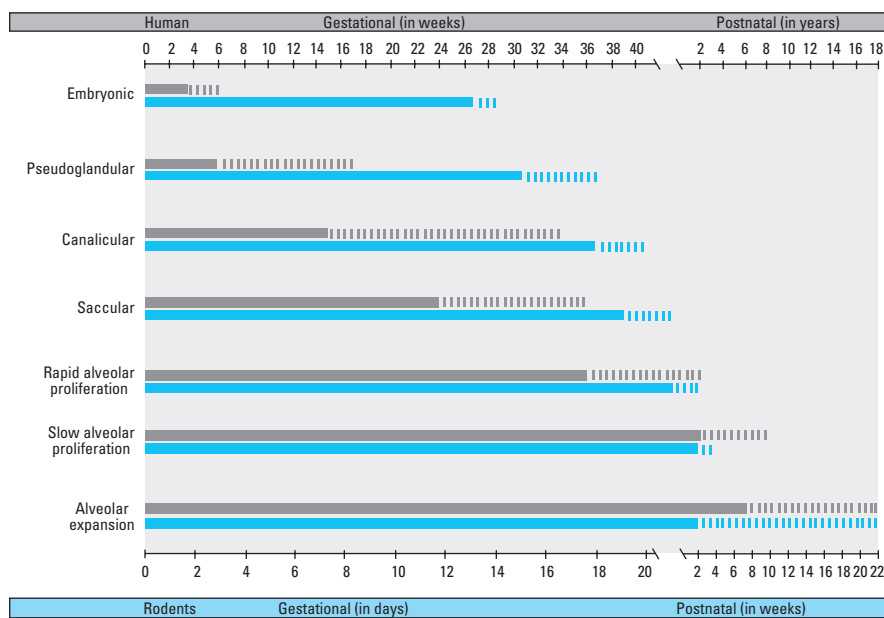

Figure 5. Respiratory system development in humans and rodents. Discrete maturational windows for prenatal and postnatal development are shown using hashed bars and compared for humans and rodents. These discrete maturational windows represent periods in respiratory system development that may have differential vulnerabilities to respiratory toxicants (Dietert et al. 2000; Pinkerton and Joad 2000). The solid bars represent time to these initial maturational stages. Figure reproduced from Faustman et al. (2003) and reprinted with permission of the Institute for Risk Analyses and Risk Communication at the University of Washington.

to differences in the manifestation of impact or outcome. Other benchmarks include the formation and innervation of the thymus as well as the seeding of the thymus by waves of lymphoid cells. Exposures timed such that they target different waves of lymphoid cells important in thymic-dependent T-lymphocyte maturation is another area for potential differential impact. Developmental changes involving peripheral lymphoid organs such as the spleen may also be important for consideration of the timing of exposures compared with risk of immunotoxicity.

These events are all initiated prior to birth in rodents and humans. However, postnatal processes are also important for complete maturation. These immune developmental stages could also provide windows of differential immune sensitivity to toxicants when compared with exposure of the fully matured adult immune system. Among changes occurring largely during the postnatal period in rodents and humans are the maturation to complete immunocompetence and the establishment of immune memory.

An example of differential immune system outcome based on the life stage in which exposure occurred is seen in studies evaluating Pb. The heavy metal Pb is a known immunotoxicant capable of producing numerous immune changes including depression of cell-mediated immunity. A hallmark of Pb-induced immunotoxicity in adult rodents is suppression of the delayed-type hypersensitivity (DTH) response (McCabe et al. 1999). This functional change is likely linked to the capacity of Pb to shift immune response capabilities away from Th1 (helper type-1 T cell)-dependent responses toward Th2-dependent responses (Heo et al. 1997; McCabe and Lawrence 1991). Exposure of rats throughout gestation to levels of Pb that do not alter maternal immune function can produce persistent depression of the DTH response in both juvenile and adult offspring (Bunn et al. 2001a; Chen et al. 1999). However, the timing of exposure appears to be an important factor. Pulsed exposure of dams to Pb during late gestation (days 15–21) results in offspring with depressed DTH response function (similar to the complete gestational exposure). The same Pb exposure earlier in gestation (days 3–9) fails to alter DTH response function in the offspring (Bunn et al. 2001b).

These findings of temporally dependent differential immunotoxic outcome have been extended to other species. For example, exposure of chickens *in ovo* to a single administration of Pb on embryonic day 12 causes depressed DTH function in juvenile chickens. However, exposure of embryos to the same level of Pb only 3 days earlier, producing identical blood Pb levels at hatching, fails to alter DTH function in the offspring (Lee

et al. 2001). It has been hypothesized that the development of the thymus may be key to these differential effects after Pb exposure. This example suggests that comparable environmental exposures during different windows of development have the potential to produce qualitative differences in immune system outcomes.

Nervous system. Figure 7 shows a comparison of nervous system development in humans and in rodents. After neurogenesis each neuronal cell continues to mature through a process of migration, settling to a specific location and extending projections to a designated target site. In many cases such as for the external germinal layer, this process of migration continues well after birth and in the human can continue for 7 months to 2 years after birth. Earliest synapses develop during the embryonic period, and by 10 weeks immature synapses are present. Cortical synapses at birth are still immature and in the human the morphological characteristics of maturity are reached between 6 and 24 months after birth. The full functional maturation of synapses may be related to the elimination of unnecessary synapses. Myelination occurs first in the spinal cord by the end of the first trimester in the human and proceeds in a caudocranial fashion. At birth the brain is immature with regard to the extent of myelination, with prominent myelination present in the brain stem, cerebellar white matter, posterior limb of the internal capsule, thalamus, and the basal nuclei. In the human, the rate of myelin deposition is the greatest in the first 2 years after birth. In the rodent this is comparable to the first 35–40 days of life. Nervous system

malformations can arise from alterations of neurogenesis, changes in the timing of migration, and perturbation of migratory mechanisms and synaptic development.

Excellent examples exist that demonstrate concordance of functional changes in behavior across primates and humans when appropriate age-specific comparisons are conducted (Paule et al. 1988; Slikker et al. 2000). For example, performance of behavioral tasks in humans and primates that are designed to monitor learning, short-term memory, color and position discrimination, time perception, and motivation are indistinguishable. Depending upon task and end point, the behavior of young adult monkeys is identical to that of children over the age range of 4–12 years. Of significance is that performance on many of these tasks is highly correlated with IQ in children (Paule et al. 1999a). Interesting age-related effects have been seen in functional outcomes for the nervous system. For example, chronic marijuana smoke exposure in peripubescent male monkeys resulted in an amotivational-like syndrome similar to that reported for human subjects (teenagers or young adults only—never reported for mature adult humans) (Paule et al. 1999b; Schulze et al. 1988).

Lessons learned from life-stage examples. Several observations can be made based on these examples. First, for each biological system there were multiple windows of susceptibility. Second, windows of susceptibility were frequently identified throughout childhood. In fact, for many cross-species comparisons, birth was a rather arbitrary milestone in the developmental process. Third, the windows of susceptibility were defined upon the basis of distinct
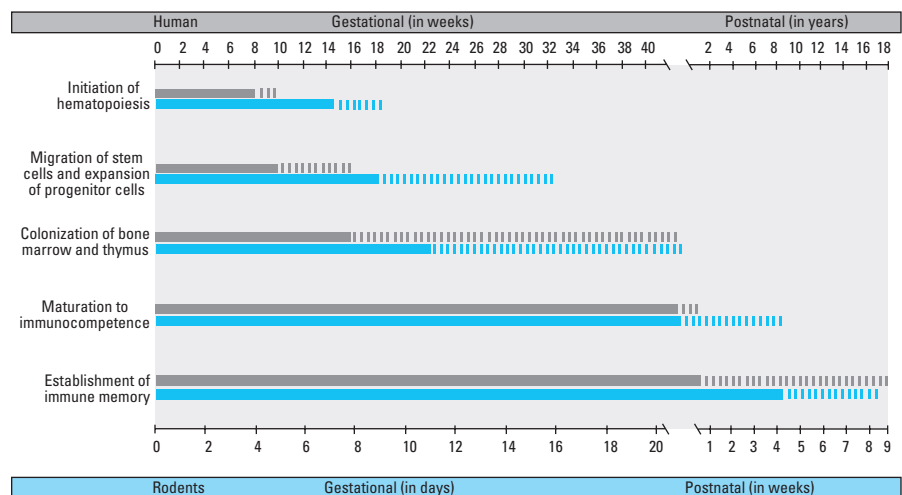

Figure 6. Immune system development in humans and rodents. A comparison of critical stages for immune system development is shown for humans and rodents. Hashed bars represent discrete development steps in the formation of the mature immune system and represent periods where differential susceptibilities to immunotoxicants could be expected (Holladay and Smialowicz 2000). Solid bars show time to "sensitive window" in each species. Figure reproduced from Faustman et al. (2003) and reprinted with permission of the Institute for Risk Analyses and Risk Communication at the University of Washington.

development processes and many of these processes were common across windows for different biological systems (e.g., apoptosis relevant for both neurological and immunological development).

Even with the limited number of systems evaluated, it is apparent that large data gaps exist in the completeness of our understanding of these processes and particularly in identifying which developmental windows represent real or hypothesized windows of susceptibility. The lack of functional data complicates interpretation of animal-to-human comparisons and precludes the translation of developmental observations into public health–relevant end points.

An End Point Example: Cancer

Another way to look at the impact of life stage on toxicity is by focusing on an end point rather than an organ or functional system. Some data from animal studies are available on cancer susceptibility across life stages. In the monograph from a previous ILSI workshop, McConnell (1992) compared outcomes of cancer bioassays conducted at various exposure times throughout gestation. In general, conclusions from such reviews were

that usually the timing of exposures did not affect tumor type (no qualitative differences), but quantitative differences in dose–response relationships and the incidence of tumors were observed. However, a disclaimer was given that there was a limited number of studies, with few chemicals tested in a consistent manner at varying times across gestation and postnatal developmental periods. That observation was essentially reconfirmed by another ILSI RSI working group focusing on research needs (ILSI RSI 1996). Thus, a comprehensive database from which to make a comparative evaluation is still lacking.

An important exception to this generalized statement is the example of acute T-lymphocytic leukemia. A high percentage of these leukemias is due to a V(D)J recombinase-mediated deletion (tal^d) or translocation (t-1:14) that can occur only in the fetus or in children (Finette et al. 1997). Somatic cell gene mutations that arise through an aberrant differentiation process are limited to cell or life stages where the process is normally operative. An example is the V(D)J recombination mechanism that normally functions to rearrange variable (V), diversity (D), and junctional (J) regions of immunoglobulin (Ig) and

T-cell receptor (TCR) genes in B and T lymphocytes, respectively. B cells differentiate in the bone marrow throughout life in humans. However, normal T-cell differentiation is limited to the thymus gland in fetuses and children and is complete by late adolescence. Aberrant functioning of the V(D)J recombinase mechanism may be induced by environmental agents such as passive exposure to tobacco smoke (Finette et al. 1997). This results in rearrangements of genetic segments other than those of the Ig or TCR genes in developing lymphocytes. Some of these aberrant rearrangements constitute the chromosome deletions and translocations that characterize lymphoid malignancies. Thus, qualitative differences in cancer outcome are possible after age-specific alterations (c.f., Olshan et al. 2000).

Developmental Processes and Interactions

Developing tissues and organs, especially during the prenatal stages of life, participate in common complex interactions that permit, encourage, and control cellular processes. These dynamic processes include differentiation, proliferation, migration, secretion, and apoptosis. As an example, Figure 8 shows the temporal differences in one of these processes (proliferation) across various brain regions during nervous system development in the mouse. A different temporal pattern would be seen if apoptosis were plotted against brain regions; in some regions it appears simultaneously with proliferation, and in some brain regions it appears at a later developmental time.

For the most part, those tissue populations that engage in developmentally important interactions share a set of characteristics, which may make them vulnerable to perturbations by outside influences. The set of characteristics includes the following:

- Populations of cells interact, as opposed to individual cells.
- The interacting populations of cells experience developmentally different histories; that is, they experience divergence in their differentiation pathways.
- The interacting populations are in proximity to each other.
- One population of cells (e.g., the inducer) transmits a message of developmental importance (usually considered to be a signal molecule) during a finite period.
- The second (responding) population of cells must be capable of receiving and responding to the signal (i.e., must be competent); the state of competence is maintained for a finite period of time.

The latter three characteristics involve one of the signal transduction pathways. A recent report by the NRC (2000) has identified 17 signal transduction pathways that are
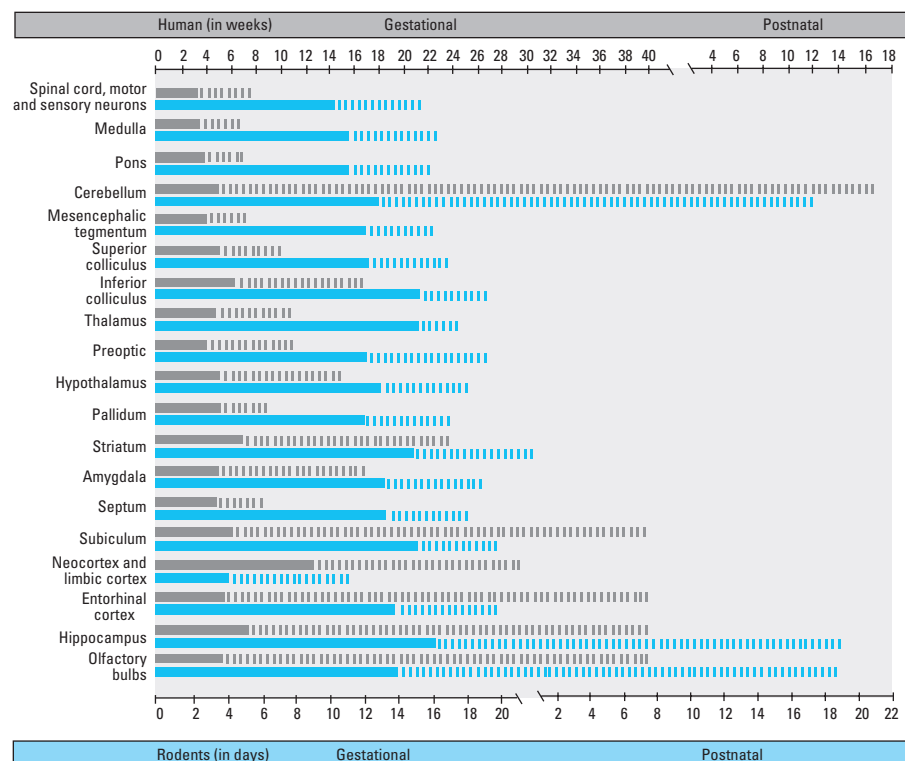

Figure 7. Nervous system development in humans and rodents. Each set of hashed bars represents the time of origin for each structure of the nervous system. Solid bars represent time to structural development for each nervous system structure (Rice and Barone 2000; Rodier 1977). After neurogenesis each cell continues to mature through the process of migration, settling to a specific location and extending projections to designated target sites. In many cases such as for the external germinal layer, this process of migration continues well after birth and in the human can continue for 7 months to 2 years after birth. Figure reproduced from Faustman et al. (2003) and reprinted with permission of the Institute for Risk Analyses and Risk Communication at the University of Washington.

highly evolutionarily conserved across multiple phyla and that appear to be able to explain most if not all relevant signaling pathways in development. The developmental process may be derailed by *a*) disruption of these steps by altering the length of the period for, or timing of, induction or competence so that they are not contemporaneous; *b*) diminishment of the amount of available developmental message; *c*) interference with reception of the message; or *d*) prevention of appropriate activity by the responding tissue. The importance of many of these pathways has been illustrated with genetically sensitized test organisms or transgenic animal models. Because developing tissues and organs rely on such complex, temporally orchestrated interactions (see this orchestration in Figure 8 for just one of these dynamic processes, proliferation), they are exquisitely sensitive to perturbations of their environment. Additionally, because normal development proceeds from a cascade of such orchestrations, developmental processes are far more vulnerable to environmental vicissitudes than are stable, mature tissues. Furthermore, as maturation proceeds, the impact of small environmental challenges becomes increasingly subtle. This contributes to the difficulty in recognizing the effects of environmental challenges on differentiation processes that occur after most gross morphological structures have been established. Impacts of modifications in histological architecture are often manifested as changes in function and as such are more difficult to detect than alterations that occurred early in development, which are often manifested as gross malformations.

The process of development has an inherently dynamic nature, and developing systems possess and exercise multiple signaling pathways simultaneously. Furthermore, many of the pathways exert overlapping functions, especially in mammalian species. As discussed by the National Research Council (2000), this redundancy has contributed to both the plasticity of developing organisms to develop normally after challenges and also has been the reason for failure of some of the knockout models. Consequently, it is important that the developmental consequences of perturbation of any of the signaling pathways be determined and that the changes not be viewed in isolation. In Figure 9, a diagram from the National Research Council 2000 report portrays the fact that such cell-signaling processes occur at the molecular, organelle, and cellular levels but must be put into a broader context of organ, tissue, and conceptual development as well as a kinetic and dynamic context to understand both dose–response relationships and ultimate impacts on developmental outcome.

Application of Information on Life Stage and Toxicodynamics

So how can knowledge of life stage–specific toxicodynamics inform risk assessment for children? First, as illustrated for several systems and end points, an understanding of the timing and cross-species comparison of the developmental processes occurring during various life stages would inform the hazard characterization processes by identifying potentially unique times and organ systems during development. This information could

suggest specific organ systems and functional impacts that might occur if exposures were to occur during those life stages. This information would also suggest the need to evaluate the potential hazard in specific types of animal tests (Figure 3) and would provide some cross-species context for hazard characterization. It could also provide some mechanistic basis for evaluating impacts of the test agent on isolated developmental processes such as apoptosis and differentiation. Of particular importance is that life stage–specific assessment of health effects would more easily allow the assessor to link and evaluate the potential for subsequent functional alterations. Understanding the temporal and physiological interrelatedness of developmental processes would allow the evaluator to better anticipate health impacts in other biological systems and to better forecast or evaluate impacts at later life stages.

Toxicodynamics informs our understanding of toxic mechanisms and mode of action (Faustman et al. 1997, 2000). The case of atrazine illustrates the fact that knowledge of developmental dynamics can make a difference (U.S. EPA 2002). Atrazine is the most commonly detected pesticide in ground and surface water, given the volume of usage and tendency to persist and move with water. The major exposure pathway is through drinking water, and there are episodic peaks of exposure. Other pathways for exposure are through food (minimal) and in residential applications (dermal/inhalation). Atrazine has been shown to cause mammary tumors in Sprague-Dawley rats. Given the endocrine target organ site in the rodent bioassay (i.e., mammary gland tumors), studies were undertaken to determine whether a neuroendocrine mode of action was involved. It was concluded that the mammary tumors in this strain are not relevant for humans. However, the finding of disruption of the neuroendocrine system raised concerns for potential effects on the development and maintenance of the reproductive system. Subsequent studies showed that the compound alters ovarian function (cyclicity), disrupts critical reproductive processes, including delaying puberty [males: postnatal days (PNDs) 23–53; females: PNDs 22–45), pregnancy loss (gestational days 6–10), decreased dam prolactin release, and prostatitis in offspring (PNDs 1–4), and has effects on lactation (milk quality/production). Atrazine is thus a good example of a compound whose mode of action in an animal model was useful in highlighting the need to examine specific potential target organs and life stages.

In addition, toxicodynamics could provide quantitative information relevant for assessing dose response, especially dose and time
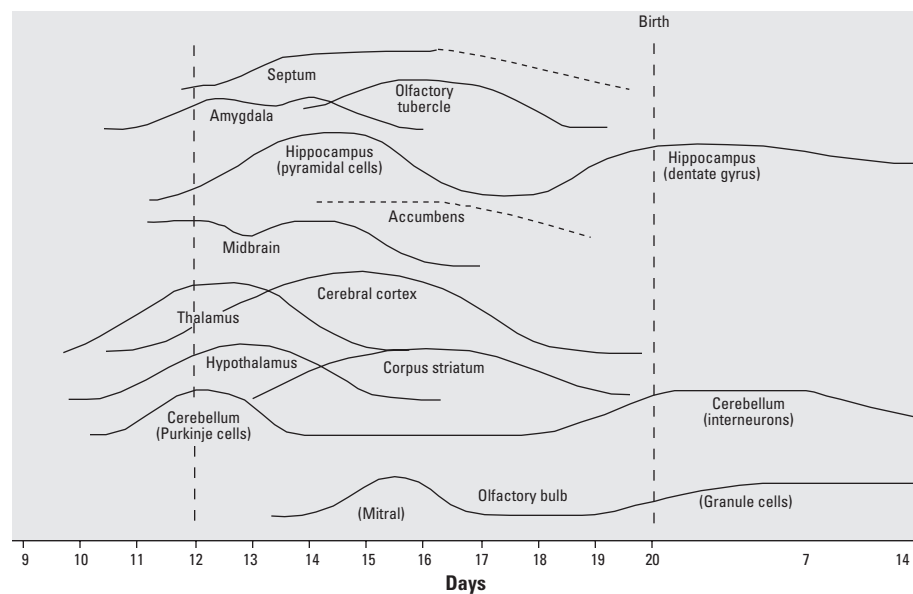

Figure 8. Patterns of neuronal proliferation in specific brain regions of mice. Illustration of overall mouse brain development showing critical windows of peak neuroepithelial cell proliferation (neurogenesis) within specific brain regions and nuclei throughout gestation. Figure reproduced from Rodier (1977) and reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

relationships. It could also begin to inform our assessments of acute versus chronic exposure impacts. It would also provide some context for linking potentially susceptible tissues with kinetic profiles to provide a basis for evaluating kinetic measurements of target-tissue doses. For example, an understanding of the underlying temporal relationships for the dynamic processes occurring in development would inform kinetic measurements such as determining whether AUC or peak *in utero* concentrations of the toxicant or metabolite are more important for our risk analysis.

An example of where life stage-specific exposure information has had an impact on quantitative dose–response assessments is seen in cases of pre-, post-, and neonatal carcinogen exposure. Anderson et al. (2000), for example, summarized published literature for transplacental and neonatal carcinogens by target tissue and time of exposure for chemical and radiation exposures and discussed a number of factors hypothesized as determining susceptibility to carcinogenic insult at different developmental stages. These susceptibility-defining factors include

> *a)* numbers of target cells at risk, *b)* sensitivity to cell killing, *c)* effects of rate of cell division. . . , *d)* ability to repair DNA damage, *e)* expansion of clones of mutated cells. . . , *f)* presence of undifferentiated stem cells, *g)* development of differentiated characteristics, including ability to carry out metabolic activation, *h)* metabolic detoxification by placenta and/or maternal tissues, and *i)* metabolic detoxification by the perinate itself. . . .

The article cites experimental evidence for each of these factors. Increasing understanding of how all these factors can impact qualitative and quantitative tissue and species specificity is needed; however, compelling examples exist for quantitative differences. For example, "in patas monkeys transplacental *N*-ethyl-*N*-nitrosourea caused more tumors than the same dose given to juvenile monkeys, confirming the quantitatively higher sensitivity of the fetuses seen for this chemical in rodents" (Anderson et al. 2000; Rice et al. 1989).

Because tumor incidence determines the slope of the dose–response curve and the Q* value (an upper bound on the slope of a cancer dose–response curve), a Q* derived from an adult animal study will have a flatter slope than that which would be derived from a study that incorporated dosing of the neonatal animal where tumor incidence is higher. The practice of amortizing exposure to a carcinogenic compound has the effect of lowering the much higher dose that children may receive during the first years of life. Taken together, the use of a Q* value that does not accurately represent the slope of the dose–response curve for young animals and the effect of amortizing children's doses may

result in a cancer risk assessment that is not adequately protective of children.

Critical Data Needs

Some specific data needs and questions are highlighted here as being particularly critical to an improved application of toxicodynamic principles in assessing risks for the developing human. These critical data needs include

- An improved understanding of the meaning (significance) of subtle effects (biomarkers) and validation of their relevance for risk assessment
- Ability to link assessments to more robust functional end points
  - Development of more end points for assessing system function that can be used in both humans and animals. This is a major issue, as the absence of these tools is a huge impediment to actually assessing the effects of exposure. Imaging techniques could be very valuable as well, especially functional magnetic resonance imaging.
  - Resources for animal-to-human correlation
- Better understanding of the toxicodynamics of public health–relevant end points, such as asthma and cardiovascular disease
- Comparison of the toxicodynamic links between the effects of acute, subchronic, and chronic exposures
- Better characterization of the development of homeostatic set points for many physiological systems
- Better understanding of repair, tolerance, and hypersensitivity in animal and human responses
- More and better diagrams across life stages
  - There needs to be a concerted effort in comparative biology/physiology to develop tables or other references for easily identifying analogous periods in development across species.

- More epidemiology studies that encompass multiple life stages (including early/developmental periods).

In addition, there is a need for multidisciplinary training for work in children's health at all levels (graduate students, postdoctoral researchers, scientists in the field) and for more multidisciplinary workshops and interactions on the assessment of children's health risks.

Summary

There are distinct life stages evident across development, with both known and hypothesized windows of susceptibility. These various life stages are based on differences in development defined by differences in relevant dynamic processes occurring at the molecular, cellular, organ, and physiological level, and these differences may define in what systems and at what magnitude an environmental impact will be manifested. There can be apparent species differences in response to environmental exposures if the dynamic processes are not compared at equivalent doses and time points across species.

Differences in developmental dynamic processes can impact all stages of the proposed children's risk assessment framework, as well as all components of the traditional risk assessment paradigm. Common dynamic processes can impact susceptibility. For example, consideration of impacts on apoptosis versus migration could provide clues as to what biological systems may be affected and at what times these impacts would be identifiable. There are also implications for animal testing, and some ways to improve our understanding of dynamic processes across species, dose, and life stage have been discussed. Finally, there is a need to improve our assessment of functional and public health–relevant effects in our testing approaches.
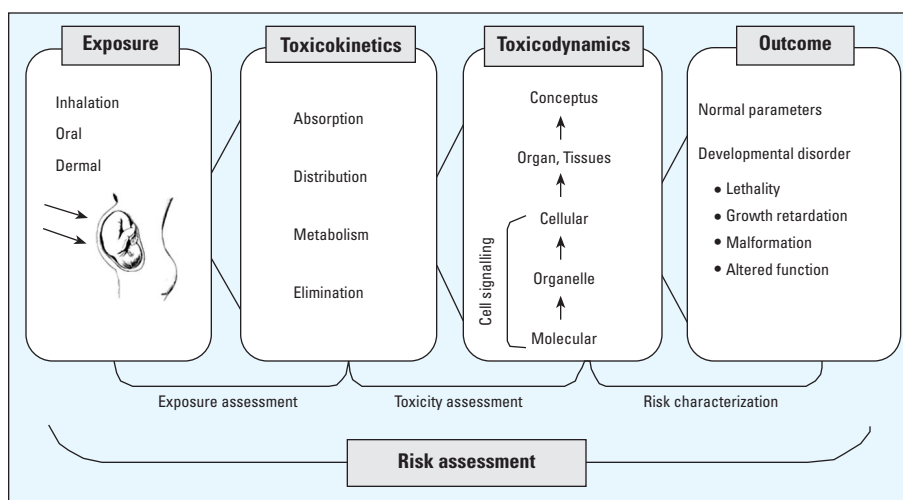

Figure 9. Levels of mechanistic inquiry for assessing the effects of a toxicant on development. Figure reproduced from NRC (2000) and reprinted with permission by the National Academy of Sciences, courtesy of the National Academies Press.

Risk Characterization and the Framework

Risk characterization, as the final step in the process of assessing children's risks, cannot succeed unless the inputs from the preceding steps are appropriately directed at the problems associated with risks during childhood.

Our definition of the life stages encompassed in the term "childhood" is broader than a dictionary definition. It encompasses not only life after birth but also embryonic and fetal development. Our definition is rooted in the concept that special risks to children are the result of actions of toxicants on developmental processes, leading to different mechanisms and/or manifestations of toxicity than in adults. These unique mechanisms and outcomes arise because the individual is developing; birth, while significant, does not mark the end of development or of the capacity for an agent to produce permanent, organizational effects on function. Therefore, from the context of developmental biology and toxicology, children's risk assessment is really developmental risk assessment, and as such must include the developmental stages that take place before birth.

There is abundant literature spanning many decades demonstrating the unique susceptibility of the embryonic period to structural teratogens. The thalidomide tragedy of the late 1950s and early 1960s demonstrated to the world that an agent can have radically different effects in the embryo than in the adult and that these effects may be permanent. Research on the developmental toxicity of lead or ethanol, to name two examples, demonstrates that the fetal and neonatal periods are also sensitive, with manifestations of toxicity being largely functional in nature with few obvious structural correlates. Epidemiologic evidence indicates that early menarche increases the risk for breast cancer; there is the potential for agents with estrogenic activity to accelerate puberty and, presumably, the risk for later effects. These are just a few examples of the unique susceptibilities of developing life stages (c.f., Landrigan et al. 2004).

But are these susceptibilities the result of mechanisms of toxicity that are themselves unique to the developing organism? There are clear examples in which the outcomes of exposure are radically different in developing life stages than in adults, so much so that the nature of the outcomes could not be predicted from observations in adults or experiments in mature animals. Whether these are attributable to different mechanisms of action is unanswerable at this point because we have too little information about toxic mechanisms, particularly during development. It is reasonable to assume that there probably are mechanisms of action specific to development,

whereas in other cases the mechanism may be the same as in adults but with a different outcome. For example, it appears clear that the effects of retinoic acid are mediated through retinoic acid receptors in embryos and adults, but the teratogenic outcome of retinoic acid exposure in the embryo is not at all similar to adult intoxication. Because of this, the possibility of unique developmental outcomes makes the problem of children's hazard identification and risk characterization an important one, irrespective of whether the mechanisms of action of a toxicant are the same as in an adult.

In the proposed framework for children's risk assessment (Figure 1), the problem formulation step focuses on the interrelationships among exposures, effects, and host factors. These considerations are consistent with the way epidemiologic data are collected and directly feed into the hazard characterization and exposure assessment steps of the classical risk assessment paradigm. Host considerations will include the life stages of concern in the assessment and also any factors that are specific to a given situation, namely, genetic, nutritional, or socioeconomic factors that may influence biological response or extent of exposure.

The end result of problem formulation is the development of a conceptual model describing the problem and indicating the possible risk assessment options. This model then guides the analysis phase. The analysis phase consists of characterizing life stage–specific exposures and health effects, namely, the content of exposure assessment and hazard characterization. The linkage between these two is a consideration of timing of development and exposure, and dosimetry. Toxicodynamics forms the basis for life stage–specific hazard characterization, and toxicokinetics is the underpinning for characterization of the timing of target-tissue exposures and dosimetry.

The purpose of the analysis phase is to produce an adequate basis for risk characterization. It is possible for the analysis phase to fail because of an inadequate conceptual model, in which case the framework allows for iterative refinement of the model and reentry into the analysis phase.

Risk characterization consists of a life stage–specific consideration of risk combined with uncertainty and variability analyses, which culminates in a narrative statement describing the nature of the risk, its likelihood under specific scenarios, and the degree of uncertainty in and confidence in the assessment. Risk assessors need to be explicit in categorizing uncertainty and variability and evaluate the use of uncertainty factors versus other methods for estimating and incorporating variability into the assessment.

The most straightforward way to determine whether there are differences between adult and developmental responses to an agent is to test for developmental effects in appropriate models and to acquire life stage–specific exposure information. The question of whether an additional uncertainty factor should be used is one that must be determined on a case-by-case basis, using a weight-of-evidence evaluation of existing data. If there are no data, or the database is deficient, then this uncertainty needs to be addressed, for example, by generating more data or by using the uncertainty factor already in place at the U.S. EPA for accommodating database deficiencies. The magnitude of the uncertainty factor(s) depends on a variety of factors associated with the database and should be assigned using a weight-of-evidence approach. Chronic, cumulative, or irreversible effects tend to be of greater concern.

Of course, there can be multiple sources of child-specific uncertainty. These can include anything from the comprehensiveness of the exposure and toxicology databases to the appropriateness of the animal model used or the strength of epidemiologic data. Only by learning more about human biology, including the potential range of responses and more about the capacity (and limitations) of animal and other experimental models to predict effects in humans, can we expect to alleviate the uncertainty represented by the uncertainty factors used in risk assessment. It should be possible to develop minimum criteria to support a good risk assessment (Moore et al. 1995). Certainly, it needs to be acknowledged that additional data may increase or decrease the reference dose for a compound. Finally, it should be noted that there are already policies in place to accommodate database deficiencies.

In the end, it is clear that the full spectrum of potential developmental effects cannot be predicted from adult data; therefore, a core data set in developing organisms is needed. Adequacy of the data set to assess the potential for risk to children should be determined after existing data on exposure (both known and anticipated scenarios) and effects are described and summarized. The overall vision for risk characterization is a meaningful, life stage–linked probabilistic calculation of risk.

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