FINAL REPORT

Workshop to Develop A Framework for Assessing Risks to Children from Exposures to Environmental Agents

ILSI Risk Science Institute
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FINAL REPORT: WORKSHOP TO DEVELOP A FRAMEWORK FOR ASSESSING RISKS TO CHILDREN FROM EXPOSURES TO ENVIRONMENTAL AGENTS

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ABOUT ILSI AND THE ILSI RISK SCIENCE INSTITUTE

The **International Life Sciences Institute (ILSI)** is a nonprofit, worldwide foundation established in 1978 to advance the understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment. By bringing together scientists from academia, government, industry, and the public sector, ILSI seeks a balanced approach to solving problems of common concern for the well-being of the general public.

ILSI is affiliated with the World Health Organization as a nongovernmental organization and has specialized consultative status with the Food and Agriculture Organization of the United Nations.

ILSI is headquartered in Washington, DC. ILSI branches include Argentina, Australasia, Brasil, Europe, India, Japan, Korea, Mexico, North Africa and Gulf Region, North America, North Andean, South Africa, South Andean, Southeast Asia, Thailand, the Focal Point in China, the ILSI Health and Environmental Sciences Institute, and a study group in Central America. ILSI receives financial support from industry, government, and foundations.

Because science is the base of ILSI's achievements, the ILSI Research Foundation was formed in 1984 to create a vehicle for ILSI to support basic scientific research in underfunded and unexplored areas. The **ILSI Risk Science Institute (RSI)**, a component of the ILSI Research Foundation, seeks to advance and improve the scientific basis of risk assessment. ILSI RSI serves as a catalyst for consensus on complex scientific issues in risk assessment by facilitating discussion and cooperation among scientists from all sectors.

ACKNOWLEDGMENTS

This report is the product of two years' effort by many individuals, who are identified in the Introduction. We note, in particular, the key roles played by the Workshop Planning Committee, the breakout group chairs and rapporteurs, and the authors of the background papers. We thank the 45 workshop participants and 9 observers for the enthusiasm and collegiality with which they accomplished their task of developing a framework for assessing children's risks. Special thanks go to two observers, Dr. LaRonda Morford (Eli Lilly & Co.) who assisted with rapporteuring the Toxicodynamics breakout group and Dr. Nancy Beck (EPA/ORD/NCEA AAAS Fellow) who helped with the preparation of the Toxicodynamics chapter.

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ILSI Risk Science Institute staff support in the organization and conduct of the workshop was ably provided by Stephanie Carter, Diane Dalisera, and Nanette Thompson, and Audrey Ray and Rita Johnson assisted with word processing in preparation of the report. Finally, the encouragement, counsel, and active participation in this project of Dr. Penny Fenner-Crisp, ILSI RSI Executive Director, is also gratefully acknowledged.

Dr. Stephen Olin, Workshop Chair
Deputy Director
ILSI Risk Science Institute

Dr. Babasaheb Sonawane, Workshop Co-chair
Chief, Effects Identification and
Characterization Group, EPA/ORD/NCEA

The views expressed in this report are those of the individual authors and workshop participants and do not necessarily reflect the views of ILSI, EPA, or any other organization. Mention of trade names of commercial products does not constitute endorsement or recommendation for use.

EXECUTIVE SUMMARY

Under a cooperative agreement with the EPA/ORD National Center for Environmental Assessment, the ILSI Risk Science Institute organized and convened a workshop July 30 – August 2, 2001 to develop a framework for assessing risks to children from exposure to environmental agents. The 45 invited participants and 9 invited observers, drawn from government, academia, industry and the public health community, represented a wide diversity of fields of expertise, ranging from developmental biology/toxicology and pediatrics to pharmacokinetics and risk assessment.

The conceptual framework created by the workshop (Figure II-1, p. 8) is based on the Problem Formulation → Analysis → Risk Characterization paradigm as applied to early life stage exposures. It recognizes the potential significance of the timing of exposures in relation to the susceptibility of the developing human, from the perspective of both toxicokinetics and toxicodynamics. It offers a systematic approach to the consideration of the factors that may influence risk during development, from conception through organ maturation (in adolescence). It acknowledges that the complexity and unique insights of a risk assessment focusing on early life stages will depend critically on the data available and the scope of the assessment.

Among the conclusions from the workshop were the following:

- There are distinct life stages during development with both known and hypothesized 'windows of susceptibility' in humans and experimental animal models. These developmental life stages are defined by differences in relevant kinetic and dynamic processes occurring at the molecular, cellular, organ, and physiological level. Interspecies comparisons must consider differences in life stages and kinetic and dynamic processes, including timing and dosimetry.

- In addition to considerations of intrinsic sensitivity of the developing human, life stage-specific behaviors, activity patterns, functions, and intakes often can lead to dramatic differences in exposures. Life stage-linked exposure assessment is a critical component of any children's environmental health risk assessment.

- In Problem Formulation, in the context of the proposed framework, defining the overall scope and objectives of the risk assessment is important for the initial assessment of life stages, exposure scenarios, and toxic effects to be considered.

- Problem Formulation produces a conceptual model of the likely key relationships between exposures and effects of the environmental agent(s) on 'host' (exposed) populations, informed by the initial identification of exposure scenarios, exposed life stages, and the known or anticipated biological effects of the environmental agent(s). The conceptual model for the risk assessment arises from and guides the collection of data in preparation for the Analysis phase.

- Toxicokinetic considerations in Analysis include agent/chemical-specific factors and life stage/age-specific factors, both of which can include effects on absorption, distribution, metabolism and excretion. Examination of these factors may reveal one or more age groups of particular toxicokinetic concern.

- Toxicodynamic considerations in Analysis include the identification of uniquely susceptible dynamic processes of concern and the functional consequences of altering these processes, and consideration of available data that may indicate differential toxicity from exposures during susceptible periods.

- Analysis of the timing of development and exposures and of the dosimetrics of the agent (including both kinetic and dynamic factors) links the characterization of life stage-specific exposures with life stage-specific effects.

- Risk Characterization for early life stage exposures may be qualitative (e.g., when quantitative data are lacking or a quantitative analysis is unnecessary) or quantitative (e.g., incorporating a PBTK or BBDR life stage-specific model) or some other semi-quantitative assessment.

- The full spectrum of potential developmental effects cannot be predicted from data on exposed adults. A core data set from studies in developing organisms is essential.

- The workshop cited a number of data resources to support the risk assessment approach outlined in the framework, including the papers developed for the workshop (Appendices 1-3), and identified critical research needs for improved assessments.

I. INTRODUCTION

"Children are not simply small adults but rather are a unique population for health risk assessment." So begins the summary of the ILSI Risk Science Institute's conference on Similarities and Differences Between Children and Adults: Implications for Risk Assessment, convened in Hunt Valley, Maryland in November 1990 (Guzelian et al, 1992). That conference summary also recognized the need for further work on the "specific application of the information presented at this conference to risk assessment methodologies," thus setting the stage for the workshop that is the subject of this report.

Nearly 11 years after the 'Similarities and Differences' conference, the ILSI Risk Science Institute (RSI) held a workshop in Stowe, Vermont, July 30-August 2, 2001 to develop a framework for assessing risks to children from exposure to environmental agents. The 45 invited participants and 9 invited observers, working in three breakout groups, focused on toxicokinetics, toxicodynamics, and risk characterization and drafted a structured approach to identifying and assessing potential risks from exposures occurring from conception through organ maturation (in adolescence). The approach is based on the problem formulation → analysis → risk characterization paradigm that has been incorporated in many risk assessment frameworks over the past decade, and is depicted in a schematic diagram (Figure II-1).

This report and its appendices comprise the primary work product from the workshop. A series of manuscripts presenting the technical content of the report and the complementary, authored background papers (included here as Appendices 1-3) also have been submitted for publication in the peer-reviewed journal *Environmental Health Perspectives.*

Preparation for the Workshop

In 1999 ILSI RSI was awarded a cooperative agreement with the National Center for Environmental Assessment (NCEA), Office of Research and Development, U.S. Environmental Protection Agency, after responding to a competitive Request for Applications. ILSI RSI and NCEA agreed that a major focus of the cooperative agreement would be methodologies for assessing children's risks, and in March 2000 ILSI RSI convened a scoping meeting to provide input on a possible workshop to develop a framework for children's risk assessment. Participating in the meeting were Dr. Bob Sonawane, Dr. Bruce Rodan, and Dr. Carole Kimmel (EPA/ORD/NCEA), Dr. Michael Firestone (EPA Office of Children's Health Protection), Dr. Michael Bolger (FDA CFSAN), Dr. Terri Damstra (WHO/ILO/UNEP International Programme on Chemical Safety), Dr. Joseph Scimeca (Pillsbury, and Chair, ILSI N.A. Food Toxicology and Safety Assessment Committee), and Dr. Stephen Olin and Dr. Isabel Walls (ILSI RSI). This committee agreed with the concept of a workshop to include both presentations and breakout groups, recognized the need for background papers prepared before the workshop, and noted the desirability of obtaining broad-based funding support. The committee also provided ILSI RSI with nominations for a Workshop Planning Committee.

In August 2000, ILSI RSI held the first meeting of the 16-member Workshop Planning Committee (see inset). Over the course of the next nine months, including a second meeting in January 2001 and many conference calls, the Planning Committee:

WORKSHOP TO DEVELOP A FRAMEWORK FOR ASSESSING RISKS TO CHILDREN FROM EXPOSURE TO ENVIRONMENTAL AGENTS

WORKSHOP PLANNING COMMITTEE

Dr. Sherlita Amler*	ATSDR/CDC
Dr. William Breslin	Eli Lilly & Company
Dr. Adolfo Correa	Center for Environmental Health/CDC
Dr. George Daston	The Procter & Gamble Company
Dr. Karen Davis-Bruno	CDER/FDA
Dr. Brenda Eskenazi	University of California, Berkeley
Dr. Elaine Faustman	University of Washington
Dr. Gary Ginsberg	Connecticut Department of Public Health
Dr. Daniel Goldstein	Monsanto Company
Dr. Susan Kess*	ATSDR/CDC
Dr. Carole Kimmel	NCEA/ORD/EPA
Dr. Philip Landrigan	Mount Sinai School of Medicine
Dr. Stephen Olin	ILSI Risk Science Institute
Dr. Bill Slikker, Jr.	Nat'l Ctr. for Toxicological Research/FDA
Dr. Ralph Smialowicz	NHEERL/EPA
Dr. Bob Sonawane	NCEA/ORD/EPA
Dr. Tom Trautman	General Mills

* Dr. Amler replaced Dr. Kess in late spring, 2001

- Developed an Outline for a Framework for Assessing Children's Risks, including a first draft of the schematic diagram
- Prepared four background papers for the workshop on:
  - Children's Health and the Environment: Public Health Issues and Challenges for Risk Assessment

- Hazard Identification and Predictability of Children's Health Risk from Animal Data
- Incorporating Children's Toxicokinetics into a Risk Framework
- Risk Assessment Practices and Challenges for Protecting Children's Health

- Generated focus questions for the workshop and for each of the breakout groups (see Appendix 4)
- Nominated experts in the key scientific disciplines and assisted ILSI RSI with the assignment of participants to breakout groups
- Worked with ILSI RSI on the program for the workshop.

In addition, all members of the Workshop Planning Committee were invited to participate in the workshop, and most were able to do so.

The Workshop

Workshop participants were selected principally for their scientific expertise and experience. Among the areas of expertise represented were developmental biology and toxicology (neuro, repro/developmental, immuno, pulmonary, general), pediatrics, genetics, epidemiology, pharmacokinetics, modeling, exposure assessment, and risk assessment. Participants were drawn from government, academia, industry and the public health community. Workshop participants and observers with their affiliations at the time of the workshop are listed in the inset (p. 4). Their current contact information is given in Appendix 5.

The workshop began with dinner on July 30, and concluded mid-afternoon on August 2, 2001. The workshop program is in Appendix 6. Dr. Olin (ILSI RSI) chaired the workshop, with Dr. Sonawane (EPA/ORD/NCEA) as co-chair. Dr. Landrigan set the stage with a presentation after dinner on the first evening. The next morning Dr. Knott summarized the conclusions from the EPA Risk Assessment Forum Technical Workshop on Issues Associated with Considering Developmental Changes in Behavior and Anatomy when Assessing Exposure to Children, held in July 2000 (U.S. EPA Risk Assessment Forum, 2000), and on the second morning of the meeting informal presentations of case studies were given by Dr. Dellarco, Dr. Clewell, and Dr. Davis-Bruno to encourage discussion of the applicability of the framework. Dr. Olin presented the charge to the workshop on the first morning, delineating the workshop scope and objectives. He noted that there were some topics that were specifically NOT included in the scope: regulatory policies, evaluation of individual chemicals and critique of prior risk assessments, and he also noted that a detailed consideration of exposure-related issues was not planned in view of the prior work of the EPA Risk Assessment Forum.

WORKSHOP TO DEVELOP A FRAMEWORK FOR ASSESSING RISKS TO CHILDREN FROM EXPOSURE TO ENVIRONMENTAL AGENTS

July 30 – August 2, 2001 – Stowe, VT

PARTICIPANTS

Names	Affiliations
John Adgate	Univ. of Minnesota
Richard Albertini	Univ. of Vermont
Sherlita Amler	ATSDR
Hugh Barton	EPA/ORD/NHEERL
Matthew Bogdanffy	DuPont Haskell Laboratory
William Breslin	Eli Lilly & Co.
James Bruckner	Univ. of Georgia
Bob Chapin	DuPont Pharmaceutical
Harvey Clewell	ICF Kaiser/KS Crump Group
Elaine Cohen Hubal	EPA/ORD/NERL
Adolfo Correa	CDC
George Daston	Procter & Gamble
Karen Davis-Bruno	FDA/CDER
Vicki Dellarco	EPA/OPP
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Rodney Dietert	Cornell Univ.
Joyce Donohue	EPA/OW
Brenda Eskenazi	Univ. of California, Berkeley
Elaine Faustman	Univ. of Washington
Penny Fenner-Crisp	ILSI Risk Science Institute
Gary Ginsberg	Conn. Dept. of Public Health
Daniel Goldstein	Monsanto
Jean Harry	NIEHS
Dale Hattis	Clark University
Robert Kavlock	EPA/ORD/NHEERL
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Gary Kimmel	EPA/ORD/NCEA
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Phil Landrigan	Mt. Sinai School of Medicine
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Bette Meek	Health Canada
Stephen Olin*	ILSI Risk Science Institute
Merle Paule	FDA/NCTR
Kent Pinkerton	Univ. of California, Davis
Jennifer Seed	EPA/OPPT
Larry Sheets	Bayer Corp.
Michael Shelby	NIEHS
Wayne Snodgrass	Univ. of Texas Medical Ctr.
Diana Somers	PMRA/Canada
Bob Sonawane**	EPA/ORD/NCEA
Tom Trautman	General Mills
Isabel Walls	ILSI Risk Science Institute
Tracey Zoetis	Milestone Biomedical Associates

* Workshop Chair ** Workshop Co-Chair

OBSERVERS

Names	Affiliations
Robert Amler	ATSDR
Nancy Beck	EPA/ORD/NCEA (AAAS Fellow)
Richard Becker	American Chemistry Council
Terri Damstra	WHO/IPCS
Michael Firestone	EPA/OCHP
Steven Knott	EPA/ORD/NCEA
Ray McAllister	American Crop Protection Association
LaRonda Morford	Eli Lilly & Co.
Vanessa Vu	EPA/OPPTS/OSCP

Most of the time in the workshop was devoted to the work of the breakout groups. Participant assignments to breakout groups are shown in the inset. Breakout group chairs and rapporteurs are also identified. Observers were free to sit in on any of the breakout groups.

WORKSHOP TO DEVELOP A FRAMEWORK FOR ASSESSING RISKS TO CHILDREN FROM EXPOSURE TO ENVIRONMENTAL AGENTS		
Toxicokinetics	**Toxicodynamics**	**Risk Characterization**
Chair: Gary Ginsberg **Rapporteur:** James Bruckner **Breakout Group:** • Hugh Barton • Matthew Bogdanffy • Harvey Clewell • Karen Davis-Bruno • Dale Hattis • Dan Krewski • Kannan Krishnan • Stephen Olin • Wayne Snodgrass • Bob Sonawane	**Chair:** Elaine Faustman **Rapporteur:** William Breslin **Breakout Group:** • Richard Albertini • Adolfo Correa • John DeSesso • Rodney Dietert • Joyce Donohue • Jean Harry • Robert Kavlock • Gary Kimmel • Bruce Lanphear • Merle Paule • Kent Pinkerton • Jennifer Seed • Michael Shelby • Diana Somers • Tom Trautman • Isabel Walls	**Chair:** George Daston **Rapporteur:** Penny Fenner-Crisp **Breakout Group:** • John Adgate • Sherlita Amler • Bob Chapin • Vicki Dellarco • Brenda Eskenazi • Daniel Goldstein • Elaine Cohen Hubal • Carole Kimmel • Phil Landrigan • Melanie Marty • Bette Meek • Larry Sheets • Tracey Zoetis

The breakout group chairs (Drs. Ginsberg, Faustman, and Daston) and their rapporteurs were the keys to the success of the workshop, as they moved their respective groups through their tasks. With their leadership and the active participation of the breakout group members, the framework drafted by the Workshop Planning Committee was discussed, further developed and modified, and adopted by the workshop participants; the focus questions were addressed;

concepts, insights, conclusions and recommendations developed in breakout groups were presented and discussed in plenary sessions and revised, as appropriate, by the breakout groups; and critical data needs for improving the assessment of children's risks were identified.

The Report

Following the workshop, the chairs and rapporteurs, in collaboration with their respective breakout groups, prepared the breakout group reports. Drafts were circulated by the chairs to the breakout group members for comment and input, and the overall workshop chair assembled and edited the report. The full report was then circulated to all workshop participants and observers for comment, comments were addressed by breakout group chairs and background paper authors, and this final workshop report was prepared.

Chapters II and V of the report were drafted by the Risk Characterization breakout group. Chapter II presents the framework developed by the workshop participants, as informed by the extensive discussions in all three breakout groups and in plenary sessions during the workshop. Chapter V summarizes the Risk Characterization group's discussions. Chapters III and IV are the products of the Toxicokinetics and Toxicodynamics breakout groups, respectively.

Three of the four background papers for the workshop were further developed and modified by the authors after the workshop and are included in this report as Appendices 1-3. Although prepared specifically for the workshop and used and discussed extensively at the workshop, these papers have remained the work products of their respective authors. They are included as appendices to the report because they complement, support, and enhance the report and the framework for assessing children's risks.

This report with its appendices will be posted on the ILSI website at: http://www.ilsi.org/committees/rsi/childrensriskassessmentwkshpreport.pdf .

II. A PROPOSED FRAMEWORK FOR ASSESSING RISKS TO CHILDREN FROM ENVIRONMENTAL EXPOSURES

Over the past decade there has been a dramatic increase 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.

The objective of the ILSI Risk Science Institute 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. Detailed consideration of issues related to exposure assessment are being addressed elsewhere (e.g., U.S. EPA Risk Assessment Forum 2000). In the workshop and in this report, the term 'children' was defined to include humans from conception through organ maturation (in adolescence). 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 as necessary.

The Framework for Assessing Risks to Children from Exposure to Environmental Agents, as developed in this workshop, incorporates many of the principles and elements of other frameworks and risk assessment guidance developed by the U.S. Environmental Protection Agency over the past decade (e.g., U.S. EPA Risk Assessment Forum 1992; U.S. EPA 1997). It is responsive to, and consistent with, the directives articulated in the EPA Administrator's children's risk assessment policy guidance (U.S. EPA 1995a) and Executive Order 13045 (April, 1997).

The proposed Framework, presented in Figure II-1, is broadly analogous to frameworks previously established by U.S. EPA for use in its risk assessment/risk management process (RA/RM). 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 RA/RM 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 II-2. The integrated process presumes that before any significant effort is made to conduct a risk assessment, a planning and scoping exercise has been carried out to assure an

Figure II-1. Proposed Framework for Assessing Risks to Children from Exposure to Environmental Agents

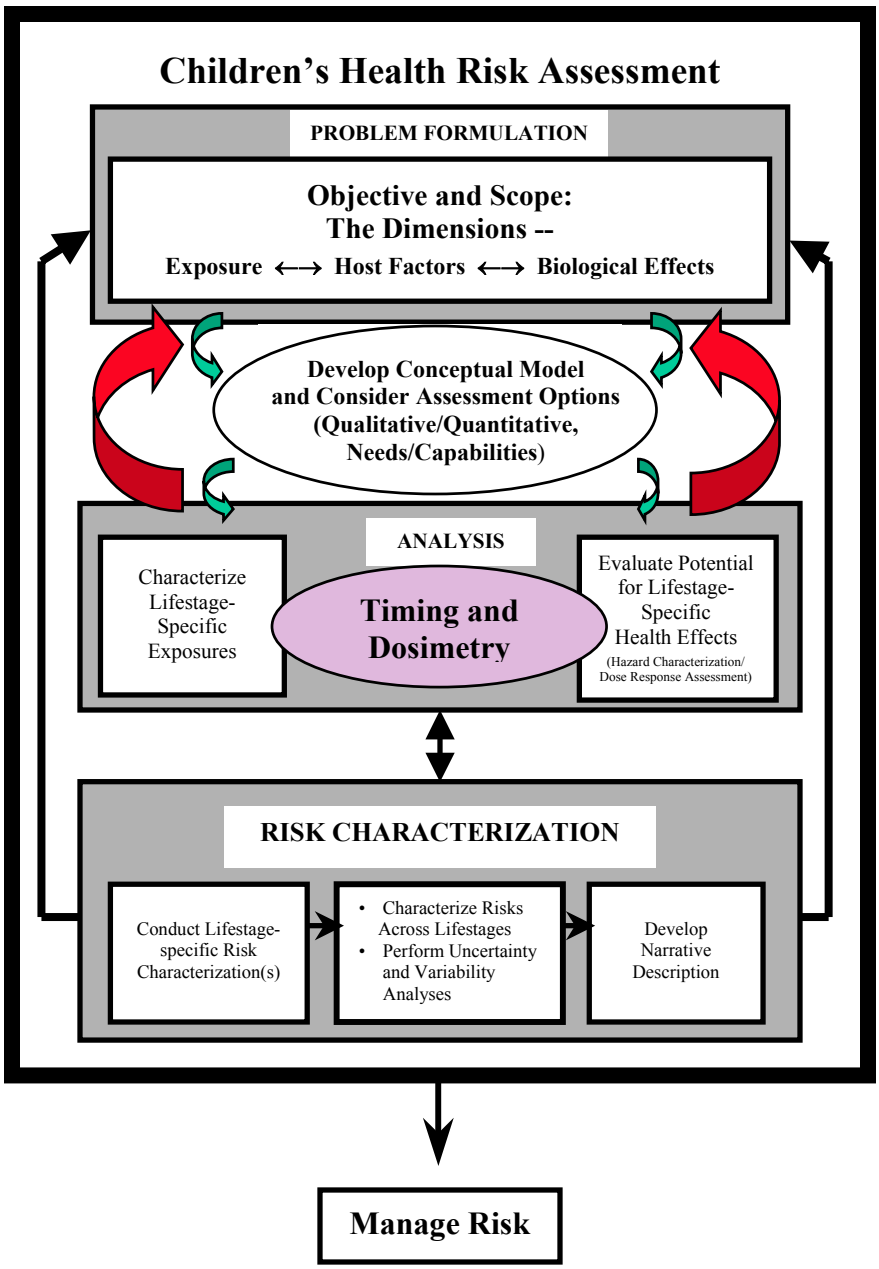
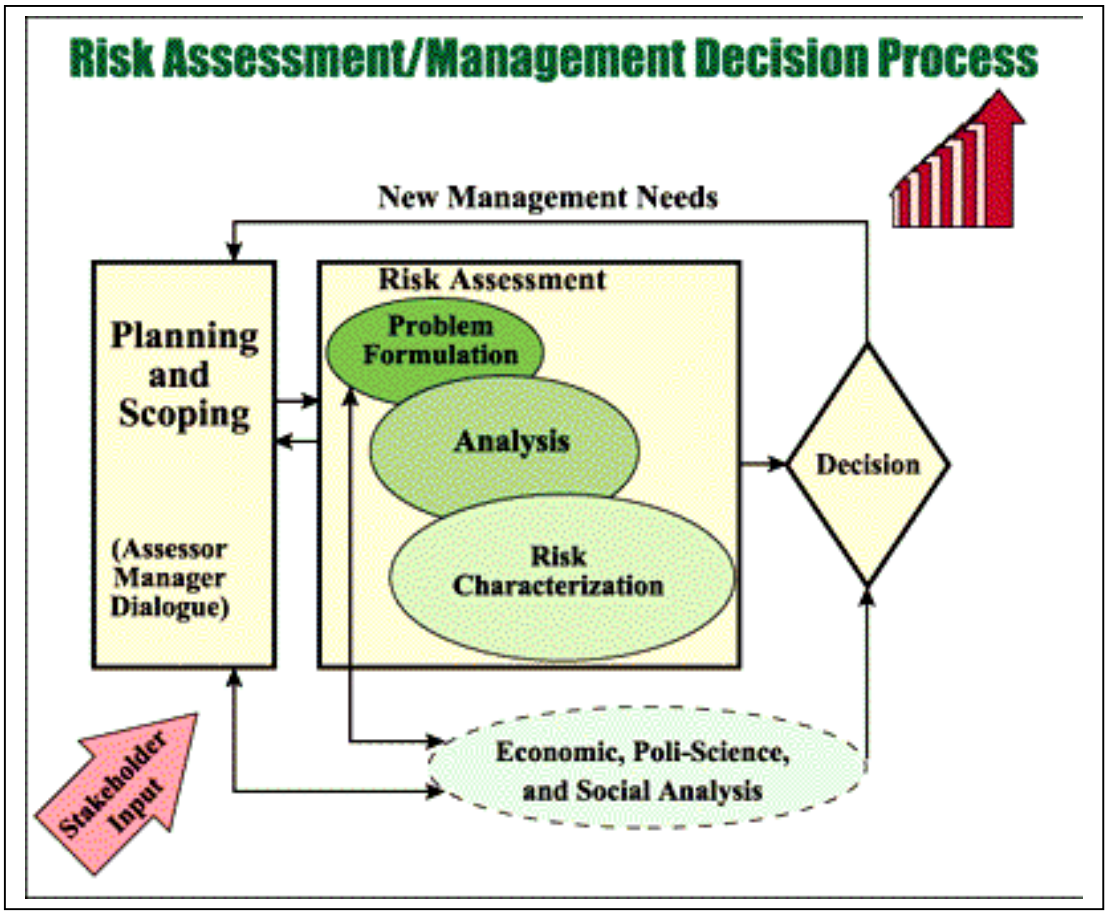


Figure II-2. Stages in the Integrated Risk Assessment Process*



*From U.S. EPA (1997)

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 endpoints, 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 Workshop participants redefined, reduced in number and/or reassigned the components of the risk dimensions and elements presented in the 1997 Cumulative Risk Assessment Guidance. The Guidance presented six dimensions for inclusion in the development of an outline: Sources, Stressors, Pathways, Population, Endpoints and Time frames. The proposed Framework includes only three dimensions: 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. The Framework merges Sources and Pathways into a single dimension: Exposure. Stressors are encompassed by Exposure and Host Factors, and Endpoints become Biological Effects. Because timing of exposure is considered as, if not more, critical than duration of exposure, Time Frames is dropped as a risk dimension, with the concept of criticality of Timing introduced in the second phase of risk assessment—Analysis. The risk dimension Population is dropped since the population ('children') has been predetermined.

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 child-bearing 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 kids of a certain age are exposed, if it's a day care center where 3-5 yr 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, 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 relationships between the Host Factors and the Biological Effects, informed by the initial identification of exposure scenarios, exposed life stage groups, and the chemical(s)' identified characteristics and toxicological endpoints 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 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 chemical's toxicokinetic profile to understand major clearance pathways and mechanisms for activation and detoxification. Evaluate the chemical's toxicodynamic effects 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 (which 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: 1) Unique Effects (are there any life stage/toxic effect combinations which represent novel toxicities that would not be seen in adult-only exposure scenarios?) 2) Quantitative Differences in Effect (are there any developmental life stages in which a greater effective exposure dose and/or greater adverse reaction is likely as compared to adults? If so, prioritize for further analysis.) 3) Lack of Adult Risk Assessment: If there is no pre-existing or relevant adult risk assessment, then continue forward 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 which 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: 1) Qualitative approaches in which the risk for one or more developmental life stage(s) is described as an additional uncertainty or source of inter-subject variability in the adult-based assessment. While such an approach may not drive the risk assessment, it could add weight to its overall conclusions. 2) Semi-quantitative approaches in which uncertainty factors are modified as needed, or a specific children's uncertainty/safety factor is considered. 3) Quantitative analysis of exposure differences in which standard exposure equations are modified to capture children's behaviors and life stage exposure variables. 4) Quantitative analysis of kinetic differences, using life stage-specific physiologically-based toxicokinetic (PBTK) approaches, if feasible and appropriate; 5) Quantitative analysis of toxicodynamic differences based upon dose-response assessment of effects in developmental life stages relative to adults. 6) Quantitative approaches to describe inter-child variability within a given life stage group. This Analytic 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. EPA's 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).

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 certain 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.

III. TOXICOKINETIC CONSIDERATIONS IN UNDERSTANDING CHILDREN'S HEALTH RISKS FROM EXPOSURE TO ENVIRONMENTAL AGENTS

Introduction

The charge to this breakout group was to evaluate how toxicokinetic (TK) analyses can be used to decrease the uncertainties and refine the analysis of children's risks. In advance of the workshop, the Workshop Planning Committee had designed a draft framework for children's risk assessments which involves a 3 step process: Problem Formulation, Analysis, and Risk Characterization. Our objective was to evaluate whether a children's toxicokinetic analysis is best conducted within such a framework and to describe the types of data and considerations that are needed at each step in the process. While the workgroup's focus was upon toxicokinetics, there was an awareness that this portion of the analysis would also rely upon exposure assessment and toxicodynamic (TD) considerations for input to risk characterization. The goal was not to provide detailed instructions or guidelines for how the TK analysis should be conducted. Instead, the focus was on creating a broad perspective which ensures that the relevant questions related to absorption, distribution, metabolism, and excretion of xenobiotics across the various developmental stages (*in utero* through adolescence) are addressed. The workgroup recognizes that every chemical and risk assessment will have unique considerations that will affect the way the assessment is conducted. The goal here is to develop a framework that is broad enough to be useful for a wide variety of children's TK analyses.

It is also important to note that risk assessments are often governed by the type of data available. It may be possible to approach children's risk assessments by an extrapolation from animal data for *in utero* or juvenile life stages if such data are available. Alternatively (or perhaps, additionally), the database may be most amenable to an extrapolation from human adults to early life stages. While the latter type of extrapolation is the primary focus of this framework document, much of what is discussed and recommended is also relevant to direct extrapolation from animal toxicity studies.

The workgroup's overall approach to the task was to: (a) identify the key TK 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 TK approaches and methods fit into the overall children's risk assessment draft framework; and (e) identify critical data needs.

In working with the three-step framework process mentioned above, the workgroup found that it offers a logical, stepwise progression that can readily incorporate the questions, approaches and tools used in TK analyses. The workgroup also found that the TK analysis would be part of the Problem Formulation and Analysis phases, with its results feeding into Risk Characterization. Therefore, the following workgroup summary is organized around the activities that would occur in the first two steps of the three-step framework process.

Problem Formulation for the TK Analysis

The initial step of Problem Formulation is to state the purpose of the assessment, followed by definition of the problem/issue being addressed and identification of potential methods and datasets that may be applied. The workgroup began this process by defining the overall goal of TK assessment, a goal applicable to children's risk assessments in general.

Overall Goal

The broad goal of TK 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. TK assessment can remove some of the uncertainty in risk assessment by replacing inter-species 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, TK assessments can take into account the range of inter-individual variability (where such distributions have been described) to show both the central tendency and upper bound estimates of internal dose. It is also important to recognize that TK assessment can help understand toxic mechanism 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 post-natal periods.

Moving beyond the general reasons for TK analysis, each assessment needs to consider the specific purpose and objectives of a TK analysis for the risk scenario being analyzed. This involves an understanding of scenario-specific factors that affect exposure and chemical-specific TD issues that affect target organ and key internal dose metrics (e.g., parent compound vs. metabolite). Problem Formulation also needs to take stock of the key TK factors that generally tend to govern internal dose, identify the types of chemical-specific and developmental data needed for a children's TK analysis, and then develop a set of analytic options for conducting the analysis. Brief descriptions of these aspects of Problem Formulation follow.

Exposure Inputs to TK Analysis

One of the key inputs to TK analysis is exposure assessment. The manner in which children are exposed governs the route of uptake (g.i. absorption, dermal penetration, respiratory tract absorption), whether first pass effects will occur (e.g., hepatic extraction before systemic circulation) after oral exposure, and which contact sites will receive the largest applied dose. The manner of exposure will also affect the dose rate, 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 exposure point concentration in diet, water, air, or soil combined with the intensity and frequency of contact typically define the exposure dose in risk assessments. This dose and the pattern of exposure become the input for TK analysis, which goes on to consider the absorption, distribution, metabolism, and ultimate excretion of the dose. Thus, the exposure scenario has a large bearing on measures of internal dose: peak concentration in blood/tissues and the total area-under-the

concentration x time curve (AUC) dose. The peak level of exposure can be an important determinant of whether saturation of metabolism, binding, or clearance processes will occur. Peak concentrations may also be a critical determinant of toxicity for acute health effects or where chronic effects are dependent upon repeatedly attaining levels of toxicant that supercede cellular defenses. The AUC dose metric is typically most relevant for assessing cumulative risks and cancer. The chemical form of the toxicant in the exposure medium (e.g., dissolved in drinking water vs. sorbed to soil particles or air particulates) can affect the chemical's bioavailability and so should be considered as part of exposure assessment and initial scoping of the TK analysis.

Exposure assessment is a particularly critical input to children's TK analysis given that, per body weight, children's exposure patterns and rates differ considerably from adults. This not only applies to inhalation rate, and ingestion of food and water, but also to intake of contaminants at ground level (e.g., soil, house dust, carpet contaminants) with which children have a greater contact rate than adults. Of course, the potential for contaminant excretion in breast milk and subsequent nursing infant exposure can be a key pathway to consider. The behaviors and physiologic factors that lead to greater exposures during childhood were recently summarized (U.S. EPA Risk Assessment Forum 2000). The exposure analyst (not the kineticist) may attempt to prioritize age groups for more detailed analysis based upon which groups appear to have the greatest exposures.

In summary, the following factors need to be extracted from exposure assessment for input to TK analysis: (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 as opposed to internal dose), which also considers body weight, breathing rate during rest and play activities, etc. Information describing these processes should be reviewed during Problem Formulation to help scope the overall assessment and ensure that exposure assessment provides sufficient information for the conduct of a TK analysis. This includes seeking data on the distribution of key exposure data across children's age groups (e.g., soil ingestion data, dietary, drinking water and inhalation distributions) to prepare for a TK analysis that can represent the range of exposures and internal doses that are possible.

TD Factors to Consider in TK Analysis

Toxicodynamics and mechanism of toxicant action have a direct bearing on how the TK analysis will be framed. The chemical's toxicity profile often provides evidence of target organ specificity of action, with some chemicals (typically highly reactive) most toxic at initial contact sites, while others exert their greatest toxicity at systemic sites. To the extent possible, TK analyses attempt to define the dose at the target site as well as in the central (blood) compartment. Thus, the toxicity profile will help determine which compartments to include in the TK analysis. This decision is also affected by mechanism of action considerations which may show that metabolic activation at one site leads to toxicity in another. In this case, both the activating tissue and the target tissue would be included in the analysis. The mechanism of action also can determine which dose metric(s) need to be evaluated, both in terms of parent compound vs. metabolites and in terms of peak vs. AUC doses. This also becomes important in

choosing datasets for PBTK model calibration and validation. These datasets should reflect internal or excreted concentrations of the active toxicant or a surrogate (e.g., urinary metabolite) that represents throughput through the activation step.

In the absence of data to the contrary, the starting assumption in TK analysis for children would be that the target sites and dose metrics relevant to toxicity in adults (or in test animals) are also relevant to children. There may be cases where additional target sites and dose metrics may be pertinent to children, as identified in toxicity and mechanistic studies *in utero*, in juvenile animals or in children. The TD information thus focuses the assessment on a particular target site(s) (e.g., contact site vs. internal organs, vs. fetus) and dose metric(s) (chemical form and method of dose integration). It also provides information about critical metabolic pathways of activation, detoxification, and clearance. 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 TK Determinants

Regardless of whether animals or humans (adults or children) are being modeled, certain TK 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 that are most likely to drive the assessment. However, the goal of Problem Formulation is not to eliminate any TK 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.

The workgroup considered the following areas to be of key importance in any TK assessment. The ways in which these inputs can be affected by developmental stage are described under Analysis.

Absorption Factors: Absorption involves uptake of chemical from the point of contact – g.i. tract for oral exposure, upper respiratory tract and lungs for inhalation exposure, and skin for dermal exposure. While chemical absorption is one of the key determinants of internal (systemic) dose, it is also recognized that the portal of entry may be a critical target site. In those cases, another important factor is the amount of chemical per unit surface area of contact site per unit time. Children's absorptive capacities may differ from adults in certain cases. Other issues, such as contact site doses to the respiratory tract, may be especially important in children given the high rates of childhood asthma.

Distribution Factors: These factors describe the structure of a physiological model, involving compartments where absorbed chemical may reside for some period of time. It is essential to include tissues involved in chemical metabolism and elimination, tissues which represent potential storage sites (e.g., fat, muscle) and any additional tissues which are target sites for a chemical's toxicity. Even if not constructing a physiologic model, the analyst must consider how the developmental life stage influences the size of and blood flow to these compartments. Of particular importance is the rate of blood flow to the liver, the primary site of metabolism for most xenobiotics. Some chemicals are metabolized rapidly by the liver (high

hepatic extraction), and so clearance by this organ can be limited by the delivery of chemical (hepatic blood flow) rather than maximal metabolic rate (Kedderis 1997). Another key compartment is the embryo/fetus if exposure is during gestation. In this case, consideration needs to be given to both maternal (including placenta) and embryo/fetal TK factors in understanding the potential for *in utero* exposure. Permeability factors may also be of importance to children's assessments, especially with regards to the ability of chemicals to cross the placenta or the blood-brain barrier. Finally, the availability of chemical binding sites in blood (plasma proteins) may be quite different early in life and thus affect the availability of a chemical for transport across the placenta, uptake into tissues or excretion.

Metabolism Factors: For many xenobiotics, whether drugs or environmental chemicals, the ability of liver enzymes to catalyze Phase I and Phase II metabolic reactions governs the rate of removal of parent compound and generation of metabolites. Given that some metabolites can be more toxic than the parent compound, hepatic metabolism has implications for both toxicokinetics and toxicodynamics. While the liver typically has the greatest metabolizing capacity, extra-hepatic metabolism can be a key factor in determining where a chemical's toxicity is targeted. This is relevant to the case of *in utero* exposures where a number of Phase I and Phase II enzymes exist in the fetus although most of these systems are immature. This immaturity carries over into early post-natal life and this has been shown to affect the clearance of drugs in infants.

Elimination Factors: A key clearance pathway for certain water-soluble xenobiotics is renal elimination, either as unchanged parent compound or as metabolites. Large molecular weight compounds and their conjugated metabolites tend to be excreted into bile, wherein hepatobiliary cycling can extend the half-life and perhaps action of the agent. Finally, highly volatile compounds may have exhalation as a primary excretion pathway. While fetal excretion of xenobiotics is mainly via return flow to the maternal circulation across the placenta, the newborn must rely upon immature renal and hepatobiliary systems for excretion.

Hopefully it is clear from the above discussion that while certain TK factors are often important in determining internal dose, the particular pathways and factors involved for any one chemical will be specific to that chemical's ADME properties. Therefore, a children's TK analysis is highly dependent upon and must be tailored to the properties of the chemical being analyzed.

Identification of Key Chemical-specific and Age-specific TK Datasets

During Problem Formulation the data needed for the TK analysis is acquired and organized according to type of information being provided, and an initial review is conducted to evaluate the type of analysis that may be possible (see analytic options below). The data needs and sources can be organized into the following areas:

- Exposure Inputs: from the exposure assessment portion of the risk assessment

- Chemical-specific TD Data: including information on toxic mechanism and key target organs. This information can be sought via computerized searches to identify primary and secondary literature sources, together with review of chemical

monographs such as the ATSDR Toxicological Profiles, IARC monographs, and documents available from U.S. EPA (e.g., toxicologic reviews in support of IRIS).

- Chemical-specific TK Data: including data describing mechanisms of uptake by various routes of exposure, chemical properties that affect uptake and distribution (e.g., oil and water solubility, volatility, sorption to environmental media, partition coefficients), distribution information such as volume of distribution and data on localization of chemical in different body compartments/fluids, data describing sites and pathways of metabolism, major metabolites found that may be important for biomonitoring, any other metabolites that may be related to toxic mechanisms, and data on rate and pathways of excretion. Datasets describing parent compound and metabolite concentrations over time in body compartments should be specifically noted as these may be important in calibrating and validating PBTK models. Any data in children or juvenile animals, or that describe *in utero* exposure, should also be specifically noted as these data may be critical to understanding how internal dose during development can differ from that in adults. Literature searches to retrieve primary data sources are needed. If PBTK models already exist for the chemical, publications describing these models are usually an excellent source of information regarding analytic approaches and underlying data sources. The availability of a validated model for the chemical in animals, adult humans or during pregnancy will greatly expedite the ensuing TK analysis for children and the *in utero* period.

- Child-specific TK Data: The ideal is to have chemical-specific TK data for the relevant age groups, but this will rarely be the case for environmental toxicants. This is due to the lack of sequential monitoring data after defined exposure events in children. Therefore, data for surrogate chemicals in children, typically therapeutic drugs, can help the assessor understand how children's distribution, metabolism, and excretion can differ from adults. Based upon the clearance mechanisms of the drugs tested in children, it is possible to make generalizations about how active a given metabolism or excretory pathway is in children. Combining this information with *in vitro* studies of metabolic enzyme function at various life stages and with developmental physiology information that describes renal function, blood flows, compartment size, water and lipid contents, and permeability of barriers, one can develop a database of children's TK development. Resources and citations useful in developing such a database are presented in the supporting background paper for this workgroup (Appendix 3).

Listing of Analytic Options

The types of TK analyses possible for a children's risk assessment can range from qualitative to semi-quantitative to quantitative. The least data-intensive option is a **qualitative discussion** of whether internal dose in children (per unit of external dose) may be different than in adults, in which direction these differences may run (e.g., more internal exposure to parent compound but less to metabolites in neonates; or less protein binding so greater potential for high peak exposures but shorter half-life in neonates). The qualitatitive analysis might discuss the risk implications of these child-specific TK factors relative to what is normally anticipated in

non-cancer risk assessments for inter-subject TK variability (one half log or 3.16 fold). The analysis may become **semi-quantitative** if it attempts to derive child-specific TK uncertainty or adjustment factors based upon the likely size of differences between adults and children in terms of internal dose. Such assessments might take on bounding assumptions in which only one or a few factors are adjusted for children; in this case, a simplified calculation rather than a complete model would attempt to relate internal dose across age groups. For example, if it is known that glucuronidation is a key elimination pathway for a toxic metabolite, the deficient glucuronidation capacity and higher blood concentrations seen with surrogate chemicals (e.g., lorazepam, morphine) in newborns might form the basis of describing how much more internal exposure there could be to the active metabolite in neonates. **Quantitative analysis** would rely upon PBTK models to predict internal doses of environmental toxicant (or key metabolites) in children of specific age groups. Such models need to be developed and validated in children to the extent possible. In this case, many parameters would need to be described for the age groups being evaluated so that the physiology and function of these groups can be adequately represented. The distributions of parameter values across a given age group need to also be described so that a range of values or the full distribution can be loaded into the model rather than a single parameter point estimate. Use of Monte Carlo simulation software can then enable the output of the distribution of internal doses, allowing the assessor to focus on a particular level of protection (e.g., the median or the 95th percentile) and to evaluate the degree of TK variability in a more refined way.

Problem Formulation should list these options and the types of data needed for each. Unless there is an a priori reason to not conduct a full PBTK assessment (e.g., exposure pathway unlikely to be widespread or to be a significant risk driver; very little chemical-specific TK data in adult humans or animals), Problem Formulation should retrieve a broad TK database that could potentially be used in children's PBTK modeling. In this way, the assessor can evaluate what types of analyses the data will support. The gathered data will be valuable regardless of which analytic option is ultimately chosen.

Analysis of TK Data

This phase of the TK assessment reviews the chemical-specific TD and TK information described above, together with the child/age group-specific TK information. The major questions raised in this phase are: 1) How does TK affect the chemical's toxic mechanism via activation or detoxification pathways? 2) What are the key dose metrics and target organs for the chemical being analyzed? 3) What are the major *in utero* or child-specific TK factors that may alter chemical fate? 4) Based upon internal dose considerations, which age groups should be prioritized for more detailed analysis? 5) Which TK analytical methods are best suited to evaluating children's internal dose? 6) 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 TD and TK 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 TD and TK data is used to understand the TK determinants of toxicity: whether metabolism represents detoxification or activation or both (i.e., some metabolic pathways are detoxifying while others create active products). This involves identification of the various enzymes that may be important in chemical activation and removal (e.g., Phase I enzymes - specific cytochrome P450s (CYPs), peroxidases, dehydrogenases; Phase II systems - glucuronidation, sulfation, glutathione (GSH) conjugation; Other enzymes - epoxide hydrolase, serum esterases). Since an enzyme can be part of an activation pathway for one chemical and yet be detoxifying for another (e.g., glutathione S-transferases, 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.

Once the TK determinants of toxicity and detoxification are understood, the next area to review is target sites, specifically with respect to whether the TK determinants of toxicity differ at different sites. For example, perchloroethylene and trichloroethylene are believed to cause toxicity and cancer via oxidative metabolism in the liver, but to cause renal toxicity and cancer via pathways involving GSH conjugates. Therefore, the key activation and detoxification steps in each target organ should be evaluated. Toxic metabolites should be identified so that internal dose metrics can be selected for each target organ. Since target sites can be different in the fetus or child than in adults, toxicity information for early life stages is particularly important in understanding the variety of toxic endpoints and target sites that should be considered.

Other TK determinants of chemical fate should be evaluated so that 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 which should be modeled or evaluated in other ways; TK factors that control chemical distribution and excretion.

Analysis of Age Group-specific TK Factors

Numerous TK 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 TK mechanisms involved in the chemical's ADME, the life stage where exposure occurs, and the interplay of other factors which 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 TK 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 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) as 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 that are possible in early life. The following general discussion provides a framework into which chemical-specific information can be added to focus the TK 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. 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 g.i. 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, with this attributed to greater pinocytic activity of intestinal epithelium prior to closure. This non-selective 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 1993). In animals, closure occurs around the time of weaning, although the time course for this in humans has not been defined.

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 g.i. tract absorptive regions; presence of milk in stomach) (NRC 1993).

The possibility of higher g.i. uptake of ingested chemicals early in life should be evaluated within the context of the chemical's behavior 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 chemicals which are poorly absorbed (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, g.i. uptake data for these and analogous chemicals in children or juvenile animals should be sought. These efforts may allow age group-specific adjustment factors to be applied for g.i. absorption.

Respiratory Dosimetry and Absorption

<u>Particles and Aerosols</u>. 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 as compared to adults (U.S. EPA 1999, 2000; NRC 1993). 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 due to asthma or other conditions. Further, in asthma, the changes in breathing pattern and respiratory volume/resistance may create local exposure patterns that are 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 which 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 RfC development (U.S. EPA 1994; see also U.S. EPA Risk Assessment Forum 2002). Additional consideration should be given to whether there are life stages where mucociliary clearance and macrophage clearance of particles is substantially different than in 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 extra-thoracic dose in animals (where the toxicity data are obtained) to adult humans for gases with respiratory effects. This adjustment factor is based upon the difference in respiratory volume per surface area in the upper respiratory tract across species. This methodology should be extended to children of various ages by inputing upper respiratory tract surface area and inhalation volumes for these ages. Until that is available, the overall ~ 2 fold differential in inhalation volume per respiratory surface area described above may be assumed as a first approximation for reactive gases.

Non-Reactive 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 which rapidly reach steady state (e.g., perchloroethylene), the major (but not only) determinant of net uptake is the partition coefficient. In that case, increased respiration and cardiac output also leads 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 determined by the presence of carriers (e.g., hemoglobin) or lipid in blood, which can vary across species and age groups. Since partition coefficients can be determined *in vitro* from blood samples, a database of children's partition coefficients (across age and chemicals) may be possible to develop. Also, it may be possible to model uptake of these gases in children based upon datasets in adult animals and humans, with extrapolation to younger age groups based upon known differences in blood composition.

In summary, in evaluating systemic absorption of non-reactive gases in children, estimates of children's blood:air partition coefficients are important, especially at early life stages where non-exhalation clearance pathways are likely to be deficient. However, in older age groups where these systems are more functional, the blood:air coefficient together with the other physiologic and metabolic parameters need to be incorporated in models to understand how uptake may vary across age groups. For short-term uptake, simplified adjustments to absorbed dose based upon inhalation volume per unit body weight may provide a frame of reference in these age groups where modeling is not practical. While this simplified approach would not

predict internal concentrations, it would show how uptake can be a function of respiratory volume and body weight when other factors (metabolic and renal clearance) do not substantially differ between children and adults.

Dermal Absorption

The assessment should consider how child-specific factors may tend to increase absorption across the skin. 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 a high percentage of the body surface area 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. Since 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 due to immaturity of the stratum corneum (U.S. EPA 1992; Barker et al, 1987). 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 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. Exposure scenarios involving this portion of an infant's body (e.g., bathing in bath water containing contaminants) should consider this potential for increased penetration of chemicals.

A final consideration is whether chemical sorption on the exposure matrix (e.g., soil) significantly impedes dermal penetration. While 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 body (generally more water and less lipid in neonates), quantity of plasma protein binding sites (fewer in neonates, and those that do exist may be less available for xenobiotic binding than at older ages), and less intact blood brain barrier in neonates. These factors tend to increase the volume of distribution (Vd) for many chemicals in early life, which is borne out by a recent compilation of PK data in children (Appendix 3; Ginsberg et al, 2002). Higher Vd 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 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 accenuate chemical toxicity due to greater percentage of free chemical in the circulation (as suggested for lidocaine, cisplatin and other drugs: Zemlickis et al, 1994; Kakiuchi, et al, 1999). 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 (Appendix 3; Ginsberg et al, 2002). 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 as compared to mother. Thus, fetal exposure needs to be considered where maternal exposure occurs. Fortunately, there are a number of PBTK models to 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 cases where empirical data are missing (Byczkowski et al, 1994).

Metabolism Factors

The companion paper (Appendix 3) summarizes a variety of *in vitro* data (enzyme levels and function) and *in vivo* data (therapeutic drug PK 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 2E1, 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., CYP 3A7, GST-pi), but these appear to be functionally distinct from the adult enzymes and so don't compensate for their lack in early life. 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 such that some enzymatic functions (most notably CYP 1A2) appear to become somewhat more active than in adults (Appendix 3; Renwick et al, 2000; Dorne et al, 2001; Ginsberg et al, 2002). 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 as compared to 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 (approx. 6 months to 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* datasets that are available provide quantitative data on metabolic processing in children relative to adults for a wide variety of pathways and drugs (Appendix 3; Renwick et al, 2000; Ginsberg et al,, 2002; Hines and McCarver 2002; McCarver and Hines 2002). 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; however, possibly also slower removal of metabolites). It should be noted that neonate/adult differences in half-life can be large relative to the default assumptions for inter-individual PK variability (3.16 factor), especially when considering the full range of results from individual neonates (Ginsberg et al, 2002). This may warrant semi-quantitative approaches such as adjusting uncertainty factors to incorporate PK 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 PK 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 non-inducible (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 which are 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 be subject to lower exposure to reactive metabolites due to the lack of activating metabolic pathways, as is recognized for CYP2E1 in the fetus (Cresteil 1998).

The time course for the development of *in utero* metabolic capabilites may point out which gestational periods are most significant in terms of TK susceptibility via 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 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. While PBTK modeling of these elimination pathways in conjuction with other TK inputs specific to children is the ideal, one can assume that elimination via renal and biliary systems will be slower in newborns than 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 that is of particular concern from a TK perspective. These would be age groups where the TK mechanisms central to the chemical's absorption, distribution, activation, detoxification, and elimination are expected to be most different from adults. In general, neonates (both premature and full-term) through the first several months of life are most different from older age groups and adults with respect to certain absorptive properties (e.g., g.i. tract closure), distributional properties (low protein binding, high permeability of blood brain-barrier), altered body composition (in terms of water and lipid content), and metabolism and elimination function (immaturity of many systems). Therefore, this age period should be carefully considered for the possibility of substantiative changes in internal dose relative to adults. Somewhat older age groups (6 months to 2 years) are also important from a TK perspective in that these groups generally have greater hepatic extraction and shorter drug half-lives due to larger liver size per body weight (Appendix 3; Gibbs et al, 1997; Ginsberg et al, 2002). *In utero* may also be a critical life stage from a TK perspective since 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 TK considerations exist in those stages.

Changes in TK function may be defined within specific age groupings as a way to compile and organize the data. It appears that the 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. Alteratively, 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 TK 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 which appear to have the most unique TK features relative to adults (particularly the first weeks and months of life as described above).

Selection of Analytical Approach

The possible approaches for assessing TK factors as part of a children's risk assessment are: PBTK modeling, semi-quantitative 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 which take into account the numerous factors that can create TK differences between children and adults or that are calibrated against actual TK data in children. Several initial efforts (Haddad 1999; Pelekis 2001; Gentry et al, 2002) form useful building blocks and we can expect children's PBTK models to evolve over the next few years. Therefore, while 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 this type of data will not be 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 PK data in children who have been exposed to therapeutic drugs. While this will introduce uncertainties, it does not invalidate this approach.

As mentioned above, PBTK modeling in children can represent an important advance in refining the risk assessment process. This is especially true when PBTK models are combined with Monte Carlo approaches which incorporate 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 pre-determined 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 TK contribution to variability and uncertainty in the assessment can be explicit and readily expressed.

While 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 TK component. In such cases, screening level analyses may be the only realistic option. Additionally, in cases where a chemical's TK properties 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 will be worthwhile relative to the level of analysis being conducted in other portions of the risk assessment.

Semi-Quantitative 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 semi-quantitative approach. In these cases, review of the underlying chemical-specific and age group-specific databases may give reason to suspect that there may be 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 B1; 2-amino-3-imidazoquinoline – Kitada et al, 1990; Hashimoto et al, 1995), a form of cytochrome P-450 prevalent *in utero* and just after birth, this may constitute a sufficient rationale to develop at least a semi-quantitative or screening level estimate of relative internal dose in this age group. This would especially be the case if the active metabolite formed from CYP3A7 metabolism is expected to be poorly detoxified and excreted in this age group. Of course, the semi-quantitative approach would only be used if a more detailed PBTK assessment was ruled out. This screening level approach can be seen as supplementing the existing set of uncertainty factors which already exist in non-cancer risk assessment – specifically the half-log (3.16 fold) uncertainty factor for inter-individual variability in TK (Renwick 1998). While that uncertainty factor is designed to address a large array of general inter-individual differences that might affect TK 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) who might have their own unique mean and standard deviation for internal dose. The semi-quantitative 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 TK.

Since this would not be a comprehensive PBTK approach, the semi-quantitative assessment would focus upon one or several key TK 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 semi-quantitative assessment can evaluate how known differences in key TK pathways (Appendix 3; Ginsberg et al, 2002) may affect the absorption, metabolism and elimination of the chemical under analysis. The analysis would be semi-quantitative 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 TK factors may increase or decrease the influence of changes in, say, 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 the analysis is focused upon (e.g., specific CYP in children vs. adults). If these factors appear likely to negate the child/adult difference in CYP activity, then the analysis may be deemed too indeterminate for the application of a specific adjustment factor. However, if these additional factors appear to be of little consequence or to augment the age differential in CYP, then the child/adult ratio may be used as an adjustment factor directly, or with an additional uncertainty

factor. 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 TK data suggest that child/adult differences may not cause substantially more internal dose in children, or where this review indicates large areas of uncertainty, a purely qualitative approach may be warranted. This approach can summarize what is known about the chemical's TK properties vis-à-vis the development of TK 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 TK 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 TK data for environmental chemicals in children, a large number of datagaps 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 which have similar metabolism/clearance pathways and which have been tested in children).

To engage the framework there is an overriding 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 the activity of metabolism and elimination pathways for the chemical being modeled reflective of what is known about these pathways in children. 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.

Table III-1 summarizes the general TK data and modeling needs to make this framework fully feasible. 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 TK evaluation the chemical has already undergone and the degree to which it has been modeled in test animals and humans.

Table III-1. Critical Data Needs for Children's TK Framework

Data Needed	Type of Effort	Rationale
Accessible database of age-specific physiology inputs in animals/humans (cross-species ontogeny mapping)	Literature search to compile. New studies may be needed (e.g., child blood:air partition coeffs.; plasma protein binding of toxicants)	Needed for optimal PBTK modeling
PBTK model development – age/life stage-specific (*in utero*, lactational, neonatal thru puberty)	Model calibration and validation with therapeutic drugs or with toxicants in non-human primates or lower animals	Key tool for children's TK assessments
TK modeling case studies: 5 chems x 3 scenarios x 3 ages	Model predictions of internal dose in children relative to adults or animals	Demonstrate how to adjust internal dose for range of cases
Juvenile animal toxicology and TK data	New lab data - acquisition of chemical-specific data	Obtain actual data where critical; data useful for model assessment
TK submodels : Respiratory uptake - Particle deposition - Reactive gases Oral absorption	Respiratory deposition a) adapt RDDR/RGDR to early life stages b) adapt for asthmatic children Oral – develop absorption model responsive to children's g.i. function	RDDR – particle dosimetry likely different in children RGDR – gas dosimetry may differ – need to evaluate possible differences in resp. distribution of chemical dose Model development where critical need
Lung clearance mechanisms (mucociliary, macrophage)	New data for variety of particles at early ages	Can affect lung AUC dose – prioritize certain age groups/conditions
Dermal uptake	Compile existing data for drugs and env. chems. in adults and children	Need to evaluate signficance of age-dependent absorption factors
Metabolic systems (esp. GSTs, epoxide hydrolase, alcohol dehydrogenase)	Continue analysis of *in vivo*/*in vitro* data; new data may be required (e.g., Cyp3A7, GSTs); evaluate distributions and sources of variability	Need improved maturational data for selected systems of environmental relevance, especially where only sketchy data exist or for enzymes high in fetus/neonate

Table III-1. Critical Data Needs for Children's TK Framework, Cont'd

Data Needed	Type of Effort	Rationale
In utero dosimetry	Compile maternal/fetal dosimetry estimates from existing case studies; extension of existing models; evaluation of governing principles	Need evaluation of fetal dosimetry and modeling approaches
Lactational factors	Compile data and models of excretion into milk	Important to identify which types of chemicals and what mechanisms govern milk transfer

IV. TOXICODYNAMIC CONSIDERATIONS IN UNDERSTANDING CHILDREN'S HEALTH RISKS FROM EXPOSURE TO ENVIRONMENTAL AGENTS

Introduction

The charge to this breakout group was to evaluate how to use known dynamic differences in development to better understand children's susceptibility to environmental agents. Towards that end, this group focused on what, how and when such information could be used in an overall risk assessment framework for evaluating children's health risks.

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

Toxicodynamics in the Risk Assessment Framework

The dynamics breakout group reviewed the proposed framework (see Figure II-1) to ensure that dynamic considerations could contribute to and would fit within the framework. Table IV-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.

The dynamics group felt that the majority of their deliberations would be within the Analysis and Risk Characterization stages of the overall children's risk assessment framework. Thus, the primary focus of this group's report is targeted to a discussion of how dynamic considerations would inform those stages in the framework process.

Step 1: Toxicodynamic Considerations During Problem Formulation

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. Obviously, a narrow focus for the assessment could refine life stage-specific considerations and thus could define initial toxicodynamic considerations relevant for that assessment scope. Hence, the Problem Formulation stage in the framework provides the context for the breadth of subsequent toxicodynamic considerations.

The Problem Formulation stage also, by identifying chemicals or chemical classes to be evaluated, provides critical input into 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, i.e. chemicals that are known to affect apoptotic processes, then organ systems that are known to use these dynamic processes should be considered in the analysis. Also, because such apoptotic processes are known to occur at specific times in normal development, critical windows of

vulnerability could be identified. Thus, both critical time as well as target organ system could be identified for further evaluation using dynamic information.

Table IV-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
- Identify cross-species relevance of potential responses

Analysis

- Identification of uniquely susceptible dynamic processes
- Identification of developmental milestones and/or endpoints for testing/assessment
- Identification of functional consequences of processes if altered
- Illustrate the inter-relatedness 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

During Problem Formulation, when thinking about toxicodynamics, 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.

Steps 2 & 3: Toxicodynamic Considerations During Analysis and Risk Characterization

The proposed children's risk assessment framework emphasizes the need to connect toxicodynamic considerations with concurrent toxicokinetic considerations. Obviously, it is essential to understand if the parent compound or if a metabolite would be expected to reach developing tissues. Also, it would be important to know what dose levels and at what times such exposures would be expected. Hence, the dynamics group also discussed in detail the importance of timing and dosimety considerations within the Analysis phase of the framework. Dynamic considerations would also inform quantitative considerations in dose-response assessments and for risk characterization.

During the Analysis and Risk Characterization stages, when thinking about toxicodynamics, the risk assessor needs to take into consideration whether the parent compound or its metabolite(s) is the toxic agent. Timing and dosimetry should also be considered.

The dynamic breakout group first evaluated life stage considerations by developmental organ system. The group conducted a brief survey of some of the relevant resources and summarized some example organ systems with an aim at highlighting example dynamic processes occurring at various times within each system and the implication of those processes for identifying and characterizing "windows of susceptibility." Some examples of why these processes were important for children's health risk assessments were given. The final section in this report identifies "critical data needs."

Questions

Table IV-2 shows a list of questions posed to the dynamics breakout group in their workshop deliberations. These questions were designed to provide background and justification for the specific consideration of developmental dynamics in the risk assessment process. They provide some context for determining the magnitude and diversity of toxicokinetic considerations for children health risk assessments.

Table IV-2. Some Questions Considered by the Dynamics Breakout Group

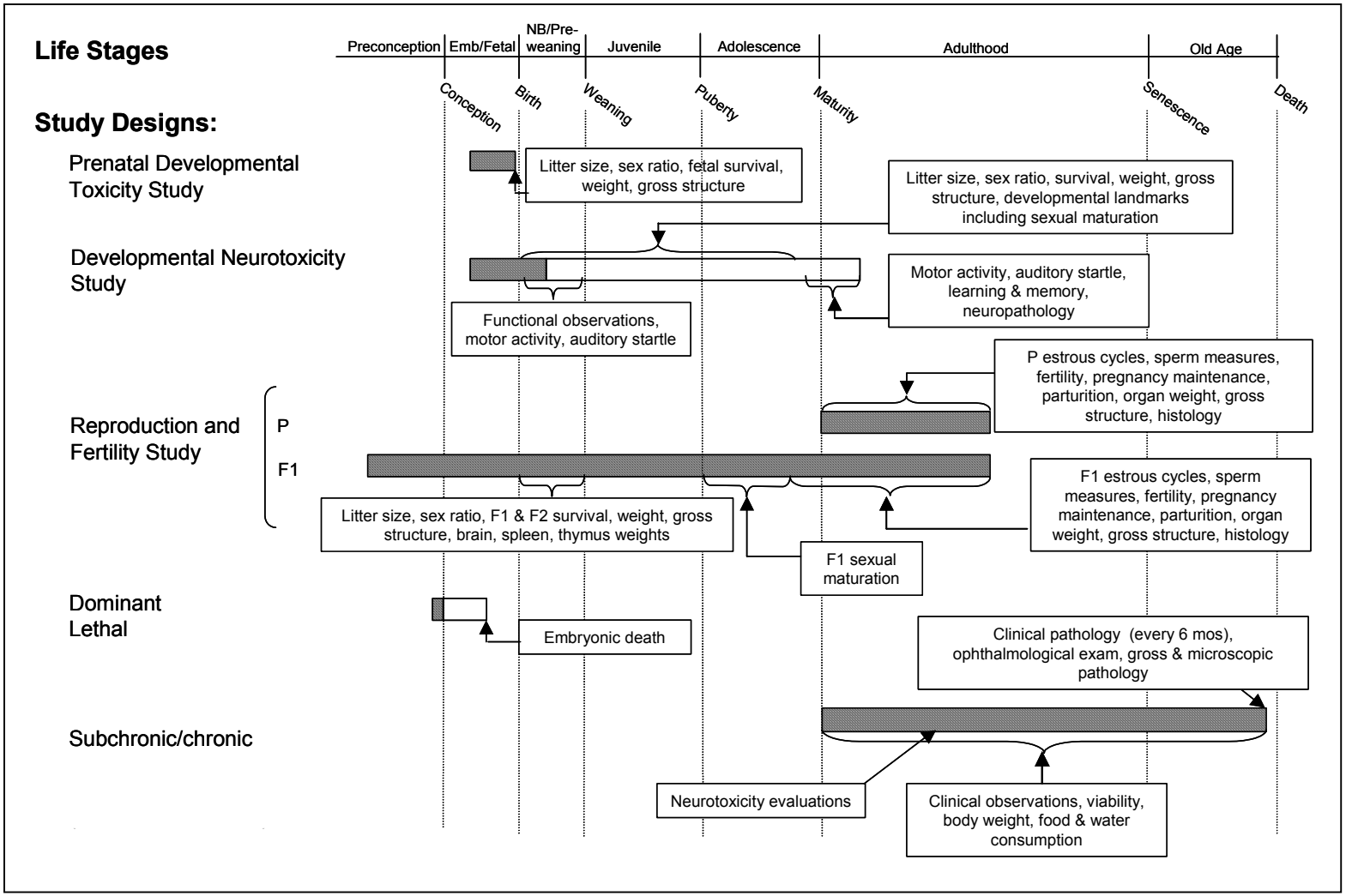
- What do we know about the dynamics of developmental processes that may make them uniquely sensitive (qualitatively and/or quantitatively) to environmental factors? Can we define the types of dynamic information that are useful for our Framework?
- Are there common characteristics of these processes?
- Are the animal tests robust enough to provide the types of dynamic information that we need? What are our qualitative and quantitative needs for these assessments?
- Can the group provide some indications of level and type of impact that might result from altering these developmental processes? How would these compare with adult dynamic processes?
- How can dynamic information support or refute proposed modes of action in animals or humans?

1. Are there distinct life stages?

The dynamics group identified a series of references and workshops that have reviewed the evidence for distinct life stages and critical windows of susceptibility in development (Adams et al, 2000; Adkins et al, 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 IV-1 shows a summary of developmental life stages including those of interest for this workshop, which range from preconception through adolescence. The dynamics group felt that it was critical to the overall risk assessment process to be able to identify distinct life stages in order to more fully identify potential windows of susceptibility. The group also felt it was important to be able to compare life stages and particular endpoints and patterns of dynamic processes across species. For their deliberations, the group used a series of general, simplified life stage events (summarized in Figure IV-1) which included conception, embryonic, fetal, newborn, neonatal, pre-weaning, weaning, juvenile, puberty, adolescence, adulthood and old age. The group thus reviewed the structure of the children's risk assessment framework to consider approaches for evaluating impacts of exposure of children at any or all these life stages.

The workgroup noted that 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 the developmental periods that occur during childhood. The workgroup felt it was not important for the framework to fully integrate all the various categorical views of childhood and major developmental periods. However, it can be important to understand how other disciplines have categorized the age periods that constitute childhood in their evaluation of data.

Figure IV-1: Exposures and Endpoints Related to Developmental Toxicity Evaluations



(Adapted from Kimmel 2001)

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 towards 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 (Institute of Medicine 1997) divide the childhood period into three major life stage categories, which are then subdivided into narrower age ranges. The three major developmental categories are: infancy, childhood and adolescence.

The infancy period is subdivided into the first and second six 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, the dynamics group recognized that exposure assessors have identified a series of different age-specific exposure windows based on age-specific behaviors and physiological considerations. For example, toddler versus neonatal differences in exposure to carpet and floor and for hand-to-mouth exposure pathways as well as dietary differences are defined by both differences in activities as well as physiological development. Exposure considerations in categorizing life stages are reviewed in the EPA children's exposure factors handbook (U.S. EPA 2000) and the 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). Child psychologists or childhood developmental specialists also define life stages using behavioral landmarks. Many other examples of discipline-based differences in childhood categorizations have been proposed.

Recognizing categorical overlap between disciplines

The group recognized that although general developmental life stages did not always match the discipline-specific exposure windows, this difference would not inhibit the ability to look at dose-response relationships for critical developmental endpoints. These differences however did highlight the need for the framework to be robust enough to allow for iterative interactions between exposures and effect analysis both in problem formulation as well as data analysis. It also identifies a need for risk assessment methods that would allow risk assessors to relate output from the exposure assessments or from discipline-specific assessments using multiple exposure times during various life stages. Thus, the timing and dosimetry relationships

shown in Figure IV-1 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

The dynamics group chose three specific organs or biological systems to evaluate and illustrate distinct life stage considerations. These three systems are 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.

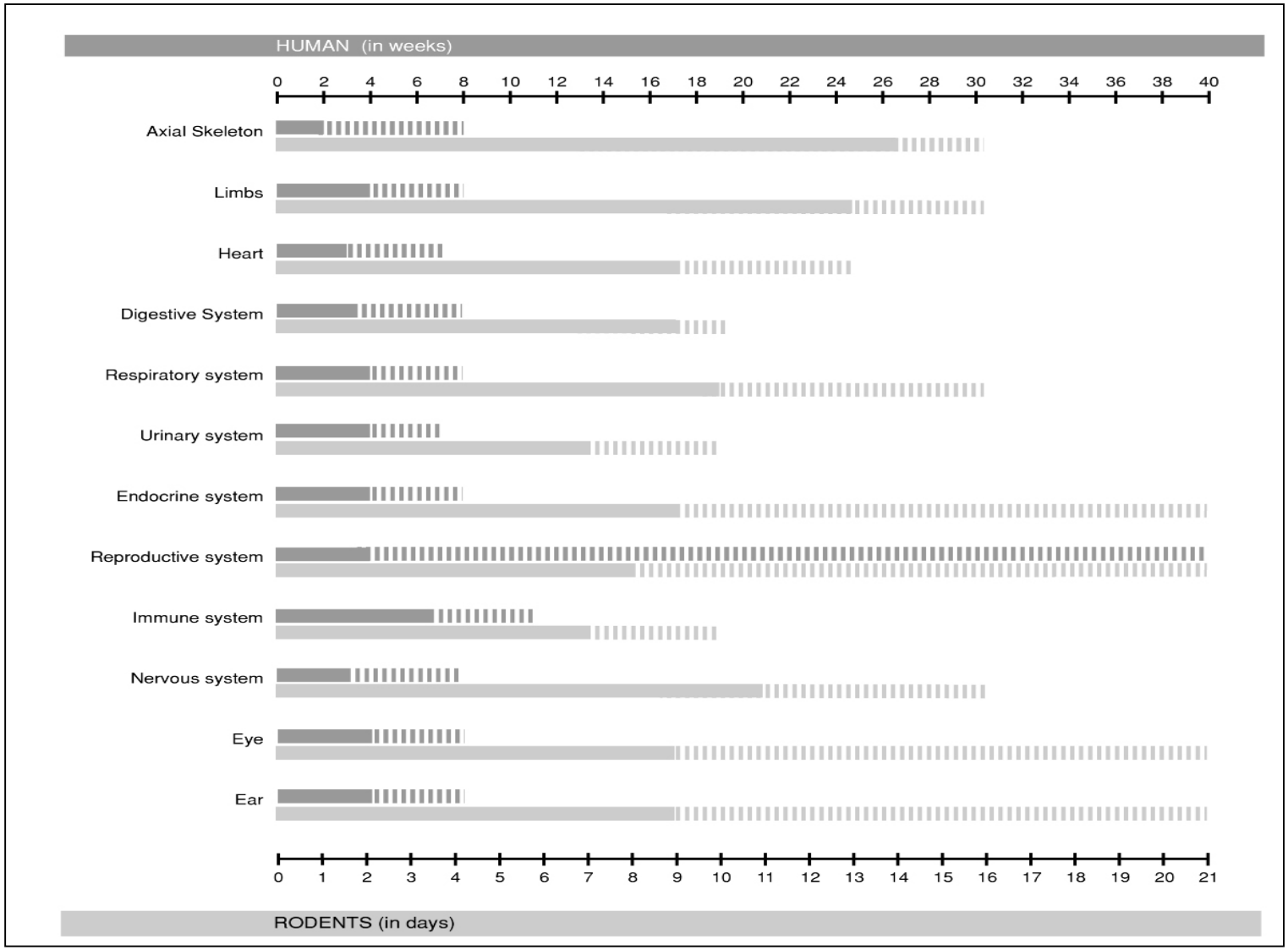
Figure IV-2 shows the initial appearance of organ systems during gestation in humans and in rodents. Note that the relative temporal initial development of each organ system, as defined by the first appearance of cellular structure of each system, is evident and can vary greatly across species.

Respiratory System

Figure IV-3 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 IV-3 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 percent of the alveoli present in the adult lung are formed following 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.

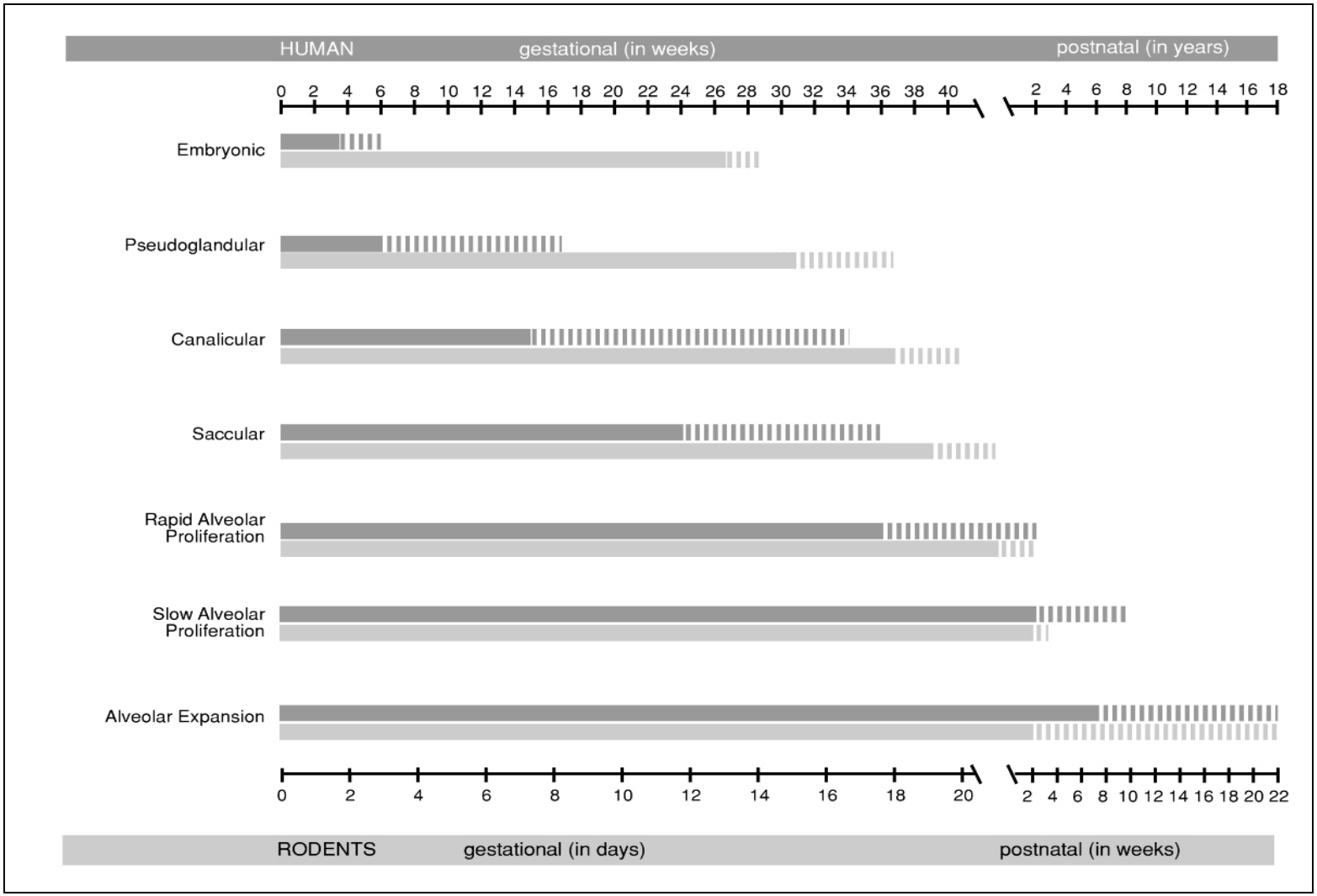
Figure IV-2: Initial Appearance of Organ Systems during Gestation in Humans and Rodents



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, since 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). Since 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 to those found in children (Smiley-Jewell et al, 1998; Plopper et al, 1994; Fanucchi et al, 1997).

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. Since 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, which occur in the prenatal period due to 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 which 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).

Figure IV-3: Respiratory System Development in Humans and Rodents



(Adapted from Dietert et al, 2000; Pinkerton and Joad 2000.)

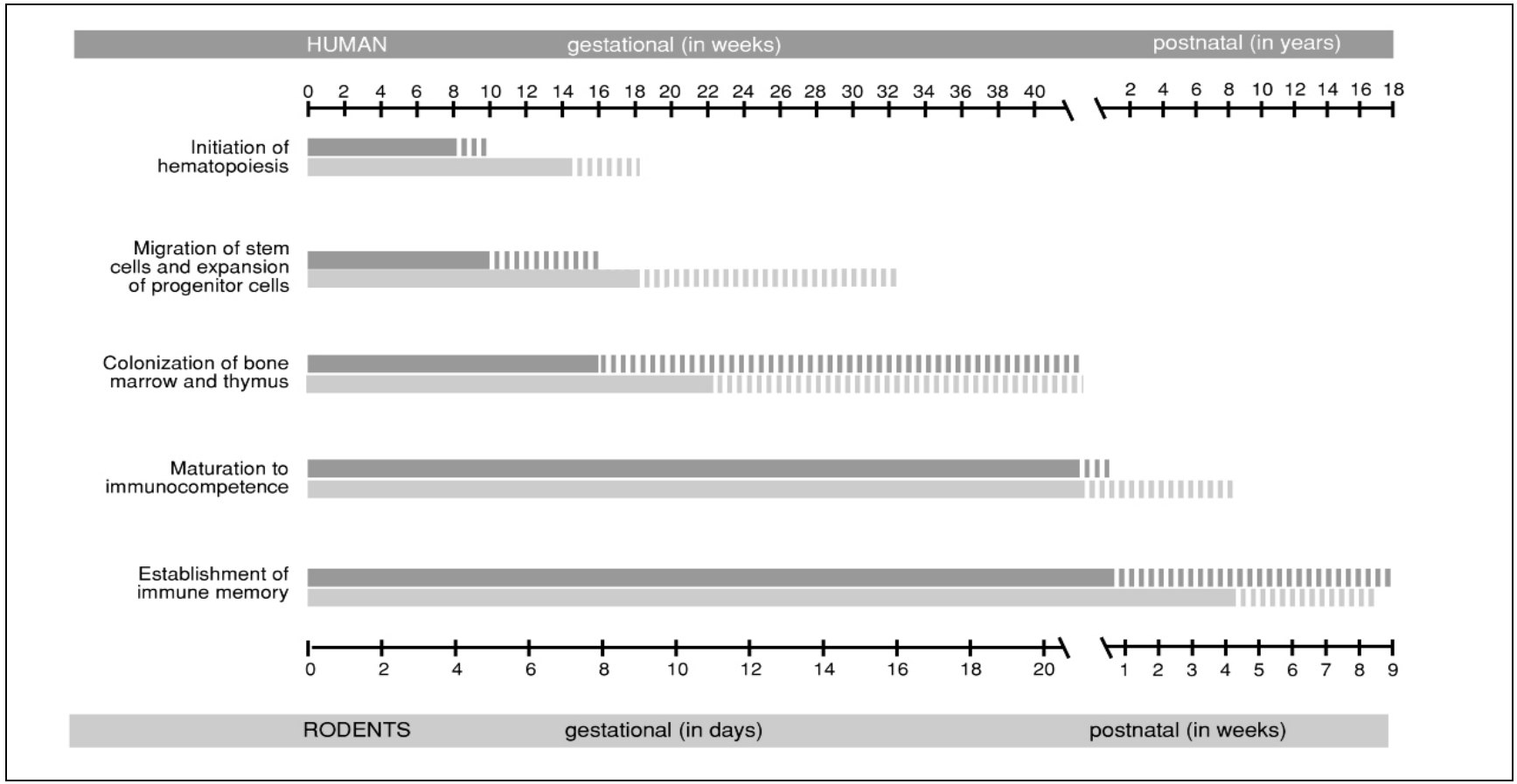
Immune system

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 IV-4 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 vs. 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 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 all are initiated prior to birth in rodents and humans. However, post-natal 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 post-natal 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 lead. The heavy metal lead (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-dependent responses toward Th2-dependent responses (McCabe and Lawrence 1991; Heo et al, 1997). 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 (Chen et al, 1999; Bunn et al, 2001a). However, the timing of exposure appears to be an important factor. Pulsed exposure of dams to

Figure IV-4: Immune System Development in Humans and in Rodents



Pb during late gestation (days 15-21) results in offspring with depressed DTH response function (similar to the complete gestational exposure). But the same Pb exposure earlier in gestation (days 3-9) fails to alter DTH response function in the offspring (Bunn et al, 2001b).

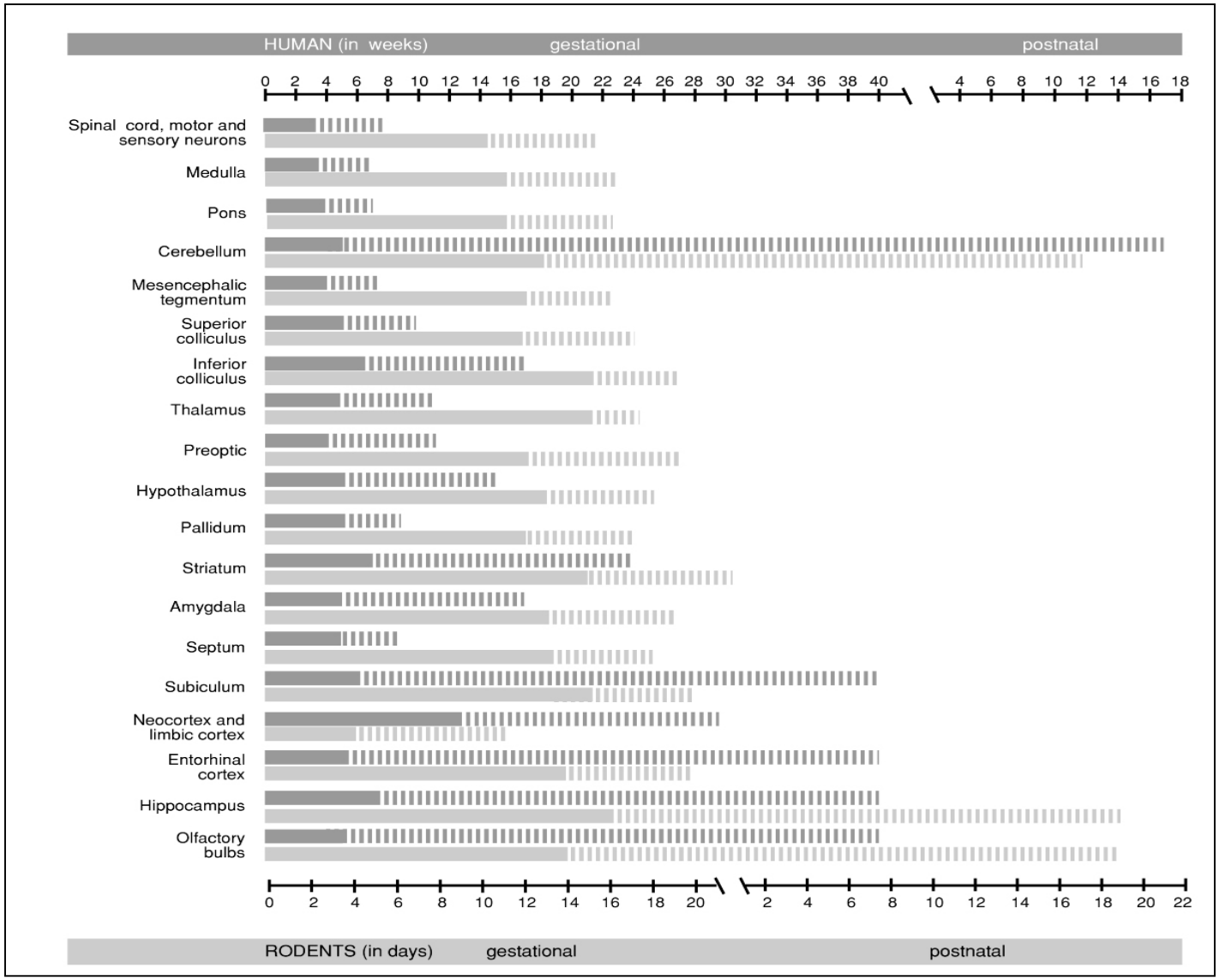
These findings of temporally dependent differential immunotoxic outcome have been extended to other species. For example, exposure of chickens *in ovo* to a single administration of Pb on embryonic day 12 causes depressed DTH function in juvenile chickens. However, exposure of embryos to the same level of Pb only three 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 following 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 IV-5 shows a comparison of nervous system development in humans and in rodents. Following 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 following 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 following 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 period in the human and proceeds in a caudocranial fashion. At birth the brain is immature in 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 following 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 which are designed to monitor learning, short-term memory, color and position discrimination, time perception and motivation are indistinguishable. Depending upon task and endpoint, 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

Figure IV-5: Nervous System Development in Humans and Rodents



correlated with IQ in children (Paule et al, 1999b). Interesting age-related effects have been seen in functional outcomes for the nervous system. For example, chronic marijuana smoke exposure in peri-pubescent 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, 1999a; Schulze et al, 1988).

Lessons Learned from Life stage Examples

The dynamics working group identified key considerations from these specific biological system 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 development processes and many of these processes were common across windows for different biological systems (i.e. apoptosis relevant for both neurological and immunological development).

As mentioned, the workshop participants chose to discuss these three example systems. However even with the limited systems evaluated, large data gaps exist in the completeness of our understanding of these processes and in particular which developmental windows represent real or hypothesized windows of susceptibility. The workshop participants also noted that functional data was often lacking and that it did not allow for the translation of developmental impacts into public health relevant endpoints. Obviously, such lack of data complicates interpretation of animal-to-human comparisons.

An Endpoint Example: Cancer

The workshop participants also felt it was important to evaluate not only windows of susceptibility based on organ system but also by common endpoint. Towards this goal, the group considered the available animal data 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 magnitude of tumors were observed. However, a disclaimer was given that there were 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 Risk Science Institute working group focusing on research needs (ILSI RSI 1996). Thus, a comprehensive comparison 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. 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, being 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. A specific example is 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). Thus, qualitative differences in cancer outcome are possible following age-specific alterations.

2. Are there common unique developmental processes?

To answer this question the workgroup participants addressed three points: 1) What processes?, 2) How/why might these particular processes be important for developmental outcomes? and 3) How would redundancy or repair affect the functional consequences of process perturbation?

What are the processes?

Developing tissues and organs, especially during the pre-natal 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 IV-6 shows the temporal differences in one of these processes (proliferation) across various brain regions during nervous system development in the rat. 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 to say, 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

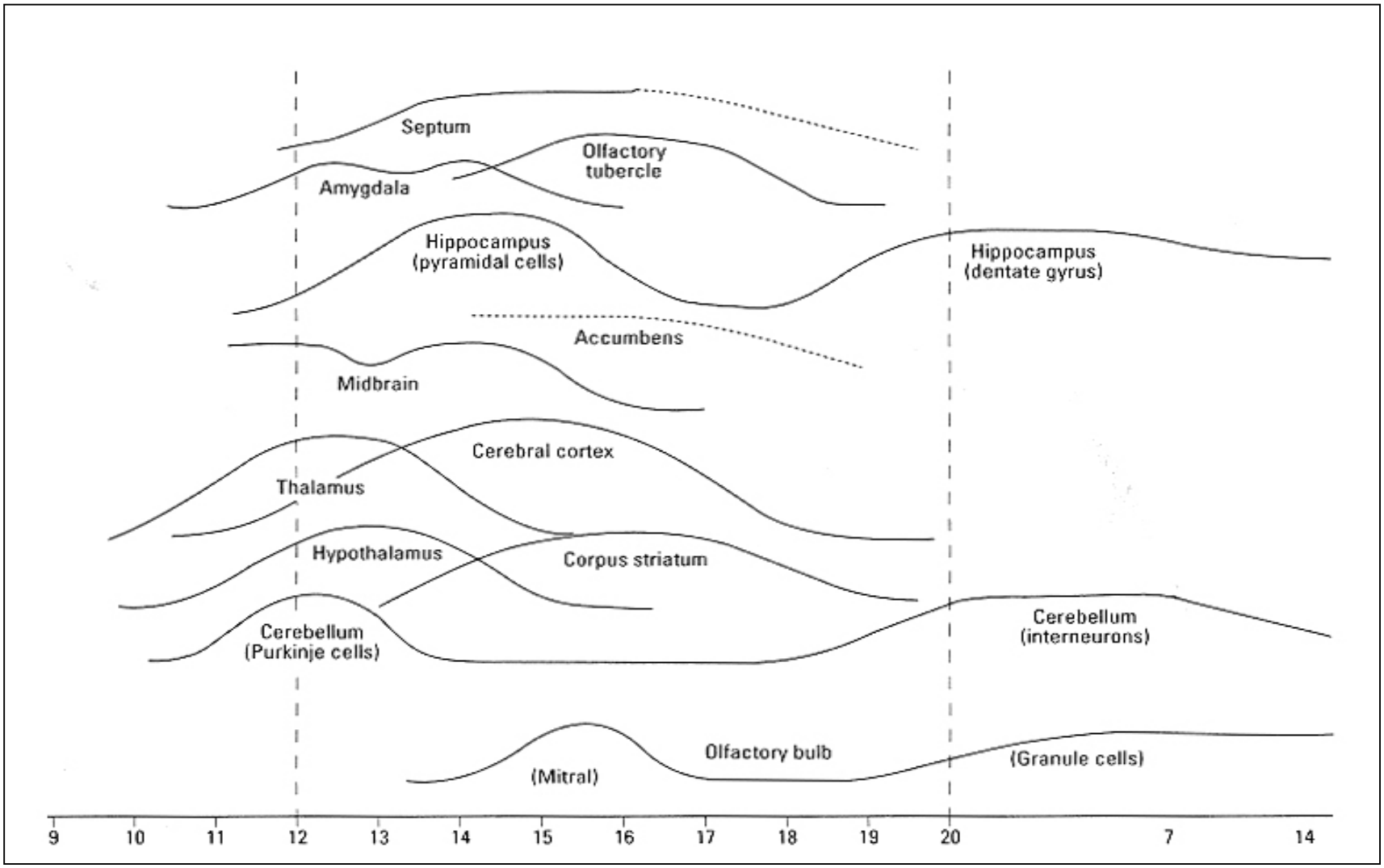
How/why might these particular processes be important for developmental outcomes?

The latter three characteristics involve one of the signal transduction pathways. A recent report by the National Resarch Council (2000) has identified 17 signal transduction pathways which are highly evolutionarily conserved across multiple phyla and which appear to be able to explain most if not all relevant signaling pathways in development. The developmental process may be derailed by: disruption of these steps by altering the length of the period for, or timing of, induction or competence so that they are not contemporaneous; diminishment of the amount of available developmental message; interference with reception of the message; or 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 IV-6 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.

How would redundancy or repair affect the functional consequences of process perturbation?

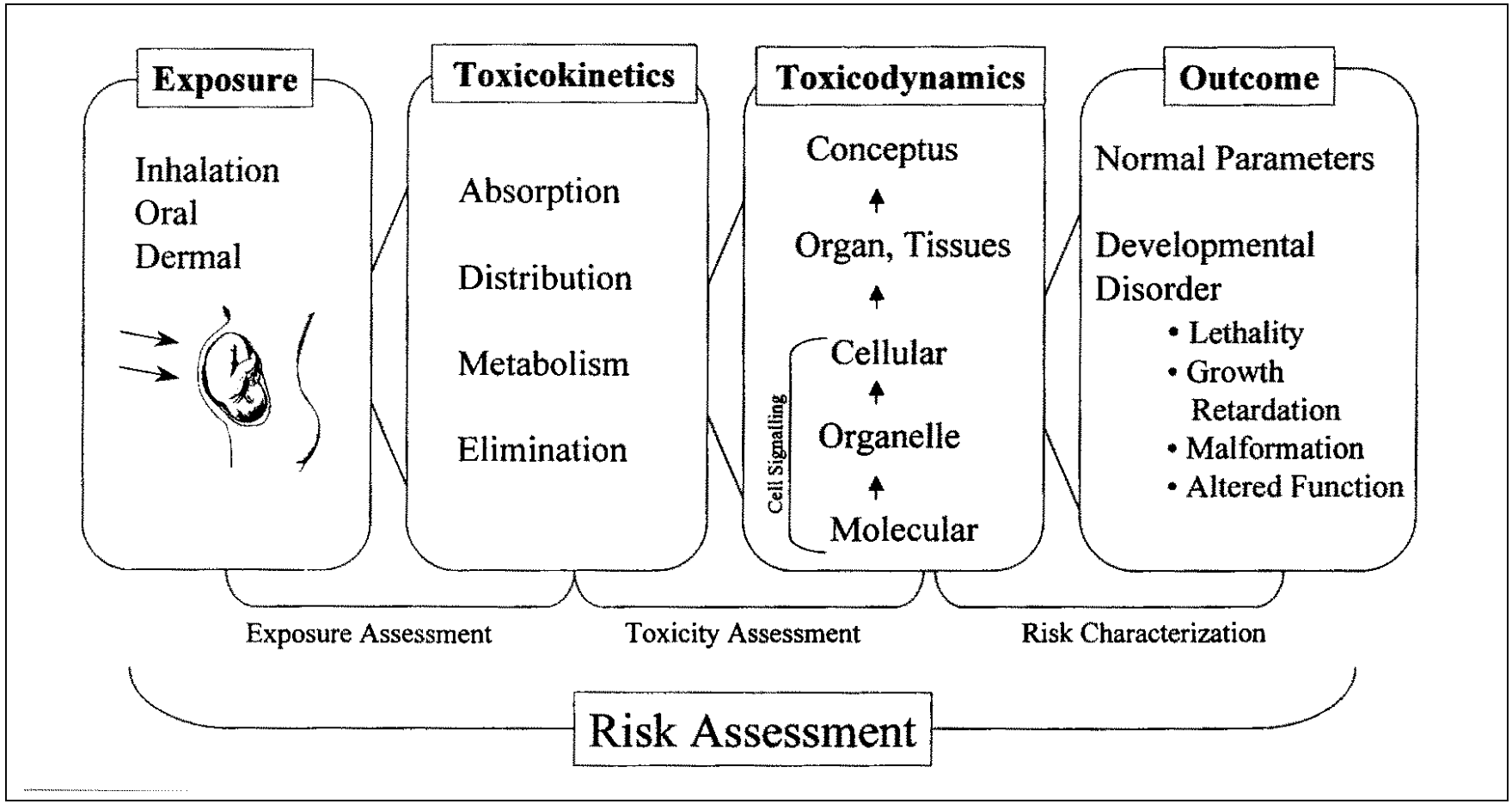
It is noted that the process of development has an inherently dynamic nature and that developing systems possess and exercise multiple signaling pathways simultaneously. Furthermore, many of the pathways have been demonstrated to exert overlapping functions especially in mammalian species. As discussed in 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 knock-out 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 IV-7, we see a detailed diagram from the National Research Council (2000) report that shows 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.

Figure IV-6: Patterns of Neuronal Proliferation in Specific Brain Regions



(Figure adapted from Rodier et al, 1977 with permission of John Wiley & Sons, Inc.)

Figure IV-7: Levels of Mechanistic Inquiry for Assessing the Effects of a Toxicant on Development



(National Research Council 2000)

3. Does knowledge about dynamic developmental processes impact our evaluation of mode of action (MOA) information?

To answer this question the dynamics breakout group addressed two issues: 1) Can we use knowledge from windows of susceptibility to support or refute proposed modes of action?, and 2) Are there different expectations for how developing systems respond to chemical impacts that would make us re-assess our proposed MOA or anticipate additional or different health impacts in children (Faustman et al, 1997, 2000)? Atrazine was used as an illustrative case study to show that knowledge of developmental dynamics did 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 residential (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: PND 23-53 & females: PND 22- 45), pregnancy loss (GD 6-10), decreased dam prolactin release and prostatitis in offspring (PND 1-4), and has effects on lactation (milk quality/production). Atrazine is thus a good an 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.

Application of Life Stage-specific Toxicodynamics in Assessing Children's Risks

The workgroup participants used the proposed framework to identify key ways in which life stage information would inform risk assessment during both the Analysis and the Risk Characterization stages. First, as was illustrated in the previous section, 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 (see Figure IV-1) 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 inter-relatedness 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.

In addition, workshop participants felt that such information would provide quantitative information relevant for assessing dose-response, especially dose and time 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 area under the curve (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 been shown to have an impact on quantitative dose-response assessments is seen in cases of pre-, post- and neonatal carcinogen exposure. Anderson et al, (2000) has summarized published literature for transplacental and neonatal carcinogens by target tissue and time of exposure for chemical and radiation exposures. This paper methodically evaluates this published literature and discusses 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 paper cites experimental evidence for each of these factors. Increasing understanding of how all these factors can impact qualitative and quantitative tissues and species specificity is needed; however, compelling examples exist for quantitative differences. For example, "in patas monkeys transplacental ENU 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).

Since tumor incidence determines the slope of the dose-response curve and the Q* value, 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. The current approach for cancer risk assessment for children and the impact of early life stage exposures needs to be re-examined to ensure adequate protection of children in cancer risk assessment.

Table IV-3 lists a number of prompts that can be useful in defining the depth of the database that is available. The dynamics breakout group discussed these prompts and felt that these or similar prompts should be included in every assessment of the potential for childhood toxicity.

Table IV-3. Assessment Questions to be Considered for Each Exposure Window

Exposure: questions to be developed in separate activities.
Is the exposure interval (potentially) relevant to the outcome of concern?
Is the relevant kinetic data (ADME and/or embryofetal) adequately defined?
Does the relevant receptor exist during the exposure interval in question?
Have the toxicodynamics been adequately defined?
Are other relevant physiologic and metabolic parameters defined?
Is this a critical period for the outcome of concern?
Is a mechanism or MOA relevant to the exposure period understood?
Does an animal model exist for the relevant effect/exposure interval?
…….Is the model adequate?
…….Is the model validated?
…….Does the model cover relevant critical period(s)
…….Is the model validated as to ontology relative to human ontology?
…….Is the model validated as to PK/PD?
Does a protocol currently exist which would allow evaluation of the outcome of concern?
…….Is the protocol adequate?
…….Does the protocol cover relevant critical periods of exposure?
…….Is the protocol model validated as above?
Is hazard characterization adequate for the interval in question?
…….Is the outcome of exposure in this time interval the same or different from adults?
…….If same, are dose-response parameters unchanged?
…….If different, are dose-response parameters defined?
…….Is uncertainty in hazard characterization quantified?
Do effects in this interval violate classical dose-response models (cellular reprogramming, etc.)?
Has variability in susceptibility been defined for this exposure interval?
Are epidemiologic data available?
…….Is this reproducible?
…….How strong is the association?
…….Is association biologically plausible?
Have extrapolation issues been adequately addressed:
…….across species?
…….across routes of exposure?
…….across dose ranges (high to low dose extrapolation)?
Are environmental interactions relevant to this time period known?
…….Disease factors
…….Lifestyle factors
…….Nutritional factors

Table IV-3. Assessment Questions to be Considered for Each Exposure Window, Cont'd

OVERALL ASSESSMENT QUESTIONS:
Given above information, is the exposure interval relevant to the outcome of concern?
Is knowledge defined above sufficient for appropriate risk assessment?
If knowledge inadequate, is a default approach necessary?
What further research is necessary to improve risk assessment for this exposure interval and outcome?

The prompts are applicable in all phases of the proposed framework. Moreover, given that the framework allows for an iterative process between the various phases, it is likely that these prompts may be reevaluated at various stages of the process. It is important to recognize that not all of the prompts will be important for every risk assessment. The risk assessor must make initial judgements about what data are relevant for the specific assessment. This should include consideration of "critical" data needs, as well as "nice to have" data needs (Moore et al, 1995). Also to be considered is the impact of the use of default values in the absence of that data.

In the Problem Formulation phase, the prompts will provide an initial evaluation of the database available, and will function together with the risk management focus for the assessment in determining what will or can be carried out in the Analysis phase.

In the Analysis phase, the prompts will provide the risk assessor with another opportunity to evaluate whether there are adequate data to conduct a risk assessment that will provide the risk manager with the information outlined in the Problem Formulation stage. As data are analyzed, the risk assessor should continue to evaluate the impact of the data on potential data needs.

In the Risk Characterization phase, the prompts will serve as a final review of the database and be important to developing a complete narrative statement that captures for the risk manager both the impact of the data that are available and the limitations of the database where data are not available. These prompts would also serve to identify where both qualitative and quantitative considerations for children's risk would be possible.

Critical Data Needs

The dynamics workgroup recognized and identified data needs throughout its deliberations, and many are implicit in the preceding sections of the workgroup report. In addition, some specific data needs and questions were highlighted 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 endpoints

- Development of more endpoints for assessing system function that can be used in both humans and animals. This is a major issue since 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 FMRI.

- Resources for animal to human correlation

- Better understanding of the toxicodynamics of "public health" relevant endpoints, 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, 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, the workgroup noted the need for multidisciplinary training for work in children's health at all levels (graduate students, post-docs, scientists in the field). The group also acknowledged the value of and need for multidisciplinary workshops and interactions, such as the present workshop.

Conclusions

The toxicodynamics workgroup conclusions are summarized in Table IV-4. The workgroup participants concluded that there were distinct life stages evident across development with both known and hypothesized "windows of susceptibility". These various life stages were based on differences in development defined by differences in relevant dynamic processes occurring at the molecular, cellular, organ and physiological level, and these differences could define in what systems and at what magnitude an environmental impact would be manifested. They identified the potential for apparent species differences in response to environmental exposures if the dynamic processes were not compared at equivalent doses, timepoints and processes 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 such as hazard characterization, dose-response and risk characterization. The workgroup identified and discussed some common dynamic processes that can impact susceptibility. For example, impacts on apoptosis versus migration would provide clues as to what biological systems and at what times these impacts would be identifiable. Given these conclusions, the group considered some of the implications for animal testing and briefly discussed some of the ways to improve our understanding of dynamic processes across species, dose and life stage. Finally, the group emphasized the need to improve our assessments of functional and public health relevant alterations in our current testing approaches.

Table IV-4. Conclusions from the Dynamics Workgroup

1. There are distinct life stages with both known and hypothesized "windows of susceptibility."
2. Dynamic differences that impart susceptibility can exist between organ systems, within organs and at the biochemical or molecular process level.
3. There can be apparent species differences in response if these dynamic processes across time and process are not considered.
4. These differences can impact all processes of risk assessment from hazard characterization and dose-response evaluation to risk characterization.
5. There are common dynamic processes that can impact susceptibility.
6. The group considered some of the animal testing protocols and identified research needs to improve our understanding of dynamic processes across species, dose and life stage.
7. The group emphasized the need to improve our assessment of functional and public health relevant alterations in our current testing approaches.

V. RISK CHARACTERIZATION AND THE FRAMEWORK

The Risk Characterization workgroup was charged with determining the data and procedures that are needed in order to ensure that risks to children are accounted for in the risk assessment process. Much of the effort of the group was devoted to refining the draft risk assessment framework that was prepared before the workshop. While risk characterization is the final step in the framework, it cannot succeed unless the inputs from the preceding steps are appropriately directed at the problems associated with assessing risks to developing individuals. Therefore, refining the early stages of the process, particularly the problem formulation step, was felt to be critical work for our breakout group.

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.

In addition to considerations of intrinsic sensitivity, exposures to toxicants may be different during development because of life stage-specific behaviors (e.g., mouthing during infancy, breastfeeding) or functions (e.g., the presence of a placenta prenatally, high respiratory rate in early childhood).

There were a number of focus questions that the breakout group was asked to address. Our responses are given below, but the important overall message is that we do consider it necessary to have developmental toxicity data and life stage-specific exposure assessments in order to adequately characterize children's risk. Specifically:

1. Have unique susceptibilities been identified associated with one or more stages of prenatal development or childhood?

Yes. 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-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. Epidemiological 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., Appendix 1).

2. **Are there mechanisms of toxicity unique to children, or are just the outcomes different?**

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 was the suspicion of the group that there probably are mechanisms of action that are specific to development, while 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.

3. **Are there behaviors that are peculiar to children that make exposure by certain routes or media more of a concern?**

Yes. A number of examples were provided in the workgroup discussions, including breastfeeding, the propensity of children of a certain age to place things in their mouths, diets consisting of a relatively narrow selection of foods, crawling or short stature, making the breathing zone much closer to the floor or ground, and many others. There are reviews available on the subject (e.g., Cohen Hubal et al, 2000).

4. **In the context of risk assessment, how should we address responses in children that are different from those in adults? For example, is an additional uncertainty factor warranted? If so, what is the nature of the uncertainty? Could there be multiple sources of child-specific uncertainty? Is the use of an uncertainty factor dependent on whether the difference between child and adult response is qualitative or quantitative? For qualitative differences in outcome, does it matter if these are mechanistically different? Does it matter whether there is likely to be a cumulative/chronic effect from exposure? What magnitude (or range) should the uncertainty factor (UF) be? What data would be necessary to alleviate the uncertainty represented by the UF?**

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, e.g., by generating more data or by using the uncertainty factor already in place at 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 epidemiological 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. Certainly, it needs to be acknowledged that additional data may increase or decrease the reference dose (RfD) for a compound. Finally, it was reiterated that there are already policies in place to accommodate database deficiencies.

5. What assumptions can be made about childhood susceptibility or childhood exposure when the database is incomplete?

It was the strong consensus of the group 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 responses to the focus questions clearly indicate the need for explicit consideration of children in human health risk assessment, and provided guidance for development of the Framework depicted in Figure II-1.

In this Framework, the Problem Formulation step focuses on the interrelationships among exposures, effects and host factors. These considerations are consistent with the way epidemiological 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; e.g., genetic, nutritional or SES 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; i.e., 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 due to an inadequate conceptual

model, in which case the Framework allows for refinement of the model and re-entry into the Analysis phase.

Risk Characterization consists of a life stage-specific consideration of risk combined with uncertainty and variability analyses, culminating 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 vs. other methods for estimating and incorporating variability into the assessment. EPA has already identified a number of areas of uncertainty that should be accounted for in the assessment. These include extrapolation from an animal species to humans, the range of variability within the human population, extrapolating from less-than-chronic exposures to continuous exposure scenarios, extrapolating to a lowest-observed-adverse-effect level on the dose-response curve, and the absence or inadequacy of key data sets. The overall vision for risk characterization is a meaningful probabilistic calculation of risk.

The workgroup developed a list of needs that if met would reduce, or at least allow us to characterize, the range of uncertainties for children's risk assessment.

For hazard characterization, these are:

- Understanding the relevance of animal models for predicting outcomes in humans
- Understanding the comparative developmental profiles in animal models and humans (are the life stages being studied in animal testing analogous to human life stages of concern?)
- Availability of data for relevant life stages
- Better methodology (e.g., in testing protocols), applied more often, on functional outcomes at relevant life stages in animal models and humans; the methods should be sensitive, specific, and account for variability in responses and norms
- Understanding of modes of action underlying toxicity
- Understanding the temporal relationships between exposure and outcomes, particularly for delayed outcomes
- Understanding of host factors that contribute to susceptibility

For toxicokinetics:

- Descriptive kinetics for the appropriate life stage(s)
- Exposure and dose-response relationships in animal models and children

For exposure assessment:

- Life stage-appropriate exposure factor data in humans
- Habits and practices (e.g., diet, behavior) of children
- Sources and pathways (e.g., patterns of use) in children
- Characterization of complex exposure scenarios, including simultaneous exposures to multiple chemicals through multiple pathways

Of these, the group listed the following as the highest research priorities:

- Understanding of critical windows of developmental susceptibility, and of comparative developmental schedules of animals and humans
- Habits and practices data (food, behavior, location, etc.) for children
- Better methodology (e.g., in testing protocols), applied more often, on functional outcomes at relevant life stages in animal models and humans; the methods should be sensitive, specific, and account for variability in responses and norms
- Understanding the temporal relationships between exposure and outcomes, particularly for delayed outcomes
- Understanding of host factors that contribute to susceptibility
- Latent sequelae of early events
- Monitoring of disease trends and exposures

REFERENCES

Adams J, Barone S Jr, LaMantia A, Philen R, Rice DC, Spear L, et al. 2000. Workshop to identify critical windows of exposure for children's health: neurobehavioral work group summary. Environ Health Perspect 108:535-544.

Adkins B, Mueller C, Okada CY, Reichert RA, Weissman IL, Spangrude GJ. 1987. Early events in T-cell maturation. Annu Rev Immunol 5:325-365.

Anderson LM, Diwan BA, Fear NT, Roman E. 2000. Critical windows of exposure for children's health: cancer in human epidemiological studies and neoplasms in experimental animal models. Environ Health Perspect 108:573-594.

Anderson LM, Jones AB, Riggs CW, Kovatch RM. 1989. Modification of transplacental tumorigenesis by 3-methylcholanthrene in mice by genotype at the Ah locus and pretreatment with beta-naphthoflavone. Cancer Res 49:1676-1681.

Barker N, Hadgraft J, Rutter N. 1987. Skin permeability in the newborn. J Invest Dermatol 88:409-411.

Bogdanffy MS, Daston G, Faustman EM, Kimmel CA, Kimmel GL, Seed J, et al. 2001. Harmonization of cancer and noncancer risk assessment: proceedings of a consensus-building workshop. Toxicol Sci 61:18-31.

Bunn TL, Parsons PJ, Kao E, Dietert RR. 2001a. Gender-based profiles of developmental immunotoxicity to lead in the rat: assessment in juveniles and adults. J Toxicol Environ Health 64:101-118.

Bunn TL, Parsons PJ, Kao E, Dietert RR. 2001b. Exposure to lead during critical windows of embryonic development: differential outcome based on stage of exposure and gender. Toxicol Sci 64:57-66.

Byczkowski JZ, Kinkead ER, Leahy HF, Randall GM, Fisher JW. 1994. Computer simulation of the lactational transfer of tetrachloroethylene in rats using a physiologically-based model. Toxicol Appl Pharmacol 125:228-236.

Chen S, Golemboski KA, Sanders FS, Dietert RR. 1999. Persistent effect of *in utero* meso-2,3-dimercaptosuccinic acid (DMSA) on immune function and lead-induced immunotoxicity. Toxicology 132:67-79.

Clewell HJ, Gearhart JM, Gentry PR, Covington TR, VanLandingham CB, Crump KS, et al. 1999. Evaluation of the uncertainty in an oral reference dose for methylmercury due to interindividual variability in pharmacokinetics. Risk Anal 19:547-558.

Cohen Hubal EA, Sheldon LS, Burke JM, McCurdy TR, Berry MR, Rigas ML, et al. 2000. Children's exposure assessment: a review of factors influencing Children's exposure, and the data available to characterize and assess that exposure. Environ Health Perspect 108:475-486.

Cresteil T. 1998. Onset of xenobiotic metabolism in children: toxicological implications. Food Addit Contam 15:45-51.

Dietert RR, Etzel RA, Chen D, Halonen M, Holladay SD, Jarabek AM, et al. 2000. Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary. Environ Health Perspect 108:483-490.

Dorne JLCM, Walton K, Renwick AG. 2001. Uncertainty factors for chemical risk assessment: human variability in the pharmacokinetics of CYP1A2 probe substrates. Food Chem Toxicol 39:681-696.

Fanucchi MV, Buckpitt AR, Murphy ME, Plopper CG. 1997. Naphthalene cytotoxicity of differentiating Clara cells in neonatal mice. Toxicol Appl Pharmacol 144:96-110.

Faustman E, Bartell S. 1997. Review of noncancer risk assessment: applications of benchmark dose methods. Human Ecol Risk Assessment 3:893-920.

Faustman EM, Ponce RA, et al. 1997. Experimental approaches to evaluate mechanisms of developmental toxicity. In: Handbook of Developmental Toxicology (Hood R, ed). New York:CRC Press, 14-41.

Faustman EM, Silbernagel SM, Fenske RA, Burbacher TM, Ponce RA. 2000. Mechanisms underlying children's susceptibility to environmental toxicants. Environ Health Perspect 108:13-21.

Finette BA, Poseno T, Vacek PM, Albertini RJ. 1997. The effects of maternal cigarette smoke exposure on somatic mutant frequencies at the hprt locus in healthy newborns. Mutat Res Fund Mol M 377:115-123.

Garland M, Szeto HH, Daniel SS, Tropper PJ, Myers MM, Stark RI. 1998. Placental transfer and fetal metabolism of zidovudine in the baboon. Pediatric Res 44:47-53.

Gentry PR, Covington TR, Anderson ME, Clewell HJ 3rd. 2002. Application of a physiologically based pharmacokinetic model for isopropanol in the derivation of a reference dose and reference concentration. Regul Toxicol Pharmacol 36:51-68.

Gibbs JP, Murray G, Risler L, Chien JY, Dev R, Slattery JT. 1997. Age-dependent tetrahydrothiophenium ion formation in young children and adults receiving high dose busulfan. Cancer Res 57:5509-5516.

Ginsberg GL, Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, et al. 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicol Sci 66:185-200.

Guzelian PS, Henry CJ, Olin SS (eds). 1992. Similarities and Differences Between Children and Adults: Implications for Risk Assessment. Washington, DC:ILSI Press.

Haddad S, Restieri C, Krishnan K. 1999. Physiological modeling to characterize adult-children differences in pharmacokinetics. The Toxicologist 48:Abstract # 657.

Hashimoto H, Nakagawa T, Yokoi T, Sawada M, Itoh S, Kamataki T. 1995. Fetus-specific CYP3A7 and adult-specific CYP3A4 expressed in Chinese hamster CHL cells have similar capacity to activate carcinogenic mycotoxins. Cancer Res 55:787-791.

Heo Y, Lee WT, Lawrence DA. 1997. *In vivo* the environmental pollutants lead and mercury induce oligo T cell responses skewed toward type-2 reactivities. Cell Immunol 179:185-195.

Hines RN, McCarver DG. 2002. The ontogeny of human drug metabolizing enzymes: Phase I oxidative enzymes. J Pharm Expt Ther 300:355-360.

Holladay SD, and Smialowicz RJ. 2000. Development of the murine and human immune system: differential effects of immunotoxicants depend on time of exposure. Environ Health Perspect 108:463-473.

ILSI RSI. 1996. Research needs on age-related differences in susceptibility to chemical toxicants: A report prepared by an ILSI Risk Science Institute working group.

Institute of Medicine. 1997. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC:National Academy Press, 31-35.

Juchau MR. 1980. Drug biotransformation in the placenta. Pharmacol Ther 8:501-524.

Kakiuchi Y, Kohda Y, Miyabe M, Momose Y. 1999. Effect of plasma alpha1-acid glycoprotein concentration on the accumulation of lidocaine metabolites during continuous epidural anaesthesia in infants and children. Int J Clin Pharmacol Ther 37:493-498.

Kedderis GL. 1997. Extrapolation of *in vitro* enzyme induction data to humans *in vivo*. Chemico-Biological Interactions 107:109-121.

Kimmel CA. 2001. Using mechanistic data from developmental and molecular biology for developmental toxicity risk assessment. Continuing Education course: Improving risk assessment for human developmental defects: The promise of recent advances in developmental biology and genomics. Society of Toxicology meeting, San Francisco, CA, March 25-29.

Kitada M, Taneda M, Ohi H, Komori M, Itahashi K, Nagao M, Kamataki T. 1989. Mutagenic activation of aflatoxin B1 by P-450 HFLa in human fetal livers. Mutation Res 227:53-58.

Krishnan K, Andersen ME. 1998. Physiological pharmacokinetic models in the risk assessment of developmental neurotoxicants. In: Handbook of Developmental Neurotoxicity (Slikker W, Chang L, eds). New York:Academic Press, 709-725.

Lee JE, Chen S, Golemboski KA, Parsons PJ, Dietert RR. 2001. Developmental windows of differential lead-induced immunotoxicity in chickens. Toxicology 156:161-170.

Lemasters GK, Perreault SD, Hales BF, Hatch M, Hirshfield AN, Hughes CL, et al. 2000. Workshop to identify critical windows of exposure for children's health: reproductive health in children and adolescents work group summary. Environ Health Perspect 108:505-509.

Martonen TB, Musante CJ, Segal RA, Schroeter JD, Hwang D, Dolovich MA, et al. 2000. Lung models: strengths and weaknesses. Resp Care 45:712-736.

McCabe MJ Jr, and Lawrence DA. 1991. Lead, a major environmental pollutant, is immunomodulatory by its differential effects on CD4+ T cell subsets. Toxicol Appl Pharmacol 111:13-23.

McCabe MJ Jr, Singh KP, Reiners JJ Jr. 1999. Lead intoxication impairs the generation of a delayed hypersensitivity response. Toxicology 139:255-264.

McCarver DG, Hines RN. 2002. The ontogeny of human drug metabolizing enzymes: Phase II conjugation enzymes and regulatory mechanisms. J Pharm Expt Ther 300:361-366.

McConnell EE. 1992. Comparative responses in carcinogenesis bioassays as a function of age at first exposure. In: Similarities and Differences Between Children and Adults: Implications for Risk Assessment (Guzelian PS, Henry CJ, Olin SS, eds). Washington, DC:ILSI Press, 66-78.

Miller MS, Jones AB, Park SS, Anderson LM. 1990. The formation of 3-methylcholanthrene-initiated lung tumors correlate with induction of cytochrome P4501A1 by the carcinogen in fetal but not adult mice. Toxicol Appl Pharmacol 104:235-245.

Moore JA, Daston GP, Faustman E, Golub MS, Hart WL, Hughes C Jr, et al. 1995. An evaluative process for assessing human reproductive and developmental toxicity of agents. Reprod Toxicol 9:61-95.

National Research Council (NRC). 1993. Pesticides in the diets of infants and children. Washington, DC:National Academy Press.

National Research Council (NRC). 2000. Scientific frontiers in developmental toxicology and risk assessment. Committee on Developmental Toxicology. Washington, DC:National Academy Press.

Paule MG, Cranmer JM, Wilkins JD, Stern HP, Hoffman EL. 1988. Quantitation of complex brain function in children: preliminary evaluation using a nonhuman primate behavioral test battery. Neurotoxicology 9:367-378.

Paule MG, Chelonis JJ, Buffalo EA, Blake DJ, Casey PH. 1999. Operant test battery performance in children: correlation with IQ. Neurotoxicol Teratol 21:223-230.

Paule MG, Meck WH, McMillan DE, McClure GY, Bateson M, Popke EJ, et al. 1999. Symposium overview: the use of timing behaviors in animals and humans to detect drug and/or toxicant effects. Neurotoxicol Teratol 21:491-502.

Pelekis M, Gephart LA, Lerman SE. 2001. Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. Regul Toxicol Pharmacol 33:12-20.

Pinkerton KE, Joad JP. 2000. The mammalian respiratory system and critical windows of exposure for children's health. Environ Health Perspect 108:457-462.

Plopper CG, Weir AJ, Nishio SJ, Chang A, Voit M, Philpot RM, et al. 1994. Elevated susceptibility to 4-ipomeanol cytotoxicity in immature Clara cells of neonatal rabbits. J Pharmacol Exp Ther 269:867-880.

Pryor JL, Hughes C, Foster W, Hales BF, Robaire B. 2000. Critical windows of exposure for children's health: the reproductive system in animals and humans. Environ Health Perspect 108:491-503.

Renwick AG. 1998. Toxicokinetics in infants and children in relation to the ADI and TDI. Food Addit Contam 15(suppl):S17-S35.

Renwick AG, Dorne JL, Walton K. 2000. An analysis of the need for an additional uncertainty factor for infants and children. Regul Toxicol Pharmacol 31:286-296.

Rice D, and Barone S Jr. 2000. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108:511-533.

Rice JM, Rehm S, Donovan PJ, Perantoni AO. 1989. Comparative transplacental carcinogenesis by directly acting and metabolism-dependent alkylating agents in rodents and nonhuman primates. IARC Sci Publ 96:17-34.

Schulze GE, McMillan DE, Bailey JR, Scallet A, Ali SF, Slikker W Jr, et al. 1988. Acute effects of delta-9-tetrahydrocannabinol (THC) in rhesus monkeys as measured by performance in a battery of cognitive function tests. J Pharmacol Exp Ther 245:178-186.

Selevan SG, Kimmel CA, Mendola P. 2000. Identifying critical windows of exposure for children's health. Environ Health Perspect 108:451-455.

Slikker W, Hill DE, Young JF. 1982. Comparison of the transplacental pharmacokinetics of 17ß-estradiol and diethylstilbestrol in the subhuman primate. J Pharmacol Exp Ther 221:173-182.

Slikker W Jr, Beck BD, Cory-Slechta DA, Paule MG, Anger WK, et al. 2000. Cognitive tests: interpretation for neurotoxicity. Toxicol Sci 58:222-234.

Smiley-Jewell SM, Nishio SJ, Weir AJ, Plopper CG. 1998. Neonatal Clara cell toxicity by 4-ipomeanol alters bronchiolar organization in adult rabbits. Am J Physiol 274(4 Pt 1):L485-L498.

Suchy FJ, Bucuvalas JC, Novak DA. 1987. Determinants of bile formation during development: ontogeny of hepatic bile acid metabolism and transport. Semin Liver Dis 7:77-84.

U.S. EPA. 1992. Dermal exposure assessment: principles and applications. EPA/600/8-91/011B. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 1992. Framework for ecological risk assessment. EPA/630/R-92/001. Washington, DC:U.S. Environmental Protection Agency, Risk Assessment Forum. Available: http://cfpub.epa.gov/ncea/raf/rafrprts.cfm [accessed 6 December 2000].

U.S. EPA. 1994. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. EPA/600/8-90/066F. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 1995a. Policy on evaluating health risks to children. Washington, DC:Science Policy Council, U.S. Environmental Protection Agency. Available: http://www.epa.gov/osp/spc/memohlth.htm [accessed 2 December 2002].

U.S. EPA. 1995b. Policy for risk characterization at the U.S. Environmental Protection Agency. Washington, DC:Science Policy Council, U.S. Environmental Protection Agency. Available: http://www.epa.gov/osp/spc/rcpolicy.htm [accessed 2 December 2002].

U.S. EPA. 1997. Guidance on cumulative risk assessment. Part 1: Planning and scoping. Washington, DC:Science Policy Council, U.S. Environmental Protection Agency.

U.S. EPA. 1999. Guidelines for carcinogen risk assessment. Draft, July. NCEA-F-0644. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 2000. Child-Specific Exposure Factors Handbook. NCEA-W-0853. 01 Jun 2000. Washington, DC:U.S. Environmental Protection Agency. Available: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=17880 [accessed 6 December 2002].

U.S. EPA Risk Assessment Forum. 2000. Summary report of the technical workshop on issues associated with considering developmental changes in behavior and anatomy when assessing exposure to children. EPA/630/R-00/005. Available: http://www.epa.gov/ncea/raf/workshops.htm [accessed 2 December 2002].

U.S. EPA Risk Assessment Forum. 2002. A review of the reference dose and reference concentration processes. EPA/630/P-02/002F. Available: http://cfpub.epa.gov/ncea/cfm/nceawhatnew [accessed 9 April 2003].

U.S. EPA. 2002. The grouping of a series of triazine pesticides based on a common mechanism of toxicity. Washington, DC: Office of Pesticide Programs. Available: http://www.epa.gov/oppsrrd1/cumulative/triazines/triazinescommonmech.pdf [accessed 6 December 2002].

Weiss B. 2000. Vulnerability of children and the developing brain to neurotoxic hazards. Environ Health Perspect 108:375-381.

Wester RC, Maibach HI, Surinchak J, Bucks DAW. 1985. Predictability of *in vitro* diffusion systems. Effect of skin types and ages on percutaneous absorption of trichlorban. In: Percutaneous Absorption (Bronaugh RL, Maibach HI, eds). New York:Marcel Dekker, 223-226.

Zemlickis D, Klein J, Moselhy G, Koren G. 1994. Cisplatin protein binding in pregnancy and the neonatal period. Med Pediatric Oncol 23:476-479.

Zoetis T, Walls I (eds). 2003. Principles and Practices for Direct Dosing of Pre-weaning Mammals in Toxicity Testing and Research. Washington DC:ILSI Press.

APPENDIX 1

Children's Health and the Environment: Public Health Issues and Challenges for Risk Assessment*

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1.0 Introduction

The protection of human health against disease and injury caused by toxic chemicals in the environment is the ultimate goal of risk assessment and risk management. Historically, risk assessment focused on adult exposures and toxicities and gave little consideration to vulnerable life stages, such as fetal development and early childhood. An emphasis on adult cancer risk and the evolution of methodologies for estimating cancer risks that are different from the methods used to assess other health effects tended to further diminish the importance for risk assessment of children's exposures and outcomes. In addition, the use of default factors based on the healthy young adult did not account adequately for the unique exposures and sensitivities of fetuses, infants and children (Landrigan and Carlson 1995).

In the past decade, stimulated especially by the 1993 National Research Council report on *Pesticides in the Diets of Infants and Children* (National Academy of Sciences 1993), recognition has grown that children are a group within the population who have unique exposures and special vulnerabilities to chemical toxicants. It is now understood that children require an approach to risk assessment that considers their particular characteristics. The present report developed by the International Life Sciences Institute (ILSI) with support from the U.S. Environmental Protection Agency (EPA) is intended to consider the elements and structure of a child-protective approach to risk assessment.

The purpose of this introductory chapter is to establish the context for a discussion on child-protective risk assessment by: 1) summarizing information on the vulnerability of children to chemicals in the environment; 2) presenting a rationale, based on considerations of public health and preventive medicine, for developing an approach to risk assessment that considers the unique exposures and special sensitivities of infants and children; and 3) outlining issues of importance for assessing environmental health risks to children.

The word "children" is used throughout this paper to include all stages of development (fetuses, infants and children) from conception to age 21 years.

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2.0 The Historical Development of Child-Protective Risk Assessment

The NRC Report on Pesticides in the Diets of Infants and Children

The publication in 1993 of the National Research Council's (NRC) report on Pesticides in the Diets of Infants and Children (National Academy of Sciences 1993) was a critical event in raising awareness of the importance for risk assessment of children's environmental health. This report elevated concern on a broad national level about children's special vulnerabilities to environmental agents. It made clear that protection of the health of vulnerable populations would require a new approach to risk assessment.

The NRC report recommended an approach to risk assessment that moved beyond consideration of "average" exposures based primarily on adult characteristics to one that accounted for the heterogeneity of exposures and for potential differential sensitivities at various life stages, particularly during prenatal development, infancy and childhood. The NRC report built on guidelines that EPA had published for developmental toxicity risk assessment in 1986 and revised in 1991 (U.S. Environmental Protection Agency 1986, 1991). It built also on other published documents such as the International Life Sciences Institute's (ILSI) report on *Similarities and Differences between Children and Adults: Implications for Risk Assessment* (Spielberg 1992).

Infants and children were identified in both the NRC and ILSI reports as groups within the population who require special consideration in risk assessment because of: 1) their unique patterns of exposures to environmental hazards, and 2) special vulnerabilities (the word "children" is used throughout this paper to include all stages of development (fetuses, infants and children) from conception to age 21 years). The NRC report noted that "children are not little adults." It called for the development of new risk assessment methods that would incorporate better data on children's exposures to chemicals, along with improved information on the potentially harmful effects of chemicals during fetal development, infancy and childhood (National Academy of Sciences 1993).

To "provide a more complete characterization of risk", the NRC Committee recommended use of exposure distributions rather than point estimates. It noted that levels of exposure could differ by several orders of magnitude between children and adults. The NRC report recommended also that exposure assessment methods be expanded to consider exposures to multiple chemicals with multiple routes of exposure (National Academy of Sciences 1993).

To enhance characterization of the susceptibility of children, the NRC Committee recommended the development of physiologically based pharmacokinetic models that could describe the unique features of young, developing humans. To assess the long-term and delayed effects of early exposures, the Committee recommended that "it would be desirable to develop bioassay protocols that provide direct information on the relative contribution of exposures at different ages to lifetime risks." The Committee called for further development of "appropriate toxicological tests for perinatal and childhood toxicity" to address issues not addressed in current testing guideline protocols (National Academy of Sciences 1993).

The NRC Committee concluded that "in the absence of data to the contrary, there should be a presumption of greater risk to infants and children. To validate this presumption, the Committee recommended that "the sensitivity of mature and immature individuals should be studied systematically to expand the current limited database as to relative sensitivity."

To provide enhanced protection to children during vulnerable periods of early development, the NRC Committee recommended that a child-protective uncertainty factor of up to 10-fold be considered in risk assessment "when there is evidence of developmental toxicity and when data from toxicity testing relative to children are incomplete." The Committee noted that it had long been standard practice at EPA and FDA to divide the no-observed-effect-level (NOEL) obtained in animal test results by an uncertainty factor of 100-fold in establishing a reference dose (RfD) for toxic effects other than cancer or heritable mutation. The Committee noted that this 100-fold factor is comprised of two separate factors of 10-fold each: one allows for uncertainty in extrapolating data from animals to humans; the second accommodates variation within the human population. The Committee acknowledged that "this latter uncertainty factor generally provides adequate protection for infants and children."

Nevertheless, the Committee expressed concern that the standard 100-fold safety factor may not always be sufficient to account for unique susceptibilities at particularly sensitive stages of early development. The Committee was also concerned about the great gaps that currently exist in developmental toxicity testing data for many chemicals. It was for these reasons that the NRC Committee recommended consideration of a third, child-protective uncertainty factor in risk assessment.

Food Quality Protection Act of 1996

Following publication of the NRC report, the concept that children should be considered more vulnerable to pesticides than adults in the absence of evidence to the contrary was adopted by Congress. In 1996, by unanimous vote of both Houses, the concept was incorporated into the Food Quality Protection Act (FQPA) (Food Quality Protection Act of 1996), the principal federal statute governing the use of pesticides in agriculture (Table A1-1).

FQPA incorporates most of the recommendations of the NRC report. It requires that standards for agricultural pesticides be set at levels sufficiently strict to protect the health of infants and children. It directs EPA to use an additional tenfold margin of safety in assessing the risks to infants and children to take into account the potential for pre- and postnatal toxicity, particularly when the toxicology and exposure databases are judged to be incomplete. The statute authorizes EPA to replace this default 10X "FQPA safety factor" with a different factor only if, based on reliable data, the resulting margin would be adequate to protect infants and children. The requirement for the FQPA safety factor was intended by the Congress to be a stimulus to the generation of data on developmental toxicology and on early life exposures.

Recently, the EPA published its final guidance on the application of the additional FQPA safety factor in risk assessment (U.S. Environmental Protection Agency 2002a). The Agency will apply the additional factor at the beginning of the risk assessment process, but recognizes that the intent of the FQPA safety factor overlaps with several uncertainty factors traditionally used to account for data gaps and concerns in the risk assessment process. These include default

10-fold factors to account for: 1) the lack of a NOAEL (LOAEL to NOAEL factor); 2) the lack of chronic data for setting the chronic Reference Doses (RfDs) and Reference Concentrations (RfCs) (subchronic to chronic factor); and 3) inadequacies or gaps in the database considered minimal for setting RfDs/RfCs (database factor). In most cases, EPA is of the opinion that these factors will be sufficient to account for the concerns related to children's health. If, however, the adequacy and appropriateness of the toxicity assessment or the exposure assessment are judged to be insufficient, a child-protective factor will be applied to the RfD, and the resulting FQPA-adjusted RfD is termed the population-adjusted dose (PAD).

According to a report issued by Consumers Union in February 2000, EPA had applied (an) additional safety factor(s) for 104 out of 273 (38%) pesticides when evaluating acute and/or chronic exposure between August 1996 and early 2001. For the organophosphorous insecticides, one of EPA's high priority categories for review, (an) additional safety factor(s) was/were applied for 26 of 49 substances in the evaluation of acute and/or chronic exposures (Consumers Union of the United States Inc. 2001).

Table A1-1. Major provisions of the Food Quality Protection Act of 1996

Requires that standards for pesticide residues in food be health-based. Standards must be set at levels that ensure a "reasonable certainty of no harm."
Exposure and vulnerabilities of infants and children must be specifically considered in establishing pesticide residue standards.
When insufficient data exist to assess the special exposures and/or vulnerabilities of infants and children, an additional tenfold safety factor must be considered in setting standards.
Consideration of the potential benefits of pesticides must be limited.
All pesticide standards must be reviewed every ten years.
Endocrine effects of pesticides must be systematically evaluated in toxicity testing.

3.0 Children's Unique Vulnerability to Toxicants in the Environment

The detailed analysis of children's exposures to environmental chemicals undertaken by the NRC established that children's heightened vulnerability to chemicals arises from four sources (National Academy of Sciences 1993):

Children have disproportionately heavy exposures to environmental agents

Children drink more water, eat more food and breathe more air pound-for-pound of body weight compared to adults. For example, children in the first six months of life drink seven times as much water, while children ages one through five years eat three to four times more food on a body weight basis than the average adult. The air intake of a resting infant is twice that of an adult. The implication of these findings for health is that children will have substantially heavier exposures than adults to any environmental contaminants present in water, food and air (National Academy of Sciences 1993). Two additional characteristics of children further magnify their exposures: 1) their hand-to-mouth behavior; and 2) their play close to the ground.

Children's metabolic pathways, especially in fetal life and in the first months after birth, are immature

Children's ability to metabolize, detoxify, and excrete environmental agents is different from that of adults. In some instances, children are better able than adults to deal with environmental agents (National Academy of Sciences 1993; Spielberg 1992), because they cannot make the active metabolite required for toxicity. In other instances, children are less well able to deal with toxic chemicals and thus are more vulnerable to them (National Academy of Sciences 1993; Spielberg 1992). Differences in metabolism exist also between prenatal and post-natal life, and may vary over the course of pregnancy. An additional source of vulnerability in fetuses and young children is that the blood- brain barrier is not fully developed and therefore xenobiotics may be more easily able to enter the central nervous system (Rodier 1995).

Developmental processes are easily disrupted during rapid growth and development before and after birth

Rapid growth and development occur during embryonic and fetal life as well as in the first years after birth. In the brain for example, billions of cells must form, move to their assigned positions, and establish precise connections with other cells (Rodier 1995). Development of the endocrine and reproductive organs is guided by a complex and precisely timed sequence of chemical messages. If cells in an infant's brain are destroyed by chemicals, if connections between neurons fail to form, or if false signals are sent to the developing reproductive organs by endocrine disruptors, there is the possibility that neurological or reproductive dysfunction will result (Bellinger et al, 1987; Needleman et al, 1990; Jacobson and Jacobson 1996). Because children have more future years of life than most adults, they have more time to develop chronic diseases that may be triggered by early exposures. Many diseases that are caused by toxic agents in the environment require decades to develop.

Many of those diseases, including cancer and neurodegenerative diseases, are thought to arise through a series of changes within cells that require many years to evolve from earliest initiation to actual manifestation of illness. Exposures to environmental agents early in life, including prenatal exposures, appear more likely to produce chronic disease than similar exposures encountered later (Gray et al, 1991; Ekbom et al, 1997). Thus, there are likely to be critical windows of exposure even for these chronic diseases that need to be further explored.

4.0 Changing Risks at Different Ages

Children are exposed to toxic agents through the air they breathe, the water they drink, the foods they eat, the medications they consume, and through the wide variety of environments they inhabit, including their homes, day-care centers, schools, and motor vehicles. Children have unique routes of exposure that have no parallel among adults, and the routes of exposure and the resulting risks to health differ in different stages of childhood. Contact with toxic agents can occur *in utero* through trans-placental transfer of chemicals from mother to fetus; it can occur via breast milk in nursing infants; and it can occur in early childhood via hand-to-mouth transfer of toxic chemicals. Analysis of children's varying patterns and pathways of exposure to environmental agents and the resulting health effects at various stages of development is an essential prerequisite to formulation of a child-protective approach to risk assessment.

Examples of Unique Vulnerability in Pregnancy

In pregnancy, especially in the first trimester, maternal use of certain medications has been linked to a number of adverse effects in humans. The first of these outcomes to be recognized were anatomical birth defects. Examples of these unique risks include the following:

- In 1961, a sudden increase in the frequency of limb reduction defects, phocomelia in particular, in West Germany and Australia was associated with maternal prenatal use of the sedative-hypnotic thalidomide (Lenz 1962; Taussig 1962; McBride 1961). Removal of the drug from the market led to a substantial decrease in the frequency of limb reduction defects.

- *In utero* exposure to aminopterin, an antagonist of folic acid, has been associated with anencephaly, meningocele, hydrocephalus, and cleft lip and palate (Thiersch 1952; Warkany et al, 1959).

- *In utero* exposure to the anticonvulsant diphenylhydantoin has been associated with a broad spectrum of abnormalities, including orofacial clefts, nail and digital hypoplasia, growth abnormalities, and mental deficiency (Speidel and Meadow 1972; Fedrick 1973; Monson et al, 1973).

- *In utero* exposure to valproic acid, another anticonvulsant, has been associated with neural tube defects and heart, craniofacial, and limb anomalies (Kallen et al, 1989).

- *In utero* exposure to the anticoagulant coumadin has been associated with hypoplasia of the nasal cartilage, chondrodysplasias, and atrophy of the optic nerves (Warkany 1976).

- *In utero* exposure to isotretinoin (13-cis-retinoic acid), an analogue of vitamin A used to treat cystic acne, associated with a characteristic pattern of malformations, including abnormal ear development, a flat nasal bridge, mandibular hypoplasia, cleft palate, hydrocephalus, neural tube defects, and conotruncal heart defects (Lammer 1985).

- Administration of diethylstilbestrol (DES) to pregnant women to prevent miscarriage has been linked to various genital abnormalities in their offspring (Bongiovanni et al, 1959; Gill

et al, 1959) as well as to the development of adenocarcinoma of the vagina of their daughters in their late teens and early twenties (Herbst et al, 1971; O'Brien et al, 1979).

Many teratogenic agents were also found to cause neurological and other functional disorders in children that are not necessarily associated with gross structural alterations. For example, isotretinoin causes profound mental retardation in many children, and about half of those children do not have any major malformations (Adams and Lammer 1993, 1995). DES causes a variety of reproductive problems in addition to vaginal adenocarcinoma, including infertility and poor pregnancy outcomes (Kaufman et al, 2000). Most recently, exposures to thalidomide and valproic acid in early pregnancy have been linked to autism (Stroomland et al, 1994; Moore et al, 2000).

Examples of Unique Risks in Early Childhood

Heavy Metals. Despite a decline in exposures over the past two decades (Brody et al, 1994; American Academy of Pediatrics Committee on Environmental Health 1998), resulting principally from removal of lead from gasoline, lead exposure continues to occur *in utero* and among preschool-aged children in the United States. A national survey (conducted from October 1991 to September 1994) indicated that an estimated 940,000 preschool children had blood lead levels above the Centers for Disease Control and Prevention (CDC) intervention level of 10 µg/dL; nearly 275,000 had blood lead levels greater than 15 µg/dL and nearly 85,000 had greater than twice the CDC intervention level (20 µg/dL) (Centers for Disease Control and Prevention 1997). Today, lead-based paint in older homes is the most common source of lead exposure in children (Centers for Disease Control and Prevention 1997). Exposures can occur through ingestion of paint chips or dust from deteriorating surfaces, from chewing on painted cribs, or through inhalation of lead paint dust, as can occur during home renovation. Children exposed prenatally to blood lead levels as low as 10 µg/dl, and possibly even as low as 5 µg/dl, (Lanphear et al, 2000) may have delayed early mental development (Bellinger et al, 1987). Further, chronic low-level lead exposure during childhood may result in a decreased IQ, reading and learning disabilities, attention deficits, and persistent behavioral problems (Needleman et al, 1990, 1996). The fact that toddlers are the age-group at highest risk of lead poisoning is a direct consequence of their unique hand-to-mouth behavior coupled with the fact that their brains are still undergoing growth, development, myelination and differentiation.

Prenatal exposure to methyl mercury has been shown to have adverse effects on neurodevelopment. This is an age-specific, unique risk that results from transplacental transfer of mercury from maternal blood to the fetal brain. Mercury deposited from the atmosphere into lakes, streams and oceans can be converted into organic mercury compounds, which accumulate in fish and biomagnify as they move up the food chain to reach highest levels in top predator fish species and marine mammals. These compounds are lipid-soluble and are well absorbed from the gastrointestinal tract (Clarkson 1972). Methyl mercury crosses the placenta and is excreted in breast milk. Consumption of fish with high levels of methyl mercury by pregnant women in Minamata Bay, Japan, in the 1950s was associated with cerebral palsy in their offspring (Harada 1968). When people in Iraq consumed grain treated with a methyl mercury fungicide between 1959 and 1972, thousands were poisoned (Bakir et al, 1973). In both of those episodes, mothers who were asymptomatic or showed mild toxic effects gave birth to infants who appeared normal at birth, but in whom psychomotor retardation, blindness, deafness, and seizures developed over

time (Amin-Zaki et al, 1979). To further assess the susceptibility of the fetus and infants to the neurotoxic effects of methyl mercury, longitudinal studies are being conducted to evaluate the long-term subclinical effects among children whose mothers' diets include large amounts of fish or marine mammals containing methyl mercury (Davidson et al, 1998; Grandjean et al, 1997, 1997; Crump et al, 1998). A report from the National Academy of Sciences based on an analysis of three prospective studies of the children of fish-eating woman has concluded that low-level exposures to methyl mercury *in utero* can have adverse effects on neurobehavioral development (National Academy of Sciences 2000). EPA has issued a new risk assessment for methyl mercury, setting the chronic oral RfD at 0. 01 ug/kg/day (U.S. Environmental Protection Agency 2001). This RfD is based on the finding of developmental neuropsychological impairments in children from the Faroe Island epidemiology study (Grandjean et al, 1997, 1997) and on supporting data from the New Zealand study (Crump et al, 1998).

Environmental Tobacco Smoke

Environmental tobacco smoke (ETS) is a complex mixture of chemicals generated during the burning and smoking of tobacco products. Chemicals present in ETS include irritants and systemic toxicants such as hydrogen cyanide and sulfur dioxide, mutagens and carcinogens such as benzo(a)pyrene, formaldehyde and 4-aminobiphenyl, and the reproductive toxicants nicotine, cadmium, and carbon monoxide (Jenkins et al, 1992). Of children in the United States 11 years and younger, forty-three percent live in a home with at least one person who smokes (Pirkle et al, 1996). Exposures of children to ETS produce a range of effects, some of which are unique to early life and others that are analogous to the effects produced by ETS in adults.

Prenatal exposure to ETS affects fetal growth and is associated with a 20-40% elevated risk of low birth weight or "small for gestational age" (California Environmental Protection Agency 1997; Haddow et al, 1988; Eskenazi et al, 1995; World Health Organization 1999). The primary effect observed, reduction in mean low birth weight, is small in magnitude (25-50 grams). However, if the distribution of birth weight in a population of babies is shifted downward by ETS exposure, infants who are already compromised may be pushed into higher risk categories. Since low birth weight is associated with increased infant morbidity and mortality, exposure to ETS is likely to augment such burden. Exposure to ETS during infancy has been associated with an increased risk of sudden infant death that is independent of low birth weight or prematurity (Taylor and Sanderson 1995; Klonoff-Cohen et al, 1995).

In children, ETS exposure affects the upper and the lower respiratory tract. Infants and young children are at particular risk of exposure to ETS because of the small diameter of their airways, and because pound-for-pound they breathe more air than adults. Infants who are exposed to ETS in their home environment have a 1.5 to 3-fold increased risk of lower respiratory infection compared to unexposed children (Hall et al, 1984; McConnochie and Roghmann 1986; Ogston et al, 1987). The risk of lower respiratory infection associated with ETS is highest among infants under 3 months of age (Wright et al, 1991). Children whose parents smoke also are more likely to develop middle ear effusion as measured by tympanometry (Reed and Lutz 1988; Strachan et al, 1989) and chronic respiratory problems (cough, phlegm, or wheezing) (Mannino et al, 1996). Children of parents who smoke are more likely to develop asthma, and those with asthma are more likely to experience more severe disease (Weitzman et al, 1990; Martinez et al, 1992; Chilmonczyk et al, 1993). Childhood exposure to ETS affects

lung growth and development, as measured by small but significant decrements in pulmonary function tests (Lebowitz et al, 1992; Wang et al, 1994; Cullinan and Taylor 1994; Cunningham et al, 1994). Since early lung development is important in terms of future respiratory health, these results suggest that ETS may have adverse long-term effects on children's respiratory health that warrant further investigation through longitudinal studies.

Air Pollutants

Various indoor air pollutants are associated with respiratory disorders in children, and these pollutants include particles, gases, vapors, allergens and molds (Spengler 1991; U.S. Environmental Protection Agency 1994; Lambert and Samet 1996). In the home, common sources of air pollutants, other than tobacco smoking, include gas and wood stoves, and furnishings and construction materials that release organic gases and vapors, some of which contain formaldehyde. Combustion of natural gas results in the emission of nitrogen dioxide (NO_2) and carbon monoxide (CO). Cooking or heating with wood results in the emission of several substances in the form of liquid (suspended droplets), solids (suspended particles), and gases such as NO_2 and sulfur dioxide (SO_2) (Lambert and Samet 1996). The aerosol mixture of very fine solid and liquid particles or "smoke" contains particles in the inhalable range (i.e., < 10 μm in aerodynamic diameter, PM_{10}). Exposure to CO disrupts oxygen transport, may result in symptoms that mimic influenza, including fatigue, headache, dizziness, nausea and vomiting, cognitive impairment, and tachycardia (Lambert and Samet 1996). Exposure to high levels of NO_2 and SO_2 may result in acute mucocutaneous irritation and respiratory effects, and chronic exposure to relatively lower levels has been linked to asthma and respiratory irritation (Speizer et al, 1980; Morrow 1984; Neas et al, 1991). Exposure to particles in wood smoke may result in irritation and inflammation of the respiratory tract, manifested as rhinitis, cough, wheezing, and worsening of asthma (Morris et al, 1990; Robin et al, 1996). Whether adults exposed to similar levels of wood smoke have a different probability of severe lower respiratory illnesses is unclear.

Indoor environments also contain a number of aeroallergens that trigger asthma episodes in children, including allergens of house dust mites, cats, and cockroaches (Cullinan and Taylor 1994). In urban dwellings, house dust mite and cockroach allergens are important in both onset and worsening of asthma symptoms (Cullinan and Taylor 1994). Cockroach allergens have been found to be common in the homes of inner city children with asthma (Call et al, 1992; Rosenstreich et al, 1997). Recent clinical and epidemiologic studies indicate that exposure to molds or dampness may increase the risk of respiratory symptoms among children (Delfino et al, 1997; Dales et al, 1991; VerHoeff et al, 1995). Whether the increasing prevalence of asthma morbidity in children in recent years is due to an increased prevalence of exposure to aeroallergens, molds or dampness or to a combination of those indoor factors with changing patterns of ambient air pollution is unclear.

Ambient air pollution levels have also been shown to be associated with asthma and other respiratory morbidity in children. Daily fluctuations in PM_{10} have been associated with increased emergency room visits for asthma (Schwartz et al, 1993; Rennick and Jarman 1992), hospital admissions for asthma (Montealegre et al, 1993), decrements in peak flow rates in normal children (Neas et al, 1995), increased respiratory symptoms (Forsbert et al, 1993), and increased medication use in children with asthma (Pope 1991). Ozone has been of particular concern since it provokes airway inflammation at very low levels (Aris et al, 1993). In addition,

ozone increases airway reactivity (Hortsman et al, 1990; Kreit et al, 1989), and there is evidence to suggest that ozone may potentate the effects of allergens (Molfino et al, 1991). Ozone levels have been related to increases in asthma admissions and emergency room visits among children in Atlanta (White et al, 1994), New Jersey (Cody et al, 1992), and Mexico City (Romieu et al, 1995). A study of the effect of increases in ambient ozone levels on summer day camp children and workers in New Jersey suggests that children may be more sensitive than adults to increases in ambient ozone levels (Cody et al, 1992). In this study, increases in ozone concentrations above 120 ppb were associated with an increase in respiratory symptoms and a decline in peak expiratory flow rate among children, but with no change in respiratory symptoms or pulmonary function among camp workers. Further studies are needed to determine whether the risk of respiratory effects from ambient air pollutants differs between children and adults and, if so, whether such differences reflect differences in exposure or in sensitivity.

The potential impact of air pollution on asthma morbidity in a community is exemplified by a report from CDC that examined hospitalizations for asthma in the Atlanta metropolitan area during the summer of 1996 in the weeks before, during and after the summer Olympic Games. This analysis found that the rate of asthma hospitalization declined during the two weeks of the Games, when citizens of Atlanta heeded an advisory from the Mayor to improve air quality by not driving and by instead using public transportation. After the Games ended, motor vehicle traffic increased, air quality declined, and asthma hospitalization rates rebounded (Friedman et al, 2001).

5.0 Diseases in Children of Known or Suspected Environmental Origin

Patterns of disease among children in the industrially developed nations today are quite different from those of generations past (Haggerty and Rothman 1975). The traditional infectious diseases have largely been controlled: smallpox is eradicated, polio is nearly gone, measles is under control, diphtheria and tetanus are rarities, and cholera has virtually disappeared. The expected life span of a baby born in the United States today is more than two decades longer than that of an infant born at the beginning of the twentieth century (Haggerty and Rothman 1975). Similar "epidemiologic transitions" from infectious to non-infectious diseases are occurring at various rates today in many nations around the world as those countries strengthen public health programs, control the classic infectious diseases and move towards industrial development. In other countries, sadly, the increasing incidence of HIV positivity and of AIDS threatens to undo those gains.

Children today are at risk of disease caused by environmental hazards not encountered by previous generations. Over 85,000 synthetic chemical compounds, most of which have been developed since World War II, are now registered for commercial use in EPA's Toxic Substances Control Act (TSCA) inventory. There are currently 2,800 chemicals produced in quantities of one million pounds or more per year (Goldman and Koduru 2000). These high-production-volume (HPV) chemicals are those with the potential to be most widely used in foods and consumer products and to be most widely disseminated in the environment. Fewer than half of these HPV chemicals have been tested for their potential toxicity to humans, and fewer still for their toxicity to children (Goldman and Koduru 2000; National Academy of Sciences 1984).

Thus, their potential hazards to children's health and development are largely unknown (Schaefer 1994).

Diseases of great importance to children in America today that are thought, or at least suspected, to be caused or aggravated by chemicals in the environment include the following:

Asthma

A recent study by the National Center for Health for Health Statistics (NCHS) that surveyed data on self-reports of asthma, physician's office visits for asthma, emergency room visits for asthma, and hospitalizations for asthma, provides evidence for increases in prevalence of asthma in the United States during the past 15 years, particularly among children (Centers for Disease Control and Prevention 1995a). Estimates of the average rates of asthma prevalence increased over time across all age groups. Asthma mortality also increased. Children experienced some of the higher rates of morbidity as measured by self-reports, office visits, emergency department visits and hospitalizations for asthma. These increases were particularly evident in urban localities. In New York and in other major cities, asthma has become the leading cause of admission of children to hospitals and the leading cause of school absenteeism (Centers for Disease Control and Prevention 1995b). The increasing prevalence of asthma and the higher asthma morbidity among children in the United States, albeit still unexplained, suggest that, compared to adults, children are more likely to develop asthma and asthma exacerbations and/or be exposed to environmental or other factors that cause or trigger asthma episodes.

Childhood Cancer

The reported incidence of childhood cancer has increased substantially in the United States in the past two decades (Devesa et al, 1995). Although death rates are down, as a consequence of early detection and vastly improved treatment, data from the National Cancer Institute show that the reported incidence of acute lymphoblastic leukemia (ALL) increased by 27.4% from 1973 to 1990, from 2.8 cases per 100,000 children to 3.5 per 100,000. Since 1990, ALL incidence has declined in boys, but continues to rise in girls (Robison et al, 1995). From 1973-1994, the incidence of brain cancer increased by 39.6%, with nearly equal increases in boys and girls (Schechter 1999). In young men, 20-39 years of age, the incidence of testicular cancer in the years 1973-1994 increased by 68% (Devesa et al, 1995; Robison 1995).

Neurodevelopmental Disorders

Neurodevelopmental disorders, including learning disabilities, dyslexia, mental retardation, attention deficit disorder and autism are widespread and affect 5-10% of the four million babies born in the United States each year. Some clinical investigators have reported that prevalence is increasing, but existing data are not of sufficient quality to either sustain or refute that position (American Academy of Pediatrics 2001). Causes are largely unknown, but *in utero* and early life exposures to lead (Bellinger et al, 1987; Needleman et al, 1990), mercury (National Academy of Sciences 2000), PCBs (Jacobson and Jacobson 1996), certain pesticides (Eskenazi et al, 1999; Wiles and Campbell 1993; Whitney et al, 1995; Campbell et al, 1997) and other environmental neurotoxicants are known or thought to contribute (National Academy of

Sciences 1992). A recent report from the National Research Council concluded that 3% of developmental disabilities are the direct consequence of neurotoxic environmental exposures and that another 25% arise out of the interplay of environmental factors and individual genetic susceptibility (environment was defined broadly in this report and included diet, alcohol, tobacco and other "life-style" factors, as well as toxic chemicals) (National Research Council 2000).

Endocrine Disruption

Endocrine disruptors are chemicals that have the capacity to interfere with the body's hormonal signaling system (Harrison 2001; Longnecker et al, 1997). Effects of these chemicals, which have been hypothesized to include cancer (Kogevinas et al, 1997), decreased fertility (Longnecker et al, 2002), birth defects of the reproductive organs (Peterson et al, 1993; Paulozzi et al, 1997), altered sex ratios (Mocarelli et al, 2000), neurodevelopmental impairment (Jacobson and Jacobson 1996), thyroid dysfunction and diabetes (Longnecker and Daniels 2001) have been observed in cell systems *in vitro* (Birnbaum 1994), in experimental animals exposed to specific chemicals in the laboratory (Gary et al, 2000), and in wildlife populations in several broadly contaminated ecosystems such as the Great Lakes (Colborn et al, 1996) and Lake Apopka in Florida (Guillette et al, 1994).

Evidence for the effects of endocrine disruptors on human health is less abundant than for evidence of effects in wildlife or *in vitro*, but data are accumulating. There are some data linking precocious puberty with PBB among girls exposed during gestation and breast-feeding (Blanck et al, 2000a, 2000b). Based on animal studies, the embryo, fetus, and neonate, and the pre-pubertal period would appear to be at particularly high risk of adverse consequences following exposure to endocrine disruptors (Selevan et al, 2000). Early exposures to these compounds have the potential to alter anatomic structures and may influence the subsequent course of endocrine functioning (Longnecker and Danels 2001), neurological development (Jacobson and Jacobson 1996) and sexual development (Longnecker and Daniels 2001; Birnbaum 1994; Gray et al, 2000; Colborn et al, 1996; Guillette et al, 1994; Blanck et al, 2000a, 2000b; Selevan et al, 2000; Euling and Kimmel 2001). Through the Food Quality Protection Act of 1996, Congress has mandated more extensive screening of chemical compounds to assess their potential for endocrine disruption (Food Quality Protection Act of 1996).

6.0 A Framework for Assessing Environmental Health Risks to Children

Protection of children's health against the adverse effects of exposures to toxic chemicals will require a modified approach to risk assessment that moves beyond the use of "average" levels of exposure and risk and that goes beyond consideration of the 60- or 70-kilogram adult (U.S. Environmental Protection Agency 1999, 2002b). To account for the unique exposures and special vulnerabilities of infants and children (National Academy of Sciences 1993), this new approach will need to include the following elements:

1. *Improved Exposure Assessment*

Additional data are needed on children's patterns and levels of exposures to chemicals in the environment. Because exposures vary by age, this information will need to be collected within different age groups.

All sources of exposure need to be considered in evaluating the potential risks of environmental chemicals to infants and children (National Academy of Sciences 1993). Models need to be developed that can account for children's simultaneous exposures to multiple chemicals of differing potency via multiple routes of exposure. These models also need to be able to assess the cumulative effects of chemicals that may have either synergistic or antagonistic actions.

Exposure estimates need to be constructed differently depending on whether acute or chronic effects are of concern. The incorporation of biomarkers into data collection may be useful.

Most importantly, it is essential to examine the full distribution of children's exposures to chemicals in the environment. Point estimates of average are no longer sufficient. Appropriate mathematical models must be constructed, such as Monte Carlo models, that can permit the combining of various data sets and thus permit examination of full exposure distributions (National Academy of Sciences 1993).

2. *Enhanced Toxicity Testing*

New, more sensitive approaches to chemical toxicity testing are needed that can reliably detect the unanticipated developmental consequences of exposures during critical windows of prenatal and postnatal vulnerability (Selevan et al, 2000) on systems that have not been adequately or thoroughly addressed in the past, e.g., the nervous, immune, respiratory reproductive, cardiovascular, and endocrine systems.

Extensive past experience has demonstrated that infants and young children are uniquely vulnerable to certain chemicals that are relatively innocuous to adults. To detect unanticipated consequences of early exposures to chemicals, it will be necessary to develop new approaches to assay prenatal, perinatal and childhood toxicity (U.S. Environmental Protection Agency 1986, 1991) and to apply these approaches widely. For certain classes of chemicals it may be necessary to undertake studies in which chemicals are administered to experimental animals either *in utero* or shortly after birth and the subjects then followed over their entire lifespan. For other classes of compounds, it may be necessary to expose animals throughout the life span. The approach should attempt to replicate the human experience and may enhance detection of delayed effects (National Academy of Sciences 1993).

Improved functional tests of neurobehavioral, immune, endocrine and reproductive toxicity are of great importance for detecting outcomes other than anatomic malformations (U.S. Environmental Protection Agency 1986, 1991). These functional assessments need to be applied on a more routine basis, especially when data from other

studies, e.g., adult target organ toxicity, or multigeneration studies, raise concerns about possible effects on functional systems in children that have not been thoroughly evaluated in more routine testing protocols.

3. *New Toxicodynamic and Toxicokinetic Models*

The physiological and biochemical characteristics of children that influence metabolism and disposition of chemicals at different stages of development need to be considered in risk assessment. Physiological parameters such as tissue growth rates and biochemical parameters such as enzyme induction may differentially affect the responses of infants and children to environmental chemicals at different developmental stages (Ginsberg et al, 2002; Cresteil 1998).

Pharmacokinetic models that account for the unique physiologic characteristics of infants, children and adolescents need to be developed. Physiologically-based pharmacokinetic models can be used to estimate the dose of toxic metabolites reaching target tissues at different developmental stages (O'Flaherty 1997; Welsch et al, 1995).

4. *An Outcomes-Oriented Approach to Hazard Assessment*

The pathogenic mechanisms of environmentally-induced disease in children need to be elucidated at functional, organ, cellular, and molecular levels (Whitney et al, 1995; Campbell et al, 1997; Birnbaum 1994). These assessments could be undertaken in conjunction with toxicity testing of chemicals and also in the context of epidemiologic studies. Clinical and epidemiologic studies offer an excellent vehicle for studying etiologic associations between environmental exposures and pediatric disease (Bellinger et al, 1987; Needleman et al, 1990; Jacobson and Jacobson 1996). A strong case can be made for the need to establish a major multi-year prospective epidemiological study of children's health in relation to environmental exposures as a means of identifying and characterizing the consequences of multiple, early, low-level exposures, as called for in the Children's Health Act, 2000 (Berkowtiz et al, 2001).

5. *Application of Uncertainty and Safety Factors that Specifically Consider Children's Risks*

Children must be presumed to be more vulnerable to environmental toxic agents than adults in the absence of data to the contrary, as was specifically recommended by the NRC Committee on Pesticides in the Diets of Infants and Children (National Academy of Sciences 1993). That Committee called for the incorporation of an additional child-protective uncertainty factor into risk assessment to account for this greater vulnerability, particularly in the absence of data, and the Food Quality Protection Act mandated the application of an additional margin of safety for children's risk in the case of pesticides (Food Quality Protection Act of 1996). Traditional approaches to risk assessment are now being modified to more carefully and explicitly account for risks to children (U.S. Environmental Protection Agency 2002a). However, a number of data gaps in exposure assessment and in developmental toxicity must be addressed through the development and implementation of additional testing guideline protocols (U.S. Environmental

Protection Agency 1999, 2002b), the acquisition of better information on children's exposure patterns and sources, and the undertaking of basic research both on mechanisms of underlying development and on chemical interactions of environmental agents with developing organ systems (National Research Council 2000).

7.0 Conclusion

The protection of children against toxic chemicals in the environment is a major challenge to modern society (Schaefer 1994). Children are not merely a "special" vulnerable group within our population, but rather they are the current inhabitants of a developmental stage through which all future generations must pass. Protection of the health of fetuses, infants and children is essential for sustainability of the human species.

The current challenge to risk assessment stems from two facts: 1) that hundreds of new chemicals are developed every year, and released in varying quantities into the environment; and 2) that the majority of these chemicals are not adequately evaluated prior to commercial introduction for their potential toxicity, for their potential effects on development, nor for their possible interactive effects with other chemicals (Goldman 2000; National Academy of Sciences 1984). The need, in this context, is to design approaches to risk assessment that account for the unique exposures and sensitivities of children and that also will stimulate enhanced research in developmental toxicity. The ultimate goal is to formulate policies that will protect children against potential toxic agents and allow them to grow, develop, and reach maturity without incurring neurobehavioral impairment, immune dysfunction, reproductive damage, or increased risks of cancer as a consequence of environmental exposures in early life.

The protection of children against toxic chemicals in the environment will require fundamental and far-reaching revisions of current approaches to surveillance, toxicity testing and risk assessment. No guidance document on risk assessment that fails to consider the unique exposures and special susceptibilities of fetuses, infants and children can today be considered adequate to protect human health.

References to APPENDIX 1

American Academy of Pediatrics Committee on Environmental Health. 1998. Screening for elevated blood lead levels. Pediatrics 101:1072-1078.

American Academy of Pediatrics. 2001. The pediatrician's role in the diagnosis and management of autistic spectrum disorder in children. Pediatrics 107:1221-1226.

Adams J, Lammer EJ. 1993. Neurobehavioral teratology of isotretinoin. Reprod Toxicol 7:175-177.

Adams J, Lammer EJ. 1995. Human isotretinoin exposure: the teratogenesis of a syndrome of cognitive deficits. Neurotoxicol Teratol 17:386.

Amin-Zaki L, Majeed MA, Elhassani SB, Clarkson TW, Greenwood MR, Doherty RA. 1979. Prenatal methylmercury poisoning. Clinical observations over five years. Am J Dis Child 133:172-177.

Aris RM, Christian D, Hearne PQ, Kerr K, Finkbeiner WE, Balmes JR. 1993. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. Am Rev Respir Dis 148:1363-1372.

Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, al-Rawi NY, et al. 1973. Methylmercury poisoning in Iraq. Science 181:230-241.

Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. 1987. Longitudinal analyses of prenatal and postnatal lead exposure and early cognitive development. N Engl J Med 316:1037-1043

Berkowitz GS, Wolff MS, Matte T, Susser E, Landrigan PJ. 2001. The rationale for a national prospective cohort study of environmental exposure and childhood development. Environ Res 85:59-68

Birnbaum LS. 1994. The mechanism of dioxin toxicity: relationship to risk assessment. Environ Health Perspect 102:157-167.

Blanck HM, Marcus M, Hertzberg V, Tolbert PE, Rubin C, Henderson AK, et al. 2000a. Determinants of polybrominated biphenyl serum decay among women in the Michigan PBB cohort. Environ Health Perspect 108(2):147-152.

Blanck HM, Marcus M, Tolbert PE, Rubin C, Henderson AK, Hertzberg, et al. 2000b. Age at menarche and tanner stage in girls exposed *in utero* and postnatally to polybrominated biphenyl. Epidemiology 11:641-647.

Bongiovanni AM, DiGeorge AM, Grumbach MM. 1959. Masculinization of the female infant associated with estrogenic therapy alone during gestation. Four cases. J Clin Endocrinol Metab 19:1004-1011.

Brody DJ, Pirkle JL, Kramer RA, Flegal KM, Matte TD, Gunter EW, et al. 1994. Blood lead levels in the US population. Phase 1 of the Third National Health and Nutrition Examination Survey (NHANES III, 1988 to 1991). JAMA 272:277-283.

CEPA. 1997. Health Effects of Exposure to Environmental Tobacco Smoke. Office of Environmental Health Hazard Assessment – http://www.oehha.org/pdf/exec.pdf. Sacramento, CA:California Environmental Protection Agency, 3/37-3/38.

Call RS, Smith TF, Morris E, Chapman MD, Platts-Mills TA. 1992. Risk factors for asthma in inner city children. J Pediatr 121:862-866.

Campbell CG, Seidler FJ, Slotkin TA. 1997. Chlorpyrifos interferes with cell development in rat brain regions. Brain Res Bull 43:179-189

Centers for Disease Control and Prevention. 1995a. Asthma - United States, 1982-1992. MMWR Morb Mortal Wkly Rep 43:952-955.

Centers for Disease Control and Prevention. 1995b. Children at risk from ozone air pollution – United States, 1991-1993. MMWR Morb Mortal Wkly Rep 44:309-312.

Centers for Disease Control and Prevention. 1997. Update: blood lead levels – United States, 1991-1994. MMWR Morb Mortal Wkly Rep 46:141-146.

Chilmonczyk BA, Salmun LM, Megathlin KN, Neveux LM, Palomaki GE, Knight GJ, et al. 1993. Association between exposure to environmental tobacco smoke and exacerbations of asthma in children. N Engl J Med 328:1665-1669.

Clarkson TW. 1972. The pharmacology of mercury compounds. Annu Rev Pharmacol 12:375-406.

Cody RP, Weisel CP, Birnbaum G, Lioy PJ. 1992. The effect of ozone associated with summertime photochemical smog and frequency of asthma visits to hospital emergency departments. Environ Res 58:184-194.

Colborn T, Dumanoski D, Myers JP. 1996. Our stolen future: are we threatening our fertility, intelligence, and survival?: A scientific detective store. New York:Dutton.

Consumers Union of the United States Inc. 2001. A Rreport Card for the EPA. Successes and Failures in Implementing the Food Quality Protection Act. Related Tables. In: A Report Card For The EPA. (Table 2.1 and Appendix 1). http://www.consumersunion.org/food/fqpa_info.htm

Cresteil, T. 1998. Onset of xenobiotic metabolism in children: toxicological implications. Food Addit Contam 15(suppl):S45-S51.

Crump KS, Kjellstrom T, Shipp AM, Silvers A, Stewart A. 1998. Influence of prenatal mercury exposure upon scholastic and psychological test performance: benchmark analysis of a New Zealand cohort. Risk Anal 18:701-713.

Cullinan P, Taylor AJ. 1994. Asthma in children: environment al factors. BMJ 308:1585-1586.

Cunningham J, Dockery DW, Speizer FE. 1994. Maternal smoking during pregnancy as a predictor of lung function in children. Am J Epidemiol 139:1139-1152.

Dales RE, Zwanenburg H, Burnett R, Franklin CA. 1991. Respiratory health effects of home dampness and molds among Canadian children. Am J Epidemiol 134:196-203.

Davidson PW, Myers GJ, Cox C, Axtell C, Shamlaye C, Sloane-Reeves J, et al. 1998. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. JAMA 280:701-707.

Delfino RJ, Zeiger RS, Seltzer JM, Street DH, Matteucci RM, Anderson PR, et al. 1997. The effect of outdoor fungal spore concentrations on daily asthma severity. Environ Health Perspect 105:622-635.

Devesa SS, Blot WJ, Stone BJ, Miller BA, Tarone RE, Fraumeni JF Jr. 1995. Recent cancer trends in the United States. J Natl Cancer Inst 87:175-182

Ekbom A, Hsieh CC, Lipworth L, Adami HQ, Trichopoulos D. 1997. Intrauterine environment and breast cancer risk in women: a population-based study. J Natl Cancer Inst 89:71-76.

Eskenazi B, Prehn AW, Christianson RE. 1995. Passive and active material smoking as measured by serum cotinine: the effect on birthweight. Am J Public Health 85:395-398.

Eskenazi B, Bradman A, Castorina R. 1999. Exposures of children to organophosphate pesticides and their potential adverse health effects. Environ Health Perspect 107:409-419.

Euling S, Kimmel CA. 2001. Developmental stage sensitivity and mode of action information for endocrine disrupting chemicals. Sci Total Environ 274:103-113.

Fedrick J. 1973. Epilepsy and pregnancy: a report from Oxford record linkage study. Br Med J 2:442-448.

Food Quality Protection Act of 1996. 1996. Public Law 104-170.

Forsberg B, Stjernberg N, Falk M, Lundback B, Wall S. 1993. Air pollution levels, meteorological conditions and asthma symptoms. Eur Respir J 6:1109-1115.

Friedman MS, Powell KE, Hutwagner L, Graham LM, Teague WG. 2001. Impact of changes in transportation and commuting behaviors during the 1996 Summer Olympic Games in Atlanta on air quality and childhood asthma. JAMA 285:897-905.

Gray LE Jr, Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L. 2000. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58:350-365.

Gill WB, Schumacher GF, Bibbo M. 2002. Structural and functional abnormalities in the sex organs in male offspring of mothers treated with diethylstilbestrol (DES). J Reprod Med 16:147-153.

Ginsberg, G. Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, et al. 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicol Sci. 66:185-200.

Goldman LR, Koduru SH. 2000. Chemicals in the environment and developmental toxicity to children: a public health and policy perspective. Environ Health Perspect 108(suppl 3):S443-S448.

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, et al. 1997. Cognitive deficit in 7-year old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 19:417-428.

Gray LE Jr, Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L. 2000. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58:350-365.

Gray R, Peto R, Brantom P, Grasso P. 1991. Chronic nitrosamine ingestion in 1040 rodents: the effect of choice of nitrosamines, the species studied, and the age of starting exposure. Cancer Res 51(Part 2):6470-6491.

Guillette LJ Jr, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR. 1994. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ Health Perspect 102:680-688.

Haddow JE, Knight GJ, Palomaki GE, McCarthy JE. 1988. Second trimester serum cotinine levels in nonsmokers in relation to birth weight. Am J Obstet Gynecol 159:481-484.

Haggerty R, Rothman J. 1975. Child Health and the Community. New York:John Wiley & Sons.

Hall CB, Hall WJ, Gala CL, MaGill FB, Leddy JP. 1984. Long-term prospective study in children after respiratory syncytial virus infection. J Pediatr 105:358-364.

Harada Y. 1968. Congenital (or Fetal) Minamata disease. In: Study Group of Minamata Disease. Japan:Kumamoto University, 93-117.

Harrison PT. 2001. Endocrine disuptors and human health. BMJ 323:1317-1318.

Herbst AL, Ulfelder H, Poskanzer DC. 1971. Adenocarcinoma of the vagina: association of maternal stilbestrol therapy with tumor appearance in young women. N Eng J Med 284:878-881.

Horstman DH, Folinsbee LJ, Ives PJ, Abdul-Salaam S, McDonnell WF. 1990. Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. Am Rev Respir Dis 142:1158-1163.

Jacobson JL, Jacobson SW. 1996. Intellectual impairment in children exposed to polychlorinated biphenyls *in utero*. N Engl J Med 335:783-789.

Jenkins RA, Tomkins BA, Guerin MR. 1992. The Chemistry of Environmental Tobacco Smoke: Composition and Measurement. Indoor Air Research Series. Chelsea, MI:Lewis Publishers.

Kallen B, Robert E, Mastroiacovo P, Martinez-Frias ML, Castilla EE, et al. 1989. Anticonvulsant drugs and malformations: is there a drug specificity? Eur J Epidemiol 5:31-36.

Kaufman RH, Adam E, Hatch EE, Noller K, Herbst AL, Palmer JR, et al. 2000. Continued follow-up of pregnancy outcomes in diethylstilbestrol-exposed offspring. Obstet Gynecol 96:483-489.

Klonoff-Cohen HS, Edelstein SL, Lefkowitz ES, Srinivasan IP, Kaegi D, Chang JC, et al. 1995. The effect of passive smoking and tobacco exposure through breast milk on sudden infant death syndrome. JAMA 273:795-798.

Kogevinas M, Becher H, Benn T, Bertazzi P, Boffetta P, Bueno-de-Mesquita H, et al. 1997. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. Am J Epidemiol 145:1061-1075.

Kreit JW, Gross KB, Moore TB, Lorenzen TJ, D'Arcy J, Eschenbacher WL. 1989. Ozone-induced changes in pulmonary function and bronchial responsiveness in asthmatics. J Appl Physiol 66:217-222.

Lambert WE, Samet JM. 1996. Indoor air pollution. In: Occupational and Environmental Respiratory Disease (Harber P, Schenker MM, Balmes JR, eds). St Louis, MO:Mosby, 784-807.

Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, et al. 1985. Retinoic acid embryopathy. N Engl J Med 313:837-841.

Landrigan PJ, Carlson JE. 1995. Environmental policy and children's health. Future Child 5:34-52.

Lanphear BP, Dietrich K, Auinger P, Cox C. 2000. Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. Public Health Rep 115:521-529.

Lebowitz MD, Sherril D, Holberg CJ. 1992. Effects of passive smoking on lung growth in children. Pediatr Pulmonol 12:37-42.

Lenz W. 1962. Thalidomide and congenital abnormalities. Lancet 1:271-272.

Longnecker MP, Rogan WJ, Lucier G. 1997. The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBs (polychlorinated biphenyls) and an overview of organochlorines in public health. Annu Rev Public Health 18:211-244.

Longnecker MP, Daniels JL. 2001. Environmental contaminants as etiologic factors for diabetes. Environ Health Perspect 109(suppl 6):S871-S876.

Longnecker MP, Dlebanoff MA, Brock JW, Zhou H, Gray KA, Needham LL, et al. 2002. Maternal serum level of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene and risk of cryptorchidism, hypospadias, and polythelia among male offspring. Am J Epidemiol 155:313-322.

Mannino DM, Siegel M, Husten C, Rose D, Etzel R. 1996. Environmental tobacco smoke exposure and health effects in children: results from the 1991 National Health Interview Survey. Tob Control 1996 5:13-18.

Martinez FD, Cline M, Burrows B. 1992. Increased incidence of asthma in children of smoking mothers. Pediatrics 89:21-26.

McBride WG. 1961. Thalidomide and congenital abnormalities. Lancet 2:1358.

McConnochie KM, Roghmann KJ. 1986. Parental smoking, presence of older siblings, and family history of asthma increase risk of bronchiolitis. Am J Dis Child 140:806-812.

Mocarelli P, Gerthoux PM, Ferrari E, Patterson DG Jr, Kieszak SM, Brambilla P, et al. 2000. Paternal concentrations of dioxin and sex ratio of offspring. Lancet 355:1858-1863.

Molfino NA, Wright SC, Katz I, Tarlo S, Silverman F, McClean PA, et al. 1991. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 338:199-203.

Monson RR, Rosenberg L, Hartz SC, Shapiro S, Heinonen OP, Slone D. 1973. Dyphenylhydantoin and selected malformations. N Engl J Med 289:1049-1052.

Montealegre F, Chardon D, Tarrats H. 1993. Environmental factors precipitating bronchial asthma exacerbations in southern Puerto Rico: a pilot study. J Asthma 83:219-227.

Moore SJ, Turnpenny P, Quinn A, Glover S, Lloyd DJ, Montgomery T, et al. 2000. A clinical study of 57 children with fetal anticonvulsant syndromes. J Med Genet 37:489-97.

Morris K, Morgenlander M, Coulehan JL, Gahagen S, Arena VC, Morganlander M. 1990. Wood-burning stoves and lower respiratory tract infection in American Indian Children. Am J Dis Child 144:105-108.

Morrow PE. 1984. Toxicological data on NOx: an overview. J Toxicol Environ Health 13:205-227.

National Academy of Sciences. 1984. Toxicity Testing: Needs and Priorities. Washington, DC:National Academy Press.

National Academy of Sciences. 1992. Environmental Neurotoxicology. Washington, DC:National Academy Press.

National Academy of Sciences. 1993. Pesticides in the Diets of Infants and Children. Washington, DC:National Academy Press.

National Academy of Sciences. 2000. Toxicological Effects of Methylmercury. Washington, DC:National Academy Press.

National Research Council. 2000. Scientific Frontiers in Developmental Toxicology and Risk Assessment. Washington, DC:National Academy Press.

Neas LM, Dockery DW, Ware JH, Spengler JD, Speizer FE, Ferris BG Jr. 1991. Association of indoor nitrogen dioxide with respiratory symptoms and pulmonary function in children. Am J Epidemiol 134:204-219.

Neas LM, Dockery DW, Koutrakis P, Tollerud DJ, Speizer FE. 1995. The association of ambient air pollution with twice daily peak expiratory flow rate measurements in children. Am J Epidemiol 141:111-122.

Needleman HL, Schell A, Bellinger D, Leviton A, Allred EN. 1990. The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. N Engl J Med 322:83-88.

Needleman HL, Riess JA, Tobin MJ, Biesecker GE, Greenhouse JB. 1996. Bone lead levels and delinquent behavior. JAMA 275:363-369.

O'Brien PC, Noller KL, Robboy SJ, Barnes AB, Kaufman RH, Tilley BC, et al. 1979. Vaginal epithelial changes in young women enrolled in the National Cooperative Diethylstilbestrol Adenosis (DESAD) Project. Obstet Gynecol 53:300-308.

O'Flaherty EJ. 1997. Pharmacokinetics, pharmacodynamics, and prediction of developmental abnormali**ties**. Reprod Toxicol 11:413-6.

Ogston SA, Florey CD, Walker CH. 1987. Association of infant alimentary and respiratory illness with parental smoking and other environmental factors. J Epidemiol Community Health 41:21-25.

Paulozzi LJ, Erickson JD, Jackson RJ. 1997. Hypospadias trends in two US surveillance systems. Pediatrics 100:831-834.

Peterson RE, Theobald HM, Kimmel GL. 1993. Developmental and reproductive toxicity of dioxins and related compounds: Cross species comparisons. Crit Rev Toxicol 23:283-335.

Pirkle JL, Flegal KM, Bernert JT, Brody DJ, Etzel RA, Maurer KR. 1996. Exposure of the US population to environmental tobacco smoke: the Third National Health and Nutrition Examination Survey, 1988 to 1991. JAMA 275:1233-1240.

Pope CA 3rd. 1991. Respiratory hospital admissions associated with PM10 pollution in Utah, Salt Lake, and Cache Valleys. Arch Environ Health 46:90-97.

Reed BD, Lutz LJ. 1988. Household smoking exposure – association with middle ear effusions. Fam Med 20:426-430.

Rennick GJ, Jarman FC. 1992. Are children with asthma affected by smog? Med J Austr 156:837-41.

Robin LF, Less PS, Winget M, Steinhoff M, Moulton LH, Santosham M, et al. 1996. Wood-burning stoves and lower respiratory illnesses in Navajo children. Ped Infec Dis J 15:859-865.

Robison LL, Buckley JD, Bunin G. 1995. Assessment of environmental and genetic factors in the etiology of childhood cancers: the Children's Cancer Group epidemiology program. Environ Health Perspect 103(suppl 6):S111-S116.

Rodier PM. 1995. Developing brain as a target of toxicity. Environ Health Perspect 103(suppl 6):S73-S76.

Romieu I, Meneses F, Sienra-Monge JJ, Huerta J, Ruiz Velasco S, White MC, et al. 1995. Effects of urban air pollutants on emergency room visits for childhood asthma in Mexico City. Am J Epidemiol 141:546-553.

Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, et al. 1997. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 336:1356-1363.

Schaefer M. 1994. Children and toxic substances: confronting a major public health challenge. Environ Health Perspect. 102(suppl 2):S155-S156.

Schechter CB. 1999. Re: Brain and other central nervous system cancers: recent trends in incidence and mortality. J Natl Cancer Inst 91:2050-1.

Schwartz J, Slater D, Larson TV, Pierson WE, Koenig JQ. 1993. Particulate air pollution and hospital emergency room visits for asthma in Seattle. Am Rev Respir Dis 147:826-31.

Selevan SG, Kimmel CA, Mendola P. 2000. Identifying critical windows of exposure for children's health. Environ Health Perspect 108(suppl 3):S451-S455.

Speidel BD, Meadow SR. 1972. Maternal epilepsy and abnormalities of the fetus and newborn. Lancet 2:839-843.

Speizer FE, Ferris B Jr, Bishop YM, Spengler J. 1980. Respiratory disease rates and pulmonary function in children associated with NO_2 exposure. Am Rev Respir Dis 121:3-10.

Spengler JD. 1991. Sources and concentrations of indoor air pollutants. In: Indoor Air Pollution. A Health Perspective (Samet JM, Spengler JD, eds). Baltimore, MD:The Johns Hopkins University Press.

Spielberg SP. 1992. Anticonvulsant adverse drug reactions: age dependent and age independent. In: Similarities and Differences Between Children and Adults; Implications for Risk Assessment. (Guzelian PS, Henry CJ, Olin SS, eds). Washington, DC:International Life Sciences Institute Press, 104-106.

Strachan DP, Jarvis MJ, Feyerabend C. 1989. Passive smoking, salivary cotinine concentrations, and middle ear effusion in 7 year old children. BMJ 298:1549-1552.

Stromland K, Nordin V, Miller M, Akerstrom B, Gillberg C. 1994. Autism in thalidomide embryopathy: a population study. Dev Med Child Neurol 36:351-356

Taussig HB. 1962. A study of the German outbreak of phocomelia. JAMA 180:1106-1114.

Taylor JA, Sanderson M. 1995. A re-examination of the risk factors for the sudden infant death syndrome J Pediatr 126:887-891.

Thiersch JB. 1952. Therapeutic abortions with folic acid antagonist 4-aminopteroylglutamic acid amino PGA administered by oral route. Am J Obstet Gynecol 63:1298-1304.

U.S. EPA. 1986. Guidelines for the Health Assessment of Suspect Developmental Toxicants. Fed Reg 51:34028-34040.

U.S. EPA. 1991. Guidelines for Developmental Toxicity Risk Assessment. EPA/600/FR-691/001. http://cfpub.epa.gov/ncea/raf/pdfs/devtox.pdf. Fed Reg 56:63798-63826.

U.S. EPA. 1994. Indoor Air Pollution: An Introduction for Health Professionals. http://www.epa.gov/iaq/pubs/hpguide.html. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 1999. Toxicology Data Requirements for Assessing Risks of Pesticide Exposure to Children's Health - Report of the Toxicology Working Group of the 10X Task Force (April 28, 1999 draft) – http://www.epa.gov/scipoly/sap/1999/may/10xtx428.pdf. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 2001. Chronic Reference Dose for Methylmercury (MeHg) (CASRN 22967-92-6). Integrated Risk Information System – http://www.epa.gov/iris/subst/0073.htm. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 2002a. Determination of the Appropriate FQPA Safety Factor(s) for Use in the Tolerance-Setting Process. Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances – http://www.epa.gov/oppfead1/trac/science/determ.pdf. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 2002b. A review of the reference dose and reference concentration processes. Risk Assessment Forum, (Draft) – http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=51717. Washington, DC:U.S. Environmental Protection Agency.

Verhoeff AP, van Strien RT, van Wijnen JH, Brunekreef B. 1995. Damp housing and childhood respiratory symptoms: the role of sensitization to dust mites and molds. Am J Epidemiol 141:103-110.

Wang X, Wypij D, Gold DR, Speizer FE, Ware JH, Ferris BG Jr, et al. 1994. A longitudinal study of the effects of parental smoking on pulmonary function in children 6-18 years. Am J Respir Crit Care Med 149:1420-5.

Warkany J, Beaudry PH, Hornstein S. 1959. Attempted abortion with aminopterin (4-aminopteroglutamic acid). Am J Dis Child 97:274-281.

Warkany J. 1976. Warfarin embryopathy. Teratology 14:205-209.

Weitzman M, Gortmaker S, Walker DK, Sobol A. 1990. Maternal smoking and childhood asthma. Pediatrics 85:505-511.

Welsch F, Blumenthal GM, Conolly RB. 1995. Physiologically based pharmacokinetic models applicable to organogenesis: extrapolation between species and potential use in prenatal toxicity risk assessments. Toxicol Lett 82-83:539-547.

White MC, Etzel RA, Wilcox WD, Lloyd C. 1994. Exacerbations of childhood asthma and ozone pollution in Atlanta. Environ Res 65:56-68.

Whitney KD, Seidler FJ, Slotkin TA. 1995. Developmental neurotoxicity of chlorpyrifos: cellular mechanisms. Toxicol Appl Pharmacol 134:53-62.

Wiles R, Campbell C. 1993. Pesticides in children's food. Washington, DC:Environmental Working Group.

WHO. 1999. International Consultation on Environmental Tobacco Smoke (ETS) and Child Health. Consultation Report 1999. WHO/NCD/TFI/99.10. http://www.who.int/toh. Geneva, Switzerland:World Health Organization.

Wright AL, Holberg C, Martinez FD, Taussig LM. 1991. Relationship of parental smoking to wheezing and nonwheezing lower respiratory tract illnesses in infancy. Group Health Medical Associates. J Pediatr 118:207-214.

APPENDIX 2

Hazard Identification and Predictability of Children's Health Risk from Animal Data*

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Introduction

Children and adults are different physiologically and behaviorally. Children eat and drink more (based on size), play and act differently (e.g. very young children engage in more hand-to-mouth activity), are still undergoing development, and may be less or more able to metabolize and excrete certain substances. Given these differences, children and adults may differ qualitatively and/or quantitatively in how they are affected by xenobiotic exposure. Effects of xenobiotics in children may be completely different from effects from the same exposure in adults (qualitative difference). On the other hand, the effect of xenobiotic exposure may be similar between children and adults but may occur to a greater or lesser extent in the child (quantitative difference).

Children's health risk assessment is the evaluation of the potential for xenobiotic exposures to cause any adverse developmental effect, including growth retardation, malformations, functional deficits, and lethality. Risk assessment includes evaluating available toxicity data (hazard identification) and exposure information (e.g. dose, route, duration, developmental stage of exposure) to determine if a xenobiotic causes potential adverse health effects in humans. The focus of this paper is on hazard identification with respect to children's health risk.

Critical periods and important milestones in development

For the purposes of risk assessment, the human life span can be divided into a number of exposure periods: pre-conceptional (maternal and paternal), pre-implantation, post-implantation (organogenesis; 1st trimester), early and late fetal, premature infant, perinatal, neonatal (term), infant, toddler, pre-teen (pre-pubertal), and adult. Comparable periods for animal species are not as easily defined and are dependent on individual organs or systems. Furthermore, it is apparent from the work of Hoar and Monie (1981) and DeSesso (1997) that developmental events do not occur at the same chronological age across species. The developmental toxicology (Wilson 1977) and developmental neurotoxicology (Vorhees 1986; Rodier 1980) literature contains many examples of the stage specificity of structural and functional damage in laboratory animal species, which depends on developmental age. Thus, developmental age of maturation is most relevant for interspecies comparison.

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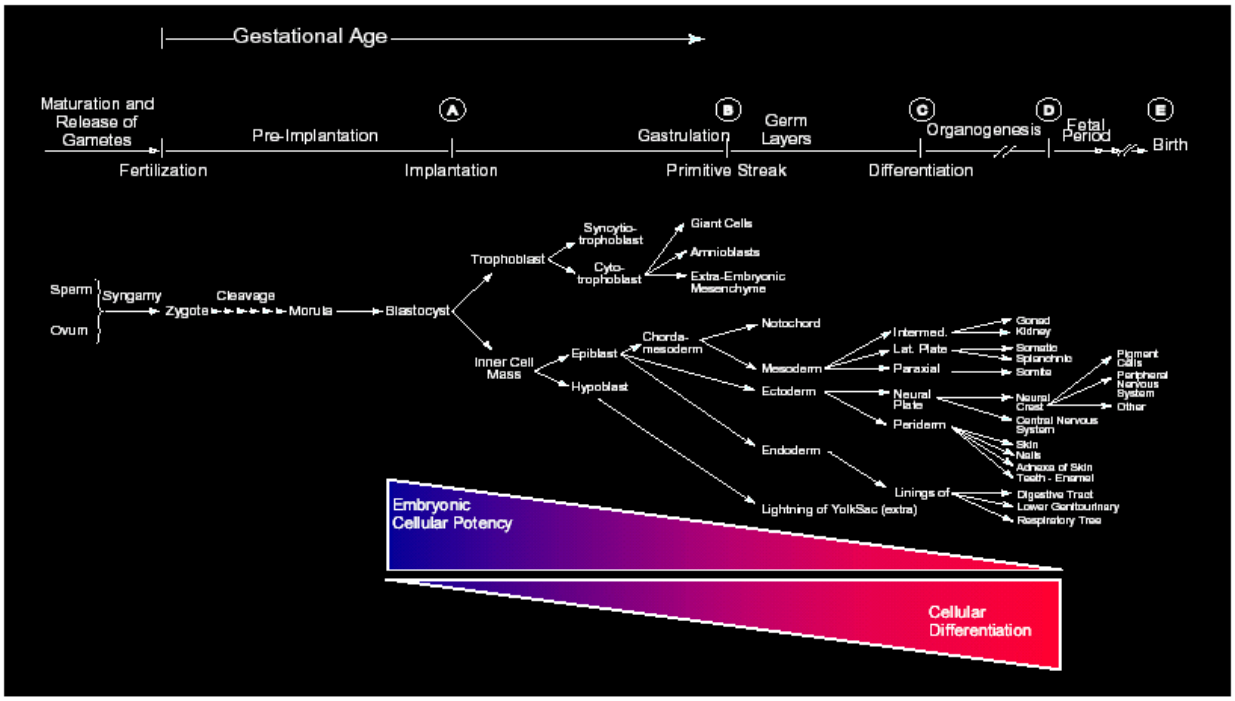
Adverse developmental effects can occur during any period of the lifespan. While it can be argued that the entire lifetime of an individual comprises the period of development, the most dramatic manifestations of development occur during the period of maturation to adult status. This period of life includes both prenatal (preimplantation, embryonic, fetal periods) and postnatal (infancy, childhood, adolescence) development. For the purposes of the current effort, the entire period of pre- and postnatal development is considered childhood and is arbitrarily defined as the period of life encompassing conception to 18 years of age in humans and from conception to sexual maturity in experimental animals. Currently, more information is known about the details of prenatal development than those of the postnatal period.

Organ system development

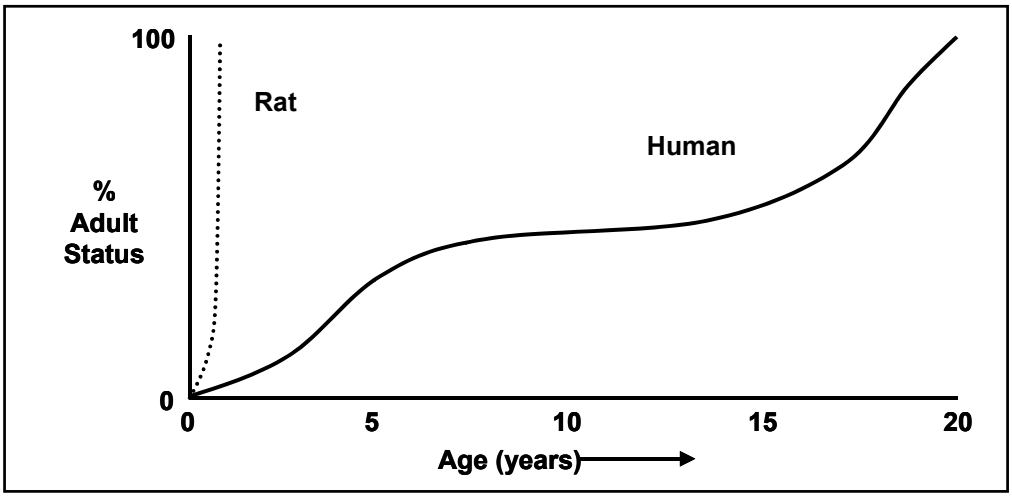
During development, cells within an organism change from a state of pluripotency (i.e., ability to develop into a great number of different tissue types) to a state of differentiation (i.e., commitment to a particular structural and/or functional role within the body). This concept of increasing cellular/organ differentiation, or specialization, throughout development is depicted in Figure A2-1. The trend of increasing differentiation with maturation occurs in all species, but the chronological timetables can be very different from species to species. That is, the

Figure A2-1. Differentiation Increases with Age of Developing Organisms



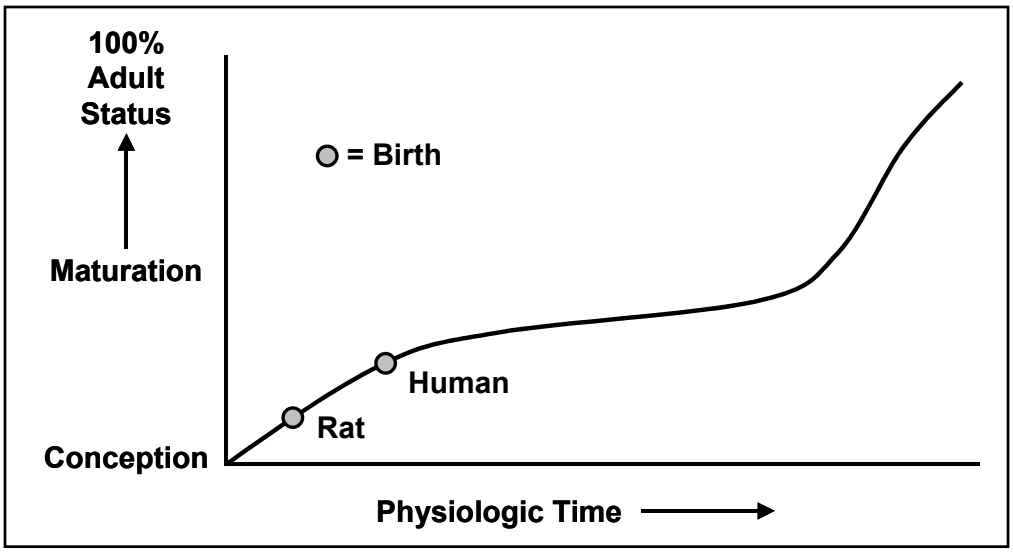
developmental time course for species with prolonged gestation periods (e.g., humans) occurs over a greater period of time than that of species with shorter gestation periods (e.g., rats), as illustrated in Figure A2-2. Humans reach adulthood in 18-25 years (depending on the criteria used to measure adult status), with bursts of developmental activity during both early childhood and

Figure A2-2. Time to Develop Adult Characteristics



puberty. In contrast, rats attain adult status very quickly. The lifespan of the two species can be scaled so that comparable stages of development are congruent, irrespective of chronological age (Figure A2-3) - this is scaling to physiological time. By doing so, it can be noted that, based on developmental stages, birth occurs much earlier in the rat than in the human. Birth is not a maturational landmark; it does not occur at the same developmental stage for each species. Rather, birth is a physiological occurrence that occurs at different developmental stages, depending on the species.

Figure A2-3. Relationship Between Extent of Maturation and Birth in Rats and Humans



In the 1820s, Karl van Baer made some observations about differentiation *among* species. Looking at prenatal development, he noted that the more general features of an embryo appear earlier than the more specialized features, and that as development progresses, different species

diverge morphologically. For example, forelimb buds appear early in development and look similar across species. Later in development, the forelimb buds of a fish become fins, and those of a bird become wings, while those of a human become arms.

It is, in part, because of the morphological and presumed physiological similarities among species during the early stages of development that prenatal toxicity testing paradigms have been used so successfully. To date, researchers have generally focused on effects mediated during the period of organogenesis, when cells and organs are undergoing early differentiation but species are still relatively similar to one another. Early in development, cells and embryos of different species react similarly to a challenge (e.g., exposure to a xenobiotic) because at those developmental stages their cells have not differentiated greatly. Later in development, the cells of one organ may react differently to a challenge than the cells of another organ, and one species may react differently than another due to developmental divergence and specialization. This concept was captured in one of Wilson's general principles of teratology: the response of a developing organism to a challenge depends on its stage of development (Wilson 1959, 1973). This principle applies to postnatal development as well.

Postnatal development

Prenatal developmental milestones have historically been morphological and well-defined (reviewed by DeSesso 1997). For the most part, especially during the embryonic and early fetal periods, milestones have been identified by the emergence/disappearance or change in form of particular structures. As development proceeds, the nascent organism takes on a progressively more mature form. Thus, the ability to discern milestones becomes more difficult, and tends towards histological or physiologically-based markers of development such as attainment of blood pressure, thermal homeostasis (resistance to cooling), closure of sutures/epiphyses, onset of glandular secretion, or appearance of cellular receptors.

In postnatal life, some milestones in nonclinical species are denoted by the appearance of particular behaviors or neurological activities (e.g., acoustic startle response, exploratory behavior, rearing, mounting, etc.). There are few reliable, comparative compendia for many peri- and postnatal milestones in typically used experimental animals and humans. Without a database of normal milestone appearance, derived from many control animals, there is little information about the normal variation in these data. This, in turn, leads to obvious difficulties in interpreting such findings as a delay in the appearance of a particular milestone. Interpretation of some behaviors is confounded because they are related to olfaction in macrosmatic species, such as rodents and carnivores, and it is not clear that these behaviors have counterparts in microsmatic species like man. Thus, the relevance of some of these findings will be problematic for use in risk assessment.

Because a generally accepted suite of postnatal developmental milestones in nonclinical species is not yet available, the critical periods for the developmental processes associated with those milestones have generally not yet been defined. Additionally, there may be more than one critical period of vulnerability and endpoint affected. For example, brain structures have different peak periods of growth. Therefore, depending on the particular time of exposure, compounds could differentially affect the structures undergoing peak development. In a review of animal studies and their clinical implications, Rodier (1980) found that exposures occurring at

different stages of brain development had different effects on brain and behavioral functions. For example, based on data from azacytidine-treated mice, hyperactivity seemed to result from insults that occurred during the middle part of neuron production (mid-prenatal insult) while hypo activity seemed to result from insults that coincided with cerebellum proliferation (early prenatal and early postnatal insults).

Although a workshop on "Critical Windows of Exposure for Children's Health" was conducted to examine the data available on critical windows for exposure during the postnatal period, detailed periods of susceptibility were not identified (Selevan et al. 2000). For reviews of the development of the systems covered and the conclusions of the individual workgroups, the reader is directed to Environmental Health Perspectives Volume 108, Supplement 3, 2000. Understanding both the fundamental biology and the temporal schedule that underlies the development of whatever milestones are eventually selected is paramount for performing well grounded, scientifically based risk assessments. Therefore, it may be desirable to begin by defining important pediatric milestones for growth and development, both global and organ-system specific. These milestones should be readily assessed by clinicians, reflect normal maturational processes, be liable to disruption when normal maturation is perturbed, and demonstrate susceptibility to pharmacologic perturbation (i.e., display properties of dose-response and time-action). Subsequently, nonclinical endpoints analogous to these pediatric milestones should be identified accordingly.

Relevance/predictability of extrapolation of animal data to children's risk assessment

Animal/human concordance has been reasonably well characterized in two primary areas pertaining to children's health: developmental toxicology and developmental neurotoxicology. Much has been written about the relevance of animal models to human risk assessment, and a few of these references are cited herein. Species comparisons of the development of physical structures and organ systems have been made by Hoar and Monie (1981) and DeSesso (1997). These comparisons enhance the predictability of the type of damage that may occur in humans following insult at various stages of development. Several studies have been conducted on the ability of animal species to identify known human teratogens. Schardein et al. (1985) found that with the exception of coumarin anticoagulants, every chemical or chemical group known to be teratogenic in humans is also teratogenic in one or more laboratory animal species. With regard to teratogenicity, animal models have been reported to demonstrate sensitivity in the range of 62-75%, a positive predictive value of 75-100%, and a negative predictive value of 64-91%, which implies that studies in laboratory animals are capable of predicting human developmental toxicity (Jelovsek et al. 1989).

Schardein and Keller (1989) developed a comparative table (Table A2-1) of fetal endpoints in the rat, rabbit, and human. Concordance with regard to growth and developmental milestones has not been as well established, primarily because it has not been established which milestones are the most appropriate to evaluate in humans or in laboratory animal species.

Table A2-1. Fetal Endpoints in Rat, Rabbit and Human

	Rat	Rabbit	Human
Body weight at term (grams)	3.5	39.8	3587
% Live births at term	93.1	90.2	86.7
% Malformations	2.1	7.3	2-3

Currently, the most abundant knowledge deals with effects of compounds administered over the course of embryofetal development in classic laboratory species. With regard to teratogenicity, the mouse and rat successfully modeled the human reaction approximately 70% of the time, while the rabbit was less likely than other species to give a false negative finding. Primates were considered to offer a high level of predictability. The ability of various species to correctly identify an agent as a human developmental toxicant with a positive response for growth retardation, death, or malformation was 98% for the rat, 91% for the mouse, 85% for the hamster, 82% for the monkey, and 77% for the rabbit (Schardein and Keller 1989).

Developmental toxicology testing – encompassing the periods of embryonic and fetal development - routinely uses two species, generally rat and rabbit. Frankos (1985) reviewed 38 known human teratogens and determined that 97% of these compounds were teratogenic in at least one animal species, 75% were positive in more than one species, and 21% were positive in all species tested. Mice identified a positive response 85% of the time, rats 80%, and rabbits 60%. Schardein and Keller (1989) determined that concomitant use of the rabbit with either the mouse or the rat enhanced the predictive potential of these individual models. They also found that concordance was almost always additive. Evaluation of postnatal developmental landmarks in laboratory animals has routinely been conducted in one species, the rats. One of the major reasons for this is that this evaluation usually occurs in the labor-intensive pre- and postnatal development study. Given the developmental toxicity history on concordance, a future consideration should be evaluation of alternate species or more than one species for developmental landmarks. Naturally, this raises questions of which species would be appropriate and, once chosen, the extent of historical data needed to validate the use of this species. These are important issues for discussion in the developmental toxicology community.

Table A2-2 indicates potential developmental landmarks that have been measured in humans and/or laboratory animals. This list is not all-inclusive, and requires input from individuals with pediatric expertise. Tests for acquisition of these landmarks may differ in humans and laboratory species, but the same basic endpoint is being measured. Representation on this list does not necessarily indicate that these tests are being conducted routinely, or that they are validated. Several areas such as hormone measurements, and evaluation of metabolism and pharmacokinetics/pharmacodynamics, are listed, but have not been well characterized in children and young animals. Evaluation of additional functional areas that develop postnatally, such as the immune and genitourinary systems, has not been extensively explored. Biomarkers for these areas are needed, and data should be evaluated for concordance between humans and laboratory animals. Surrogate markers in laboratory animals might include, for example, pinnae detachment and eye opening. What is the relevance to humans, other than a general indication that development is proceeding at a normal pace? Ability to taste and smell can be measured in

humans and laboratory animals, but is this highly relevant information to be included in a developmental landmarks test battery?

Table A2-2. Endpoint (Humans and Laboratory Animals)

Endpoint	Humans	Laboratory Animals
Survival	√	√
Growth and development		
Body weight	√	√
Skeletal development (height/crown-rump)	√	√
Pinnae detachment		√
Tooth eruption	√	√
Eye opening		√
Sensory development		
Hearing (auditory startle)	√	√
Sight	√	
Taste (taste aversion)		
Touch (tactile startle)		√
Smell (odor threshold)	√	√
Neuromuscular reflexes		
Grip strength	√	√
Muscle coordination	√	√
Gait/movement	√	√
Activity		
Spontaneous activity (hypo- and hyperactivity)	√	√
Reactivity	√	√
Sexual maturation		
Vaginal opening		√
Testes descent	√	√
Preputial separation		√
Seconday sex characteristics	√	
Hormone levels	√	√
Learning and memory		
Intelligence tests/various cognitive batteries	√	
Mazes	√	√
Avoidance behavior		√
Operant behavior	√	√
Language skills	√	
Social behavior	√	√
Metabolic capability	√	√
Pharmacokinetics/pharmacodynamics	√	√

A work group (Adams et al. 2000) specifically charged with addressing questions relevant to risk estimation in developmental neurotoxicology found that, within the context of methods used in regulatory testing batteries, extrapolation appears stronger with regard to effects on sensory and motor functioning than for cognitive or social functioning. To improve detection of learning and memory deficits, they suggested that the integrity of learning mechanisms should be further challenged through task complexity. Additionally, the workshop suggested the use of more contemporary and sensitive methods for evaluating behavior. These include adding prepulse inhibition to the startle paradigm, improving water maze learning tasks by adding reversal learning, and examining morphological and functional effects in young as well as aged animals.

Mechanistic data

The use of information regarding the locus of a compound's mechanism of action (generally a receptor), coupled with the known distribution of this target, can provide important information in the course of predicting the pharmacology or toxicology of that entity. Further, known similarities or discrepancies between the test species and humans can be used to support or refute, respectively, the relevance of nonclinical effects for pediatric risk assessment.

That said, traditional developmental milestones are considered to be the result of highly integrated processes and do not lend themselves to *in vitro* mechanistic evaluation. As with the assessment of developmental toxicology, the most expedient way to evaluate developmental milestones may be with a standardized screen to identify effects, leaving *in vitro* mechanistic evaluations in the realm of effect characterization on a case-by-case basis.

Data gaps

Data gaps, once identified, should be investigated by appropriate scientific experimentation. Despite many systems undergoing significant development during this time, one period with considerable data gaps is the peripubertal/adolescent period. For example, although prominent remodeling and maturation events occur in the brain during adolescence, little investigation in either humans or animals has occurred. Another area with limited data concerns the evaluation of functional deficits in relation to discrete windows of vulnerability. Rarely have early gestational versus late gestational versus lactational exposures been examined.

There may be a role for scientific groups (e.g. ILSI and PhRMA), as well as governmental and regulatory agencies (e.g. EPA, FDA, NTP), in funding landmark assessments for development, especially postnatal development in which there are fewer validated assays and endpoints. Alternatively, nonclinical endpoints selected empirically could be monitored prospectively for clinical relevance. Finally, needed information about a given species might be ascertained through the use of additional concurrent control groups in nonclinical postnatal testing.

Methods for assessing potential hazards to children

Nonclinical studies

Nonclinical toxicity assessments include prenatal developmental toxicity studies, fertility and reproduction studies, developmental neurotoxicity studies, and/or peri- and postnatal studies. Tables A2-3 and A2-4 are meant to summarize the overall designs of these protocols; for detailed descriptions the reader is referred to the original guidelines (EPA 1991, 1996, 1998; FDA 1994). Additionally, several reviews discuss and compare the EPA, FDA, and OECD guidelines (Collins et al. 1998; Kimmel and Makris 2001).

Generally, these protocols include extended periods of dosing to simulate long-term human exposure and development periods. For example, dosing extends the entire period of gestation (implantation to term) in the prenatal developmental study; in the developmental neurotoxicity study, dosing usually begins at implantation and continues throughout prenatal development until midway through or to completion of the preweaning period to cover major periods of nervous system development.

Table A2-3. EPA Testing Guidelines

	Prenatal Development Study (OPPTS 870.3700)	Reproduction and Fertility Effects (2-generation) (OPPTS 870.3800)	Developmental Neurotoxicity Study (OPPTS 870.6300)
Dosing Period	GD 6-20 (rat) GD 6-29 (rabbit)	**P:** 10 weeks prior to mating through weaning **F:** weaning through mating, pregnancy and lactation	GD 6- PND 10
Number of Animals	20 pregnant females	20 pregnant females	20 litters
Typical Endpoints Evaluated	• Maternal toxicity • Number of implantations and corpora lutea • Embryo/fetal mortality • Fetal weight and sex • Fetal morphology (external, visceral, skeletal)	• Estrous cyclicity • Semen quality • Mating indices • Fertility indices • Parturition • Offspring growth and viability • Reproductive landmark development (vaginal opening and preputial separation) • Reproductive organ weights and histopathology	• Offspring growth and viablity • Offspring toxicity • Developmental landmarks • Motor activity • Auditory startle habitation • Learning and memory • Neuropathology including morphometric analysis

During the past four decades, considerable effort has been expended and much experience had been gained in the area of prenatal developmental toxicology. As a result, scientific protocols to assess the potential for prenatal developmental toxicity have been designed and refined. These protocols have served us well in identifying potential hazards to developing embryos/fetuses. The rationale for these protocols was based (at least in part) on the strong morphological (and presumed physiological) similarity of mammalian offspring across species, especially at the most susceptible early stages of development, when comparative developmental stages are most similar. This period encompasses differentiation and organogenesis. As development proceeds, and especially as it is manifested during the later stages of prenatal development, differences between species with regard to tissue organization and phenotype appear and concordance of developmental schedules dissipates.

Table A2-4. FDA Testing Guidelines

	Fertility and Early Embryonic Development	Pre- and Post-Natal Development, Including Maternal Function	Embryo-Fetal Development
Dosing Period	2 weeks (females) and 4 weeks (males) prior to mating through GD 6	GD 6-PND 20	GD 6-17 (rat) GD 7-19 (rabbit)
Typical Endpoints Evaluated	• Maternal toxicity • Mating indices • Fertility indices • Number of implantations and corpora lutea • Embryo mortality	• Growth and viability • Maturation and fertility indices • Sensory functions and reflexes • Behavior (motor activity; learning and memory)	• Maternal toxicity • Number of implantations and corpora lutea • Embryo/fetal mortality • Fetal weight and sex • Fetal morphology (external, visceral, skeletal)

For the most part, assessment of postnatal toxicity has relied on the multigenerational test, which treats the mother 10 weeks prior to mating through lactation and the pups themselves after weaning to sexual maturity. The test measures the effect of treatment on the pups' survival, growth and maturation and reproductive ability. Occasionally, behavioral testing is also performed on the offspring, but functional deficits (e.g., endocrine, immune, cardiovascular, renal, hepatic, etc.) are generally not assessed in the multigenerational test. Importantly, however, multigenerational tests are conducted on relatively few environmental chemicals and are not routinely conducted in the course of nonclinical safety testing of drug products. For pharmaceuticals, the multigeneration study has been replaced by shorter-term studies designed to evaluate specific developmental stages and reproductive processes. For later stage developmental evaluations of pharmaceuticals, dams are treated from gestation day 6 through postnatal day 21 (the ICH Pre and Postnatal development paradigm), resulting in indirect exposure to the offspring. Therefore, there are gaps in the developmental intervals assessed and functional areas evaluated in conventional repeat-dose postnatal tests.

One design flaw regarding exposure to the offspring is apparent with the multigenerational test and the pre- and postnatal test, which limits their use in determining the

potential postnatal toxicity of a compound. Pups are assumed to be exposed to the compound through the mother's milk until weaning. However, exposure to the compound during the lactational period is usually not verified or measured in these studies. As a result, exposure to the offspring is uncertain and not quantified. The compound could, in fact, be excreted or sequestered by the mother, such that the offspring are not exposed to the extent assumed; alternatively, the compound could be hyper excreted in the milk, such that the offspring receive a much higher dose than expected.

Another weakness associated with the multigenerational and the pre- and postnatal testing paradigms is that differences between the metabolic capacity of adults versus offspring and differences among species in terms of organ system development are often not well characterized (and frequently are completely unknown). Neither of these factors should be overlooked, and as discussed below, both should play an important role in choosing an appropriate animal model for postnatal toxicity testing. Furthermore, understanding the developmental differences in organ systems across species is critical to study design. If exposure does not occur at the right time (i.e. during critical period(s) for exposure), then potential adverse outcomes will be missed. Additionally, if the evaluation of the developmental milestone/process does not occur at the right time (i.e. during the critical period(s) of expression/assessment), then again potential adverse outcomes will be missed.

Attributes of a successful animal model

It is difficult - if not impossible - to make an *a priori* selection of an animal model for large-scale/routine postnatal toxicity testing. Rather, one must determine the best animal model for each chemical entity/class. No one model will be appropriate for all chemicals and testing needs.

A successful (i.e., predictive) animal model must possess four important attributes. First, the model must be relevant; that is, it must accurately relate to the effects associated with chemical exposure in humans. As a prerequisite, it is necessary to evaluate the "correct" endpoints (i.e., those associated with chemical exposure). Second, the model must be sensitive, that is to say it should give clear-cut results and clearly show a dose-responsive relationship. Third, the model must produce reproducible (and thereby confirmable) results so that it can be used in multiple studies conducted by different investigators in various locations. And fourth, the model must be practical; this means it should be relatively inexpensive and not overly work-intensive.

The above attributes are required of any successful animal model. When one is studying the effect of a compound in laboratory animals with the goal of predicting effects in children, however, an additional condition must be met: The target organ of the animal model must be in the same developmental stage as that of the humans of concern. In order to meet this requirement, one must know the developmental stage of children being exposed, their metabolic capabilities, which organ systems might be targets of the study compound, and the developmental schedules of those organ systems in humans and in potential test species. Armed with such information, a researcher can choose an animal model that will be most appropriate for the case at hand.

One of the difficulties in choosing a successful animal model is the compressed developmental schedule, which occurs in animals, primarily rodents. For example, regional development of the brain proceeds in days in rodents but in weeks to months in humans. However, the sequence of events is comparable among species (Rice and Barone 2000). Another example is in relation to the reproductive system. The interval between birth and the initiation of gametogenesis differs between rodents (only a few days in absolute terms) and humans (years). This short interval in rodents limits interpretation of studies on chemicals that are thought to bioaccumulate in children and makes it difficult to use rodents to address questions of aggregate or intermittent exposures during childhood in humans. The appropriateness of other animal models (rabbits and primates) should then be explored for such studies.

Another important consideration is that some developmental events occur postnatally in rodents but occur prenatally in humans; therefore, differences in route of exposures may occur. For example, rodents have considerable postnatal development of their nervous systems while humans have more prenatal maturation; therefore, the exposures during the same period of maturation would be different (i.e. lactational transfer during the first postnatal week in rodents and transplacental transfer during the third trimester in humans).

Clinical and epidemiological studies

Traditionally, a number of approaches have been utilized to evaluate human developmental toxicity. These include case studies, randomized controlled trials, cohort studies, and case-control studies.

Case-control studies start with children with particular diseases and usually compare them to those without disease. In most cases, a case-control design would be utilized when the disease is rare such as for childhood cancer. These studies may ascertain exposures by questionnaire or by biologic or ambient samples. Inherent in the design is the assumption that the exposure preceded the disease; however, it is often difficult to ascertain this. This is particularly difficult to ascertain if the substance has a short biologic half-life or the disease has a long latency, and the inception of the disease process is unknown.

Cohort studies typically are very expensive to conduct. They are best if the exposure is more rare than the disease, or if the temporality of the exposure is difficult to define otherwise. An advantage of a cohort study is that in a single study you can examine many disease endpoints in relation to the exposure. Multiple exposures can be ascertained in both case-control and cohort studies.

It is important that all studies assess information on other covariates, i.e., variables that could confound or alter the results when not controlled for. Uncontrolled confounding can result in either an over- or an under-estimate of the effect. It is also important that information be obtained on variables that could modify the relationship between variables so that susceptible sub populations can be determined, e.g. children exposed only *in utero* may be at higher risk than those exposed only postnatally.

Successful protocols include studies that have adequate sample size, sensitive and accurate exposure measurements, control for confounding and effect modification, and sensitive

and accurate measures of disease. Many of the most successful studies investigating the relationship of exposure and disease have been birth cohort studies where exposure is ascertained during pregnancy as well as during childhood; the children are followed for a number of years. This design has been used to study lead (Needleman and Bellinger 1991), polychlorinated biphenyls (Jacobson and Jacobson 1997), environmental tobacco smoke (Eskenazi and Castorina 1999) and currently pesticides (Eskenazi et al. 1999). These studies tend to be small due to their expense, and therefore cannot examine rare disease outcomes such as birth defects or childhood cancer.

Rare diseases such as cancer or birth defects are best studied in case-control studies. Cases are often ascertained from registries such as tumor registries or birth defect monitoring programs (e.g., California and metropolitan Atlanta), and controls are selected from the neighborhood, friends, or randomly such as through random digit dialing. If exposure is ascertained by questionnaire, there are concerns about recall bias and accuracy. Recall bias and accuracy is of concern given the time period for recall and the potential differences in recall depending on the health of the child. If exposure is ascertained by biologic or ambient samples it is difficult to determine if current exposure measures accurately reflect the critical period for disease development. Nevertheless, these studies have indicated associations with environmental tobacco smoke and with home pesticide use and certain forms of childhood cancers.

Summary

Children are not adults. They differ by activities and stages of development. These differences, in turn, can affect how and when exposures to chemicals occur and the resulting responses. Historically, evaluation of developmental toxicity has focused on gestational exposures and morphological changes resulting from this exposure. Current processes for evaluating growth, survival, and morphological change due to gestational exposure are adequate. However, functional consequences of gestational exposure and postnatal exposure are not as well studied. Difficulties with our experience and knowledge base for postnatal toxicity evaluations include divergent differentiation of structure, function and physiology across species, lack of understanding of species differences in functional ontogeny, and lack of common endpoints and milestones across species. Ultimately, we need to identify relevant landmarks in children, correlate these with a relevant battery of landmarks in animals, and ensure that we have enough historical data to make sense of subsequent nonclinical testing.

References to APPENDIX 2

Adams J, Barone S Jr, LaMantia A, Philen R, Rice DC, Spear L, et al. 2000. Workshop to identify critical windows of exposure for children's health: neurobehavioral work group summary. Environ Heath Perspect 108:535-544.

Collins TF, Sprando RL, Hansen DL, Shackelford ME, Welsh JJ. 1998. Testing guidelines for evaluation of reproductive and developmental toxicity of food additives in females. Int J Toxicol 17:299-325.

DeSesso JM. 1997. Comparative embryology. In: CRC Handbook of Developmental Toxicology (Hood RD, ed.). New York:CRC Press, 111-1170.

Eskenazi B, Bradman A, Castorina R. 1999. Exposures of children to organophosphate pesticides and their potential adverse health effects. Environ Health Perspect 107:409-419.

Eskenazi B, Castorina R. 1999. Association of prenatal maternal or postnatal child environmental tobacco smoke exposure and neurodevelopmental and behavioral problems in children. Environ Health Perspect 107:991-1000.

Frankos V H. 1985. FDA perspectives on the use of teratology data for human risk assessment. Fundam Appl Toxicol 5:615-622.

Hoar RM, Monie IW. 1981. Comparative development of specific organ systems. In: Developmental Toxicology (Kimmel CA, Buelke-Sam J, eds.). New York:Raven Press, 13-33.

Jacobson JL, Jacobson SW. 1997. Evidence for PCBs as neurodevelopmental toxicants in humans. Neurotoxicology 18:415-424.

Jelovsek FR, Mattison DR, Chen JJ. 1989. Prediction of risk for human developmental toxicity: How important are animal studies for hazard identification? Obstet Gynecol 74:624-636.

Kimmel CA, Makris SL. 2001. Recent developments in regulatory requirements for developmental toxicology. Toxicol Lett 120:73-82.

Needleman HL, Bellinger D. 1991. The health effects of low level exposure to lead. Annu Rev Public Health 12:111-140.

Rice D, Barone G. 2000. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108:511-533.

Rodier PM. 1980. Chronology of neuron development: animal studies and their clinical implications. Dev Med Child Neurol 22:525-545.

Schardein JL, Keller KA. 1989. Potential human developmental toxicants and the role of animal testing in their identification and characterization. CRC Crit Rev Toxicol 19:251-339.

Schardein JL, Schwetz BA, Kenel MF. 1985. Species sensitivities and prediction of teratogenic potential. Environ Health Perspect 61:55-67.

Selevan SG, Kimmel CA, Mendola P. 2000. Identifying critical windows of exposure for children's health. Environ Health Perspect 108:451-455.

U.S. EPA. 1991. Guidelines for Developmental Toxicity Risk Assessment. Fed Reg 56:63798-63826.

U.S. EPA. 1996. Guidelines for Reproductive Toxicity Risk Assessment. Fed Reg 61:56274-56322.

U.S. EPA. 1998. Guidelines for Neurotoxicity Risk Assessment. Fed Reg 63:26926-26954.

U.S. FDA. 1994. International Conference on Harmonisation: Guideline on Detection of Toxicity to Reproduction for Medicinal Products. Fed Reg 59:48746-48752.

Vorhees CV. 1986. Handbook of Behavioral Teratology (Riley EP, Vorhees CV, eds.). New York:Plenum Press, 3-48.

Wilson JG. 1959. Experimental studies on congenital malformations. J Chron Dis 10:111-130.

Wilson JG. 1965. Embryological considerations in teratology. In: Teratology: Principles and Techniques (Wilson JG, Warkany J, eds.). Illinois:University of Chicago Press, 251-261.

Wilson JG. 1973. Environment and Birth Defects (Wilson JG, ed.). New York:Academic Press, 305.

Wilson JG. 1977. Handbook of Teratology (Wilson JG, Fraser FC, eds.). New York:Plenum Press, 47-74.

APPENDIX 3

Incorporating Children's Toxicokinetics into a Risk Framework*

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1.0 Introduction - Children Can Be a Tough Group to Figure Out

Perhaps the risk assessment community has not embraced the arena of children's risk because of the imposing task. Children are difficult to study for a number of reasons, not the least of which is a practical one: it is not ethically feasible to introduce chemicals of environmental concern, even at trace amounts, into infants and children. Thus, there is very little toxicokinetic (TK) data (that is pharmacokinetic data for environmental toxicants) in this age group. One can gather data from "natural experiments" in which children are exposed inadvertantly to a pesticide or airborne chemical. The resulting biomarker data may be somewhat useful in assessing the extent of exposure, but these are not the well-controlled TK data one needs to start building a predictive children's model. Another equally daunting problem is that children are highly diverse, stretching in definition (for the purposes of this framework) from fetal through adolescent stages and beyond. Generalizations and defaults are not possible for such a sweeping range of development. Even within a narrow age range, there can be considerable variability given the rapid and variable rate of development in early life. Thus, improving children's risk assessments through toxicokinetics is a worthwhile endeavor, but one that will be hampered by data gaps and uncertainties.

The goal of this issues paper is to outline the TK questions that need to be addressed if this area is to contribute to a children's risk assessment. We also hope to provide the reader with some background and resources that can help shed light on these questions, and then begin framing an approach which will facilitate answering these questions on a chemical-by-chemical basis. It is recognized that children's risk assessments also need to address critical exposure and toxicodynamic issues, and that in some ways these areas overlap with toxicokinetics. This paper will point out those interfaces but for this discussion will keep toxicokinetics as a separate consideration in the children's risk assessment process.

1.1 Toxicokinetics as a Key Element in Children's Risk Assessments

The importance of toxicokinetics in risk assessment has been increasing as we have learned more about how toxicokinetic processes (especially chemical metabolism) are involved in mechanisms of toxicity, and that these processes can differ markedly across species. In numerous cases, toxicokinetic analyses have allowed replacement of the tradtional dose metric: applied dose per body weight per day, by a more relevant internal dose metric that facilitates

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extrapolation of animal dose-response data to humans (Andersen et al, 1987; Bois et al, 1990; Rao and Ginsberg 1997; Hattis et al, 1993). Most commonly this is accomplished with physiologically-based pharmacokinetic (PBPK) models which simulate chemical uptake, distribution, metabolism, and excretion in both animals and humans. Through these models, adjustments are made for cross-species differences in toxicokinetics so that the animal internal dose-response profile can be applied to humans. This allows estimation of the exposure dose needed to produce toxic levels of chemicals or key metabolites in humans, the key input into risk assessments.

While progress in toxicokinetics has removed some of the uncertainty in cross-species extrapolations in risk assessment for adults, these principles have yet to be applied in any systematic manner to the risk assessment of children. This is a critical need given that children's toxicokinetics differ from adults in a number of ways: smaller body size; different ratios of fat, muscle, and water; higher breathing and metabolic rates per body weight; and immaturity of clearance systems and enzymatic reactions (Kearns and Reed 1989; Renwick 1998; Anderson et al, 1997; Besunder et al, 1988; Ginsberg et al, 2002). Another obvious difference from adults is that children are more diverse, undergoing a developmental program of growth and maturation that continuously alters the way in which chemicals are processed and cleared. Thus, incorporating children's toxicokinetics into risk assessment is complicated by the need to consider many developmental stages, ranging from *in utero* to adolescence, and by the extensive variability that can occur even within a each age group.

Pharmacokinetic differences between children and adults with respect to the clearance of therapeutic drugs have been recognized for years. These differences have spawned numerous clinical pharmacokinetic studies for the purpose of better titrating drug dosage to a particular age or body size (Morselli 1989; Anderson et al, 1997; Renwick et al, 2000; Ginsberg et al, 2002). The focus on children as a pharmacological receptor has not been matched by a similar focus on children as a toxicant receptor. In large part this is due to the lack of pediatric toxicokinetic studies as mentioned above. This leaves us with a relatively rich pharmacokinetic (PK) database for children but a nearly empty toxicokinetic (TK) database. One of the main challenges facing the children's risk assessment framework is how to combine basic information on children's physiological development with what can be learned from children's PK datasets, into an analytic process that allows us to compare internal doses between adults and children for environmental toxicants.

1.2 Proposed Building Blocks for a Children's TK Assessment

In crafting a general framework for conducting children's risk assessments, a committee organized by ILSI has suggested several phases:

1) Problem Formulation: a) scoping of the risk assessment issue/problem statement, b) presentation of the major exposure routes to children with an initial assessment of the ages in which exposures may be greatest, c) evaluation of data needs (chemical-specific, child-specific) and resources available for further analysis.

2) Data Analysis and Development of Risk Assessment Approach: a) determine which TK and TD (toxic mechanism) pathways are of most importance for the

chemical(s) being analyzed; b) determine which age groups (if any) should be prioritized for further analysis based upon the exposure, TK (internal dose) or TD (susceptibility) factors that differ most between childen and adults; c) decide which analytic approaches are warranted and feasible.

3) Risk Characterization: a) evaluate risks by specific age groups with comparison to adult exposures and risks; b) consider intra-subject variability and uncertainty in the exposure and risk estimates in describing degree of confidence; c) describe how the children's risk assessment has or has not altered the conclusions of the overall risk assessment.

These draft framework areas are a useful point of departure for considering TK issues in children's risk assessment. The questions raised under each phase will help in analyzing adult/children TK issues in a stepwise manner. An overview of this approach is provided in Figure A3-1 and described in more detail.

Figure A3-1. Outline of Toxicokinetic (TK) Assessment Process for Children

Phase I – Problem Formulation

- What type of risk assessment is being conducted?
  - non-cancer in which TK variability (typically 3.2x factor) is already considered?
  - cancer risk assessment in which no such uncertainty factor exists?
- Might children be of special concern due to TK factors such as immaturity of metabolic systems and clearance pathways?
- What data, resources and analytical approaches are available to determine whether:
  - TK factors might affect the degree of exposure (dose) in children?
  - TK factors might cause children to have greater internal exposure per unit of external dose than adults?
    - ♦ novel pathways of metabolism and disposition are possible in children such that children's responses cannot readily be predicted from adult data?

Figure A3-1. Outline of Toxicokinetic (TK) Assessment Process for Children, Cont'd

Phase II – Data Analysis and Development of Analytic Approach
• Chemical-Specific Analyis: determine which TK mechanisms govern chemical fate (activation & detoxification) • Child-Specific Analysis: determine which age groups (if any) should be prioritized base upon size of TK differences relative to adults – Resources for Children's TK/PK Data: a) *in vivo* clearance data from therapeutic drug literature b) *in vitro* enzyme data from liver bank and serum studies c) developmental literature describing changes in body composition and functions in pre- and post-natal periods d) animal TK data for the chemical being analyzed • Determine which TK approaches are most useful and feasible
Phase III – Risk Characterization
• Determine likelihood that children will receive greater internal doses of parent compound or toxic metabolite • Utilize variability/uncertainty analysis to evaluate the distribution of internal exposures possible in children Evaluate whether adjustments of the adult risk assessment are needed to account for children's TK factors (e.g., adjustment of uncertainty factors; more quantitative approaches)

2.0 Phase I. TK Questions in Problem Formulation

A children's risk assessment will likely start with a number of questions regarding age groups and exposures, TK handling of the chemical(s), and whether there are susceptible periods or unique toxic effects (TD issues). These questions and the preliminary answers developed in Problem Formulation help to scope the remainder of the analysis. A major TK question likely to arise in Problem Formulation is:

1) Can early life stages be considered as part of the overall human variability distribution and thus be within the 3.2x TK uncertainty factor? This broad question can be broken into the following more defined areas that should help the risk assessor better scope the issues of children's TK:

What types of data, resources and analytic approaches are needed to determine whether:

- 1a) children of certain ages experience higher exposures due to higher ventilation rates, cardiac outputs, or greater absorption of chemical(s)?

- 1b) children of certain ages experience a higher internal dose of parent compound or active metabolite (per unit of administered dose) than do adults?

- 1c) children of certain ages process the chemical(s) by novel TK pathways so as to generate metabolite profiles that are qualitatively different from adults?

These questions might be raised in Problem Formulation with the hope that by the end of Risk Characterization, the TK issues can be addressed and be factored into whatever risk conclusions are reached for children.

The broad, scoping TK questions raised above are now discussed to provide background information and to help us to consider what kinds of resources and analyses will be needed for their resolution.

2) Does children's TK warrant an uncertainty factor that differs from the current practice of allowing a ~3.2x factor for intersubject variability in TK?

Non-cancer risk assessments concern themselves with threshold effects, with the public health exposure level set well below the observed threshold seen in animal or high exposure human studies (U.S. EPA 1989). A series of uncertainty factors are used to attempt to conservatively cover what we don't know about risks; the uncertainty factors lower the allowable exposure level from the animal or human no observable adverse effect level (NOAEL) or benchmark dose. Prominent among these is the interindividual variability factor, typically a 10-fold factor. This factor results from a general recognition that the animal species or worker cohort from which the NOAEL comes, rarely if ever represents the range of diversity present in the human population at large. This uncertainty factor is meant to encompass variability from a wide array of sources: inherited traits and genetic polymorphisms, gender and hormonal differences, lifestyle factors such as amount of stress, exercise, tobacco and alcohol use, obesity, nutritional status, and finally disease conditions, some of which can alter the handling of xenobiotics. Related to this last factor is the ingestion of prescription and over-the-counter medications, many of which can alter the pharmacokinetics of other chemicals. Another circumstance that this inter-subject uncertainty factor is asked to cover is age, ranging from the fetal period through geriatrics.

As mentioned above, the non-cancer uncertainty factor is 10, which can be understood to consist of a half log factor (3.16x) for toxicokinetic variability and a similar half log factor for pharmacodynamic variability (Renwick 1998). Thus the inter-individual variability in TK created by the various genetic, lifestyle, physiologic state and age factors needs to fit within a 3.2-fold factor for the default to be adequate. It becomes the job of the children's risk assessor to determine whether this is so, and if not, whether a special children's uncertainty factor or other more quantitative approaches are called for.

At the heart of this question is whether children (or certain age groups of children or the *in utero* period) represent a sufficiently unique subpopulation in terms of the way xenobiotics are handled to be considered outside the variability bounds of the adult human population. This question must not only consider the central tendencies for key TK endpoints, but also the extent of variability in children. This is needed to determine whether the upper or lower ends of the

children's distribution (e.g., low-end metabolizers) are captured within the bounds created by the adult mean value and a 3.2x uncertainty factor.

It is important to point out that a similar uncertainty factor does not exist for cancer risk assessment. These assessments are generally considered conservative due to the nature of the linear low dose modeling often used, and because interspecies scaling of the unit risk factor employs a generic body weight (Garbis and Peters 1987; Hytten and Leitch 1971) function that increases human potency relative to rodents (Andersen et al, 1995; U.S. EPA 1992). However, the lack of any special consideration of children's risk in cancer assessments leaves open the question of whether the derived unit risks are appropriate for all age groups. In fact, one recent cancer risk assessment did adjust the unit risk for child-specific factors, but this adjustment was not based upon toxicokinetics (U.S. EPA 2000).

Thus, in the absence of precedents for using a children's toxicokinetic analysis to adjust cancer or non-cancer assessments, a framework is needed for how this could be done in a rationale and consistent manner. The hope is that such a process will foster an understanding of the size of adult/child differences in key TK pathways relative to the amount of inter-individual variability we have come to expect in non-cancer assessments.

To go beyond this large, overarching question, Problem Formulation needs to identify key databases, resources, and analytic approaches available for Areas 1a, 1b, and 1c as described above (TK influences on how much chemical is absorbed; TK determinants of internal dose per unit of external dose; Novel or shifting TK pathways during development). The overview of Phase II below describes resources and approaches which can be initially identified during Problem Formulation. The use of these resources will be determined in Phase II by chemical-specific factors (e.g., pathways of metabolism and clearance, toxic mechanism and identification of key dose metric), child-specific factors (e.g., *in utero* and post-natal developmental profile of key TK pathways for target chemical), and scenario-specific factors (which age groups most likely to be most exposed; which exposure routes most likely to be substantial).

3.0 Phase II: Data Analysis and Development of Risk Assessment Approach

In this phase, chemical-specific data regarding the fate of the chemical(s) being analyzed are combined with children's data relating to the developmental profile of TK systems (from *in utero* periods through adolescence). This will hopefully foster an understanding of how children of various ages are likely to handle the chemical(s). Obviously, the best case is to have metabolism and disposition data for the chemical(s) in children. However, it will be extremely rare to find such TK data. Some approaches that can be considered for filling these data gaps are the following:

3.1 Use of TK data for the chemical(s) under analysis to characterize the major fate and transport mechanisms.

Typically, animal data, and in some cases, also adult human data, will be available for characterizing the chemical's fate and disposition. Once the major TK factors governing

chemical fate are known, then the approaches which follow can be evaluated for utility and feasibility.

3.2 Use of surrogate chemicals (typically therapeutic agents) for which PK data in children exist.

Even if the target chemical(s) has not been evaluated in children, a chemical that is similarly processed may have been tested. Information for surrogate chemicals can help delineate the maturation of key pathways and how the target chemical will be handled at certain developmental stages. This section describes the kinds of datasets and resources available for this part of the Phase II assessment.

Children's PK databases have been developed over the past several years in which therapeutic drugs tested in both children and adults are identified and key PK parameters compared across ages (Renwick 1998; Renwick et al, 2000; Ginsberg et al, 2002). These databases are especially useful because the metabolism and clearance pathways of many of the drugs are known, making them useful indicators for particular pathways. For example, dextromethorphan and debrisoquine are known substrates for a particular cytochrome P-450 enzyme (CYP 2D6), trimethadione, chlorzoxazone, and halothane are markers for CYP2E1 activity, morphine is predominantly processed by glucuronidation, and a host of antibiotics are not extensively metabolized but are mostly excreted unchanged by the kidneys (Dollery 2000; Bertz and Granneman 1997; Kurata et al, 1998; Tanaka 1998).

A well-known example of child-adult differences in metabolic processing is caffeine, in which an initial N-demethylation reaction is catalyzed by CYP1A2, and a secondary N-acetylation step is catalyzed by N-acetyltransferase (Welfare et al, 2000). The CYP1A2 reaction is the primary factor governing half-life of this drug. Caffeine's half-life in newborns is 14-fold higher than in adults, which is likely the result of the immaturity of CYP1A2 at this age (Parsons 1976; Parsons and Neims 1978; Ginsberg et al, 2002; Dorne et al, 2001). The metabolic clearance of caffeine and another CYP1A2 substrate, theophylline, become more like the adult rate by 2-6 months of age, after which time the half-life becomes significantly shorter than in adults (Ginsberg et al, 2002; Dorne et al, 2001). This suggests that once CYP1A2 function approaches adult levels, the greater size and blood flow to the liver (per body weight) in young children can lead to greater enzymatic clearance of CYP substrates (Gibbs et al, 1997). However, this also means that chemicals activated to toxicants by this pathway (e.g., aromatic amines, polycyclic aromatic hydrocarbons, aflatoxin B1) may produce more active metabolite in this age group as compared to adults. Unfortunately, there are no data that can be used to directly evaluate this possibility.

Following up on the caffeine example further, this drug has been used to show that the N-acetyltransferase step is also deficient in early life. Once caffeine is demethylated by CYP1A2 it can be N-acetylated to form AFMU (5-acetylamino-6-formylamino-3-methyluracil) or excreted in urine in a non-acetylated form (1x). The ratio of AFMU to 1X has been used to phenotype the N-acetyltransferase trait in adults and children. In the case of children, the ratio of acetylated to non-acetylated metabolite is low in newborns thru 2 months of age (12% of adult ratio) but then rises to 65% of the adult ratio during the 2-6 month interval (Pariente-Khayat et al, 1991). This agrees with data showing that most newborns are slow acetylators, but that beyond 100 days, the

underlying genetic polymorphism (fast vs. slow) becomes evident (Pons et al, 1989; Szorady et al, 1987). Such data with caffeine are exemplary of the information that can be obtained from pharmacokinetic data for therapeutic agents.

Table A3-1 compiles information obtained from *in vivo* PK analyses of drugs with *in vitro* analyses of enzyme levels from blood or liver samples. The combination of the two types of information for a given clearance pathway can provide a strong indication of how the pathway's function develops in the postnatal period. *In vivo* PK data have been analyzed across chemicals that share a common mode of elimination to develop a more complete evaluation of the function of specific pathways (Ginsberg et al, 2002; Renwick et al, 2000). Table A3-1 shows data for 6 CYP enzymes, several Phase II conjugation pathways, renal and biliary clearance, other metabolic functions (e.g., epoxide hydrolase, alcohol dehydrogenase), and two esterases involved in the detoxification of organophosphate pesticides. This compilation of PK data by pathway and age group indicates a fairly consistent pattern, i.e., premature neonates, full-term neonates, and infants up to 6 months of age tend to have less metabolic and clearance capacity than adults. The two exceptions in the chart are for enzymes that are expressed primarily in the fetal and early post-natal period: CYP3A7 and glutathione transferase (GST) pi. These fetal forms are replaced during the first year of life by corresponding (but not enzymatically equivalent) adult forms. Beyond 6 months, many CYP enzymes are sufficiently active that clearance *in vivo* is actually greater than in adults. This appears to be due to the greater liver size and blood flow in children as compared to adults (Gibbs et al, 1997). Table A3-1 provides further evidence for this phenomenon in that the *in vivo* data indicate greater clearance capacity than suggested by the *in vitro* protein levels or enzyme activities for CYP1A2, 2E1, 2C9/19, 2D6, and 3A4, particularly at 6 months of age.

These overall patterns are illustrated for a specific CYP, CYP3A4 as evidenced by therapeutic drugs that depend upon this CYP for clearance (Figure A3-2) (Ginsberg et al, 2002). CYP3A4 is the major CYP in adult human liver but its function is evidently deficient in early life. This pattern appears to be widely applicable in that an analysis across 40 therapeutic drugs involving 11 different metabolic/excretory mechanisms shows a similar shift from immaturity in the earliest age categories to evidence of drug half-lives shorter than adults beyond 6 months of age (Figure A3-3).

The period of shorter half-lives in children relative to adults may represent a time in which there is faster removal of parent compound but greater formation of metabolites, which can be a concern if chemical metabolism leads to more toxic moieties. It should be pointed out that despite the possibility of more rapid activation of some compounds, lower toxicity may be experienced by children due to more rapid systemic clearnance of metabolites. Thus, predicting the health implications of shifts in xenobiotic metabolism will be chemical-specific and needs to consider both parent compound and toxic metabolites.

The period of accelerated clearance is in contrast to the earlier periods in which hepatic metabolism was slower than in adults leading to the potential for prolonged retention and higher levels of parent compound. This situation may be compounded by the slower renal and biliary function at these times. In terms of detoxification systems, the chart shows that epoxide hydrolase is active at birth but apparently only at 50% of adult function. Although the data are very limited, it appears that two forms of GST may be deficient (40-60% of adult levels) in early

life. These data may have risk implications given that GST mu is critical to epoxide conjugation with GSH and that epoxide hydrolase also appears to be slow in this age group. Thus, the newborn and very young infant may have a relative deficit in detoxifying epoxides, although compensating mechanisms (e.g., other GSTs) may exist. It should be noted that further research into the developmental profile of these detoxification systems is an important research need.

Table A3-1: Post-Natal Developmental Profile of Pharmacokinetic Functions
(Pathway Function Shown as % Adult; Abbreviations: PL – Protein Level; EA-Enzyme Activity; for others see legend)

PK Pathway	Premature Neonates	Full-Term Neonates	1 Week– 2 Month	2 - 6 Months	6 Months – 1 Yr	1-2 Years	> 2 Years
Renal Clearance		35% - *in vivo* t½ for 7 drugs[a] GFR ~20% adult[b]	28% - *in vivo* t½ for 7 drugs[a]	88% - *in vivo* t½ for 7 drugs[a] GFR ~100% adult[b]	137% - *in vivo* t½ for 7 drugs[a]	137% - *in vivo* t½ for 7 drugs[a]	185% - *in vivo* t½ for 7 drugs[a]
CYP 1A1	--[c]	--[c]	--[c]	--[c]	--[c]	--[c]	--[c]
CYP 1A2		2% - PL[e] 2% - *in vitro* EA[f] 11% - *in vivo* t½ for 2 drugs[d]	4% - PL[e] 3% - *in vitro* EA[f] 4% - *in vitro* EA[g] 23% - *in vivo* t½ for 2 drugs[d]	16% - PL[e] 9% - *in vitro* EA[f] 13% - *in vitro* EA[g] 81% - *in vivo* t½ for 2 drugs[d]	25% - PL[e] 15% - *in vitro* EA[f] 13% - *in vitro* EA[g] 175% - *in vivo* t½ for 2 drugs[d]	175% - *in vivo* t½ for 2 drugs[a]	54% - PL[e] 35% - *in vitro* EA[f] 185% - *in vivo* t½ for 2 drugs[d]
CYP 2A6		20%- nicotine t½[ff]					
CYP 2E1		13% - PL[h] 27% -*in vitro* EA[i] 27%-serum DMO/TMO[j]	22% - PL[h] 39% - *in vitro* EA[I] 27%- *in vivo* serumDMO/TMO[j]	30% - PL[h] 47% - *in vitro* EA[i]	36% - PL[h] 41% - *in vitro* EA[i] 97%-serum DMO/TMO[j]	92%-*in vivo* serum DMO/TMO[c]	82% - PL[h] 83% - *in vitro* EA[b] 92%-serum DMO/TMO[c]
CYP 2C9/19	21% - PL[k] 33%-*in vitro*EA[l]	30% - *in vivo* t½ for 1 drug[m]	29% - PL[k] 30% - *in vitro* EA[l]	38% - PL[k] 45% - *in vitro* EA[l]	36% - PL[k] 83% - *in vitro* EA[l] 182% - *in vivo* t½ for 1 drug[m]	36% - PL[k] 83% - *in vitro*EA[l] 182% - *in vivo* t½ for 1 drug[m]	130% - *in vivo* t½ for 1 drug[m]
CYP 2D6		13% - PL[n]	22% - PL[n]	34% - PL[n]	45% - PL[n]		88% - PL[n]
CYP 3A4	19% - *in vivo* t½ for 8 drugs[o]	17% - *in vitro* EA[p] 50% - *in vivo* t½ for 8 drugs[o]	29% - *in vitro* EA[p] 55% - *in vivo* t½ for 8 drugs[o]	37% - *in vitro* EA[p]	46% - *in vitro* EA[p] 200% - *in vivo* t½ for 8 drugs[o]	110%-*in vitro*EA[p] 200% - *in vivo* t½ for 8 drugs[o]	189% - *in vivo* t½ for 8 drugs[o]

Table 1. Post-Natal Developmental Profile of Pharmacokinetic Functions (Cont'd)

Post-Natal Developmental Profile of Pharmacokinetic Functions (Pathway Function Shown as % Adult)

PK Pathway	Premature Neonates	Full-Term Neonates	1 Week– 2 Month	2 - 6 Months	6 Months – 1 Yr	1-2 Years	> 2 Years
CYP 3A7		1100%-*in vitro* EA[q]	600%-*in vitro* EA[q]	300%-*in vitro* EA[q]	200%-*in vitro* EA[q]		
Epoxide Hydrolase		42% - PL[r] 50% - CBZ-E/CBZ[s]	50% - CBZ-E/CBZ[s]	50% - CBZ-E/CBZ[s]	50% - CBZ-E/CBZ[s]	50% - CBZ-E/CBZ[s]	65% - CBZ-E/CBZ[s]
Glucuron-idation	23% - *in vivo* t½ for 6 drugs[t]	34% - *in vivo* t½ for 6 drugs[t]	47% - *in vivo* t½ for 6 drugs[t]	102% - *in vivo* t½ for 6 drugs[t]	84% - *in vivo* t½ for 6 drugs[t]	84% - *in vivo* t½ for 6 drugs[t]	74% - *in vivo* t½ for 6 drugs[t]
Sulfation/Gluc.		84%-APAP clearance[u]		149%-APAP clearance[u]			70%-APAPclearance[u]
Acetylation		83% slow phenotype[v]	12%- *in vivo* caffeine N-acetylation[w]	65%- *in vivo* caffeine N-acetylation[w]			48% slow phenotype[v]
GSH Transferase		$GST\alpha_{B1}$ ~ 100% - PL[x] $GST\alpha_{B2}$ ~ 40% - PL[x] GSTmu ~ 60% - PL[x] GSTpi >2100% - PL[x]	$GST\alpha_{B1}$ ~ 100% - PL[x] $GST\alpha_{B2}$ ~ 40% - PL[x] GSTmu ~ 60% - PL[x] GSTpi >2100% - PL[x]	$GST\alpha_{B1}$ ~ 100% - PL[x] $GST\alpha_{B2}$ ~ 40% - PL[x] GSTmu ~ 60% - PL[x] GSTpi >2100% - PL[x]	$GST\alpha_{B1}$ 112% - PL[x] $GST\alpha_{B2}$ 62% - PL[x] GSTmu 93% - PL[x] GSTpi 2100% - PL[x]		121% - *in vivo* t½ of busulfan[y]
Biliary Exc.		57% - BSP clear[z]	70% - BSP clear[z]	90% - BSP clear[z]			
Serum Protein	few binding sites[aa]	few binding sites[aa]	few binding sites[aa]	Increasing # sites[aa]	increasing # sites[aa]	adult level[aa]	adult level[aa]
Serum Cholinesterase	50% - EA[bb]	57% - EA[bb]	101% - EA[bb]	91% - EA[bb]	104% - EA[bb]		
Serum Arylesterase		28% - EA[cc]	34% - EA[cc]	52% - EA[cc]	84% - EA[cc]	80% - EA[cc]	
Alcohol Dehydrogenase		55%-*in vivo* EtOH clearance[dd]	15%- *in vitro* EA[ee]		32%- *in vitro* EA[ee]		91%- *in vitro* EA[ee]

Legend for Table A3-1:

[a]Ginsberg et al. (2002) analysis of renally cleared drugs: ampicillin, cimetadine, furosemide, piperacillin, ticarcillin, tobramycin, vancomycin.

[b]Glomerular filtration rate (GFR) in ml/min/kg (Morselli, 1989; Besunder, et al., 1988).

[c]CYP1A1 protein or enzyme activity not detectable in microsomes from liver bank samples at any age indicating very low constitutive levels (Sonneir and Cresteil, 1998).
[d]Ginsberg et al. (2002) analysis of drugs cleared primarily via CYP1A2: caffeine, theophylline.

[e]Sonnier and Cresteil (1998) measured CYP1A2 protein levels via immunochemical methods in microsomes from liver bank samples (N=6 to 23 per age group).

[f]Sonnier and Cresteil (1998) measured CYP1A2 activity in microsomes from liver bank samples using methoxyresorufin as substrate (N=6 to 23 per age group).

[g]Cazeneuve et al. (1994) measured CYP1A2 activity in microsomes from liver bank samples via caffeine N-demethylation.

[h]Vieira, et al. (1996) measured CYP2E1 protein levels in liver bank microsomes by immunochemical means (N=4 - 23 per age group).

[i]Vieira, et al. (1996) measured CYP2E1 activity levels in liver bank microsomes using chlorzoxazone as substrate (N=2 for 1-10 yr old group; otherwise N=9 - 21 per age group).

[j]Tanaka (1998) measured the ratio of oxidized metabolite dimethadione (DMO) to parent drug trimethadione (TMO) in serum of children dosed at the following ages: <4wks (N=5); 1 month to 1 yr (N=5); 1-10 yrs – N=21; adult – N=20). CYP2E1 converts TMO to DMO.

[k]Treluyer et al. (1996) measured CYP2C protein levels in microsomal preparations from liver bank samples with immunochemical techniques.

[l]Treluyer et al. (1996) measured CYP2C19 enzyme activity in microsomal preparations from liver bank samples using diazepam as substrate.

[m]Ginsberg et al. (2002) analysis of tolbutamide, a drug cleared primarily via CYP2C.

[n]Treluyer, et al. (1991) measured CYP2D6 protein levels in microsomal preparations from liver bank samples with immunochemical techniques.

[o]Ginsberg, et al. (2002) analysis of drugs cleared primarily by CYP3A: alfentanil, carbamazepine, fentanyl, lidocaine, midazolam, nifedipine, quinidine, triazolam.

[p]LaCroix, et al. (1997) measured CYP3A4 enzyme activity (EA) in microsomes from liver bank samples using testosterone as substrate (n=12 newborns, 9 @ 1 wk-1 month, 13 @ 1-3 months, 11 @ 3-12 months, 2 > 1 yr, 11 adults).

[q]LaCroix, et al. (1997) measured CYP3A7 enzyme activity (EA) in microsomes from liver bank samples using dehydroepiandrosterone as s ubstrate (n=12 newborns, 8 @ 1 wk-1 month, 20 @ 1-3 months, 14 @ 3-12 months, 0 > 1 yr, 12 adults).

[r]Ratanasavanh, et al. (1991) measured epoxide hydrolase protein levels by Western blot on liver bank microsomal preparations (N=5 for 1 day to 1 wk of age; N=5 for adults).

[s]Epoxide hydrolase levels estimated by carbamazepine-epoxide (CBZ-E) to CBZ ratio in blood in different age groups at steady state after repeat drug administration in epileptic patients. Data pooled across 2 studies (Korinthenberg, et al., 1994; Pyonnonen, et al., 1977). Higher CBZ-E/CBZ ratios in children is indicative of slower EH activity since CBZ-E formation and CBZ clearance is slower in young children (Kuhnz, 1983; Eichelbaum, 1975, 1985; Ohmori, et al., 1998).

[t]Ginsberg et al. (2002) analysis of drugs cleared directly via glucuronidation : lorazepam, morphine, oxazepam, trichloroethanol, valproic acid, zidovudine.

[u]Ginsberg et al. (2002) compilation of kinetic data for acetaminophen (APAP) across 5 studies; N=7 to 24 per age group. APAP is a substrate for various conjugation reactions with sulphation predominating in early life (Levy, 1975; Besunder, et al., 1988).

[v]Pariente-Khayat, et al. (1991) measured the ratio of acetylated to non-acetylated metabolite in urine after caffeine administration in children (N=10-26). Younger age groups compared against an older age group (6 month-2 yrs) since adult data not available and since onset of rapid phenotype occurs by this age.

[w]Szorady, et al. (1987) phenotyped 100 newborns 2-3 days old as well as 100 or more subjects in older age groups using acetylation of sulfadimidine (as appears in urine).

[x]Strange, et al. (1989) measured GST protein levels by radioimmunoassay in liver bank tissue cytosols. $GST\alpha_{B1}$ levels were 70% of adult *in utero* and 112% of adult @ 5 –10 months post-natal. Therefore, approximate that newborn thru 6 months levels would be approx. equal to adult. $GST\alpha_{B2}$ protein levels were 25% of adult during the *in utero* period 62% of adult @ 5-10 months post-natal. Therefore, estimate is that newborn thru 6 months are 40% of adult. GSTmu is 22% of adult during *in utero* period and 93% of adult @5-10 months. Therefore, estimate is that newborn thru 6 months are 60% of adults. GSTpi is 5300% of adult during *in utero* period and 2100% of adult @ 5-10 months.

[y]Gibbs, et al. (1997) measured busulfan half-life in a group of 14 children (avg. age=3) relative to adults. Busulfan is metabolized predominately by $GST\alpha_{B1}$.

[z]Jusko et al. (1972) measured clearance of BSP (bromsulphphalein) in groups of 5 or more children; values in young children compared against an older child group (mean age=) since adult data not available.

[aa]Serum protein binding sites (albumin, α_1-acid glycoprotein) low in newborns but increases to adult levels by 1 year (Besunder, et al., 1988).

[bb]Data pooled across 4 studies (Lehmann, 1957; Augustinsson, 1963, Ecobichon and Stephens, 1973; Zsigmond, 1971) in which serum cholinesterase levels were measured with acetylcholine, benzoylcholine, butyrylcholine or procaine as substrate.

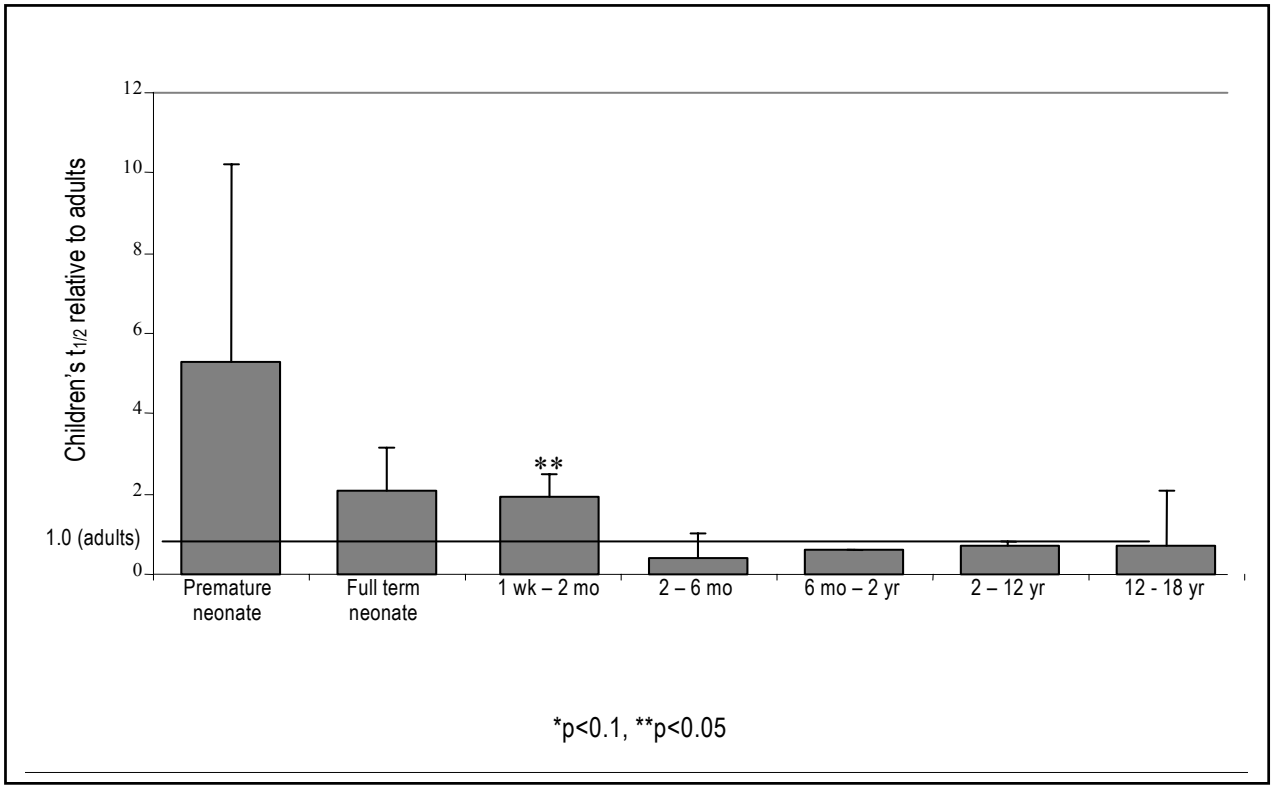
[cc]Augustinnson et al. (1963) and Ecobichon and Stephens (1973) measured serum arylesterase activity with phenyl acetate as substrate.

[dd]Idanpaan-Heikkila et al. (1972) followed the removal of ethanol from mother and newborn blood (N=6 for each age); newborns were exposed transplacentally and born with equal blood alcohol conc. as mother.

[ee]Pikkarainen, et al. (1967) measured alcohol dehydrogenase activity in liver samples from newborns 1 wk to 2 months (N=2), infants 6 month-2 yr (N=2), older children 5-15 yr (N=3), and adults (N=3). Data were 9 fetuses were approx. 25% of the newborn levels.

[ff]Dempsey, et al. (2000) measured nicotine elimination from newborns (N=5) exposed transplacentally

Figure A3-2. Analysis of Children's Pharmacokinetic Database Half-Life Results for CYP3A Substrates -- Alfentanil, Carbamazepine, Fentanyl, Lignocaine, Midazolam, Nifedipine, Quinidine, Triazolam (Ginsberg et. al 2002)



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Another potentially important deficit in children is glucuronidation during the first 2 months of life. Given the relative deficiency of this enzyme and high bilirubin levels (an endogenous glucuronidation substrate), it is possible that there will be greater internal exposure early in life to xenobiotics (or their metabolites) that require glucuronide conjugation. This is the case with the antibacterial drug chloramphenicol, in which a relative lack of glucuronidation contributed to its accumulation and toxicity (anemia) in infants (Vest 1965; Mulhall et al, 1983). In contrast, some xenobiotics can undergo conjugation with alternative cofactors which allows a shift in metabolic profile if the primary pathway is compromised or immature. This is known to occur with acetaminophen, in which case sulfation predominates early in life until glucuronidation takes over (Levy et al, 1975).

The findings described above are consistent with the pediatric PK summaries provided by Renwick's group (1998, 2000), Dorne et al. (2001) in which they demonstrate that there are a variety of drugs and age groups for which clearance in childhood is slower than in adults. There are also cases in which clearance is more rapid, particularly when the pediatric group was at least several months of age (Renwick 1998). Further, the relative size of the neonate/adult differences shown in Table A3-1 and Figures A3-2 and A3-3 are similar to that seen in a composite analysis of 36 drugs with pediatric data (Renwick et al, 2000). These overall trends may be useful in making generalizations about the development of PK functions in pediatric populations. The type of age-specific and pathway-specific PK information available for children should prove

useful in predicting how children (particularly neonates) may differ from adults with respect to internal dosimetry and ultimate risk.

Figure A3-3. Analysis of Children's Pharmacokinetic Database: Half-Life Results for Full Database – 40 Substrates (Ginsberg et. al 2002)

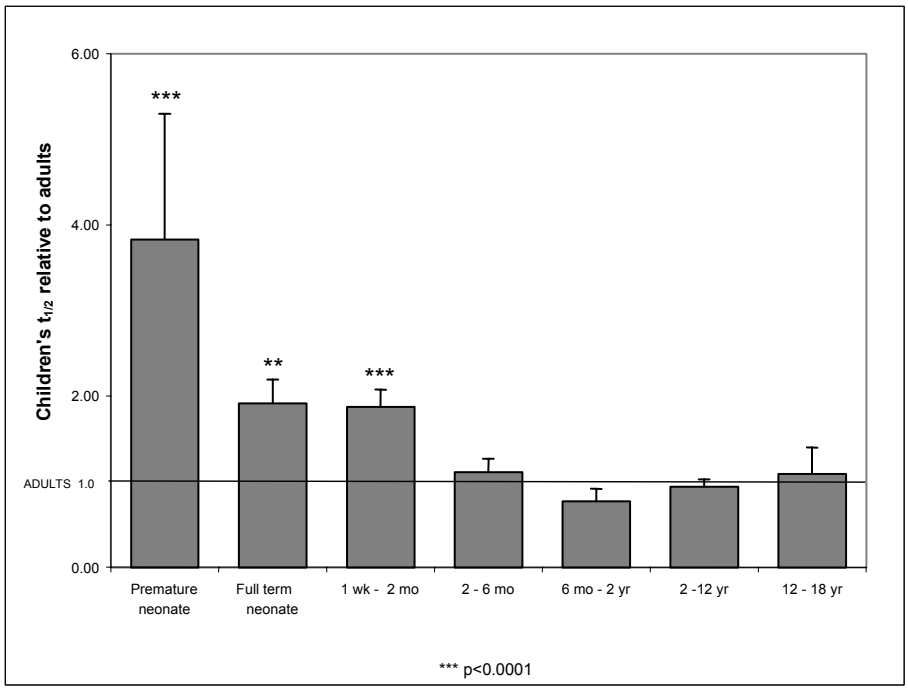


Figure reprinted with permission from Oxford University Press.

Simply knowing about the function of particular pathways may not be enough information to predict *in vivo* handling of a xenobiotic at a particular age. There are numerous TK factors involved in chemical processing and clearance including partitioning into body compartments, protein binding, respiration, and organ flows. Therefore, a more comprehensive analysis, such as PBPK modeling, may be needed to integrate the various factors at work and predict xenobiotic fate in children. The information provided in Table A3-1 combined with basic physiologic information may make PBPK modeling of children more feasible. Where this is pursued, the uncertainty and data gaps surrounding input parameters for children need to be recognized and made transparent.

While characterization of pathway function based upon indicator drugs is a useful approach, it carries the uncertainty that a drug may be processed by several clearance pathways. If one pathway is deficient at a certain age, but another is more functional, then the overall half-life or clearance rate may not be affected; instead parent compound may be shunted from the less active to the more active pathway, leading to a shift in metabolite profile. The assignment of chemicals to particular pathways is based upon the fate of the majority (generally 60% or more) of the administered dose in adult humans, as ascertained from the literature. Shifts in metabolic processing in early life would tend to obscure child vs. adult clearance differences based upon overall half-life or blood clearance. Thus, in some cases, the data in Table A3-1 and Figures A3-

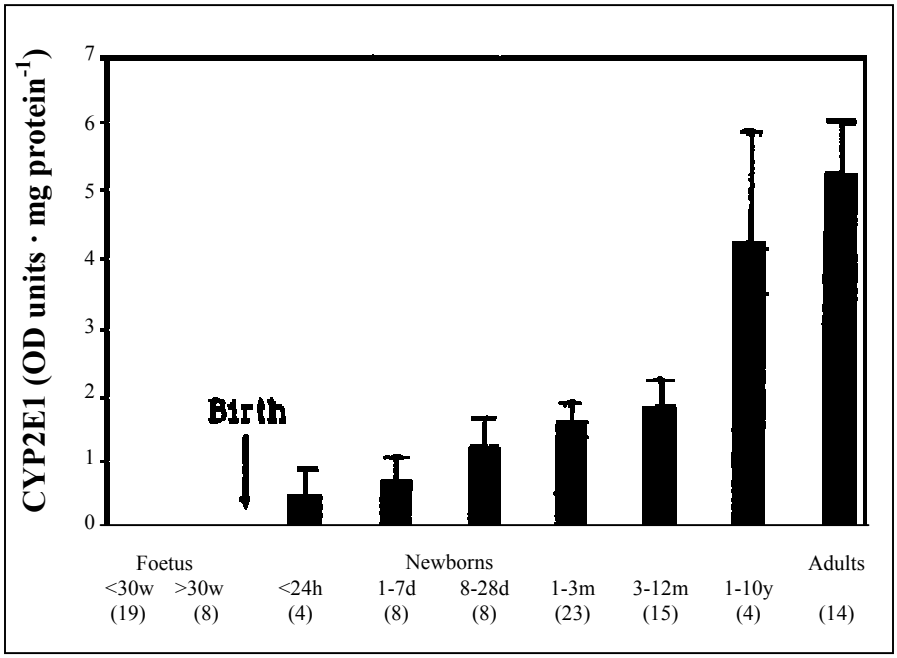
2 and A3-3 may be an underestimate of the degree of child-adult difference that might actually exist for a given pathway.

Another caveat for some of the *in vivo* datasets cited in Table A3-1 and Figures A3-2 and A3-3 is that they represent clinical PK trials in children who are not in full health. The datasets summarized in Ginsberg et al. (2002) were screened not only for sufficient numbers of subjects per age group, but also for the health of the subjects on test. Data for children who were critically ill or severely comprimised, especially with regards to hepatic or renal conditions that would affect xenobiotic handling, were excluded from the database. However, it is possible that the clinical state of the children on test may have affected factors such as volume of distribution or other PK factors in certain datasets. The overall concordance between *in vitro* and *in vivo* data shown in Table A3-1 suggests that this factor is not a systematic issue here.

3.3 Use of in vitro data to ascertain how TK pathways change during development.

The liver bank data describing CYP protein levels and enzyme activites, as well as the serum esterase data shown in Table A3-1, can be used as indicators of pathway function at particular ages. These data are made more reliable for risk assessment when there are corroborating *in vivo* half-life or clearance data for indicator chemicals. An example is CYP 2E1 function in which *in vitro* liver bank studies (Figures A3-4a and 4b) generally agree with *in vivo* drug metabolism data for the CYP 2E1 substrate, trimethadione (Figure A3-5) (Vieira et al, 1996; Tanaka 1998). These developmental profiles can be essential for estimating the degree to which 2E1-mediated metabolic activation may occur for a wide variety of environmental toxicants (e.g., benzene, chlorinated solvents, ethanol).

Figure A3-4a. Age-Related Variations of CYP2E1 Protein in the Human Liver
(Figure from Vieira et al. (1996); reprinted with permission from Blackwell Publishing)



Microsomal proteins (60 µg) were separated on a 9% SDS/PAGE and transferred to nylon membranes. CYP2E1 was immunochemically detected with a polyclonal antibody raised against rat orthologue CYP2E1: the antigen-antibody complex was visualized after addition of peroxidase-conjugated anti-(rabbit IgG) antibody using 4-chloro-1-naphthol as the dye, and quantified by scanning with an image analysis system. Results are expressed as the mean ± SE of densitometric values of immunochemically detected CYP2E1/mg protein: OD units are an arbitrary measure of the density. Groups are defined in Methods and the number of samples in each group is indicated between brackets (Vieira et al, 1996).

Figure A3-4b. Age-Related Variations of Chlorzoxazone Hydroxylation in the Human Liver (Figure from Vieira et al, (1996); reprinted with permission from Blackwell Publishing)

Microsomal samples (0.3 nmol *P*-450) were incubated with 500 µM chlorzoxazone and a NADPH-generating system. The formation of the 6-hydroxylated metabolite was monitored at 287 nm after separation by HPLC. Results are expressed as the mean ± SE of activity measured as rate of formation of 6-hydroxychlorzoxazone/mg microsomal protein. Groups are defined in Methods and the number of samples in each group is indicated between brackets (Vieira et al, 1996).

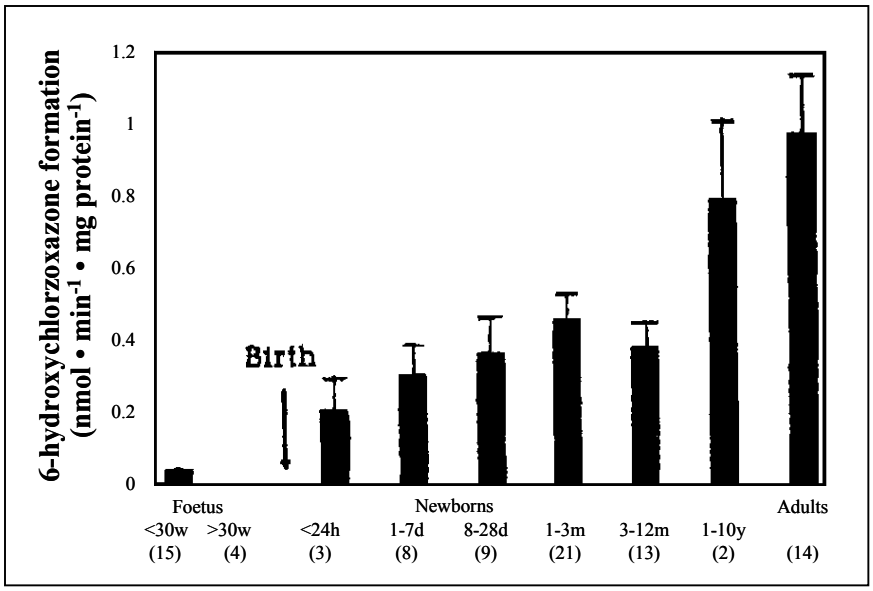
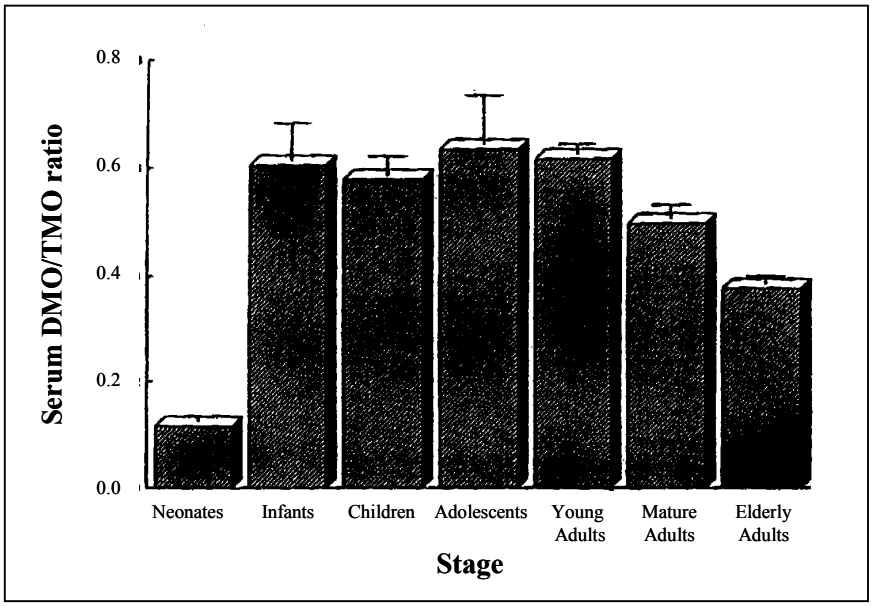


Figure A3-5. Age-Related Changes Over Seven Stages of Life in the Serum Dimethadione/ Trimethadione Ratio 4 h After Oral Administration of Trimethadione (4mg/kg)
(Figure from Tanaka (1998); reprinted with permission from Blackwell Publishing)



The seven stages were: neonates (<4 weeks; *n*=5), infants (<12 months; *n*=12), children (<10 years; *n*=21), adolescents (<20 years; *n*=3), young adults (<40 years; *n*=20), mature adults (<65 years; *n*=20), and elderly adults (>65 years; *n*=40). Mean ± SE (Tanaka 1998).

3.4 Use of developmental information to evaluate how TK can affect susceptibility during the in utero period.

The maternal-fetal environment presents unique TK considerations. The developing organism is exposed as part of the maternal system via placental transport. While maternal

factors (distribution, metabolism, excretion) govern fetal exposure, exposure to the fetus can be quantitatively or qualitatively different than in the mother. This may result from chemical accumulation in the fetal compartment (NRC 2000), or from in situ metabolism that causes fetal exposure to metabolites different from that experienced in the maternal system. Another factor is that metabolites formed in the fetus may be slower to cross the placenta than parent compounds due to their decreased lipid solubility, leading to a relative concentration of metabolites in the fetus.

The following summarizes the key TK factors during *in utero* development.

3.4.1 Placental Transport

Between the site of maternal exposure and the conceptus is a specialized set of membranes that chemicals must cross. The placenta has its origins in the conceptus, with its unique anatomical and functional features influencing chemical transfer to the developing conceptus. This set of membranes undergoes considerable change with development and also exhibits substantial species differences. As pregnancy progresses, the syncytiotrophoblast layer (in direct contact with maternal blood and relatively thick in early pregnancy) becomes progressively thinner (Garbis and Peters 1987). The cytotrophoblast layer becomes more discontinuous and the endothelium of the embryo/fetal vessels within the villi becomes thinner. Thus, as pregnancy progresses, there is closer contact between the fetal blood and the syncytiotrophoblast, that placental cell layer most important to placental function and maternal-embryo/fetal exchange. This leads to greater exchange of nutrients and chemicals across the placenta as gestation proceeds. On the macroscopic level, the effects of gestational age can be exemplified by comparing the tremendous change in the ratio of placental/fetal weight (ratio equals 4 at 10 weeks and 0.2 at 40 weeks) (Hytten and Leitch 1971).

Neither the function nor the anatomical thickness of the placenta, however, is consistently related to the number of layers separating the conceptus from the mother. Importantly, any substance in the maternal circulation can, to some extent, be transferred across the placenta unless it is metabolized or detoxified before or during its placental passage (Slikker Jr 1987; Garbis and Peters 1987).

A useful method for characterizing placental transfer across chemicals and species is the dually-perfused placenta. This is an *in vitro* preparation in which placental transfer is defined as a clearance ratio. Studies of nonmetabolized model compounds indicate decreases in placental transfer as the molecular weight of the model compounds increase (Illsley et al, 1985). Under these very controlled conditions without maternal or fetal involvement, differences in placental transfer between species are evident, with sheep being more different from human than guinea pig. These types of functional assessments help to predict placental transfer and fetal exposure after maternal dosing.

Additional factors which influence the placental transfer of chemicals are uterine/placental blood flow, placental permeability, and placental metabolism (Mirkin and Singh 1976; Miller et al, 1976; Waddell and Marlowe 1981; Mihaly and Morgan 1983; Juchau 1980a). These factors are not static during pregnancy but may change as gestation progresses.

Chemical delivery to the developing conceptus relies primarily on blood flow to the placenta. Although chemicals may transfer from mother to fetus via the amniotic fluid after crossing the amnion, the majority of agents gain access to the conceptus via placental passage (Nau and Liddiard 1978). In addition to the changes in placental blood flow that occur during gestation, changes in blood flow as a result of chemical exposure must also be considered. While it is known that experimentally induced changes in placental blood flow can alter normal development of the conceptus (Greiss and Gobble 1967; Barr and Brent 1978; Millicovsky and DeSesso 1980), the effect of such perturbations on placental transport of xenobiotics needs to be evaluated.

Placental permeability to a chemical is influenced both by placental characteristics (e.g., thickness, surface area, carrier systems and lipid/protein content of the membranes) and also by characteristics of the chemical agent (e.g., degree of ionization, lipid solubility, protein binding, and molecular weight) (Mirkin and Singh 1976; Mihaly and Morgan 1983; Welsch 1982). The rate of placental transfer is rapid for nonionized, lipid-soluble chemicals of low molecular weight (less than 1000) and is largely controlled by placental blood flow (Mirkin and Singh 1976; Mihaly and Morgan 1983). However, charged molecules such as tubocurarine have also been shown to enter the fetus (Kivolo and Saarikoski 1972; Kivolo and Saarikoski 1976). Likewise, chemicals that are highly ionized at normal blood pH, such as the salicylates, readily cross the placenta (Wilson et al, 1977). The question is thus not whether a compound crosses the placenta, but rather at what rate.

3.4.2 Maternal Considerations

A chemical must pass through and may interact with several anatomical compartments on its journey from the site of maternal exposure to the organ systems of the developing conceptus. Maternal factors act to either enhance or diminish the concentration of an active chemical in the conceptus. Maternal detoxification decreases the amount of parent compound available for placental transport, whereas maternal bioactivation may make more active chemical available. The entire set of maternal TK factors (e.g., absorption, distribution, serum binding, and elimination) also influence the concentration of active agent at the target site. Due to the physiological changes that occur during pregnancy, the influence of these maternal factors on chemical delivery may also change during gestation (Noschel et al, 1980; Cummings 1983; Bogaert and Thiery 1983; Juchau and Faustman-Watts 1983; Juchau 1995). These issues have been reviewed (Juchau and Faustman-Watts 1983; Levy 1981; Brock-Utne et al, 1980; Krauer et al, 1980; Slikker Jr and Miller 1994).

For chemicals which bind avidly to plasma proteins, this factor can retard placental transfer. In general, only the free drug crosses the membranes of the placenta (Krauer et al, 1980; Welsch 1982). Protein binding is usually reversible and there is a finite number of binding sites; thus, binding is saturable and equilibrium may be described by the law of mass action (Krauer et al, 1980; Miller et al, 1976). As long as binding is reversible, it does not prevent the chemical from crossing membranes but only slows the rate at which the transfer occurs (Levine 1973).

Table A3-2. Partial list of human placental xenobiotic and hormone metabolizing enzymes or isoenzymes

Phase	Type	Reaction (gene)	Substrate	Constitutive	Inducer	Inhibitor
I	MFO	O-de-ethylase (CYP1A1)	7-Ethoxycoumarin	(+)	Cigarette smoke	Aminoglutethimide
I	MFO	Aryl-hydrocarbon hydroxylase (CYP1A1)	PAH	(?)	Cigarette smoke	Alpha-naphthoflavone
I	MFO	Hydroxylase (CYP3A7)	Cortisol	+	_	_
I	MFO	Aromatase (CYP19)	Androgens	(+)	_	Aminoglutethimide
I	MFO	Cholesterol side chain cleavage (CYP11A)	Cholesterol	(+)	_	Aminoglutethimide
I	MFO	Estrogen catechol formation, 2-hydroxylation (CYP 1A1) 4-hydroxylation (CYP 1B1)	Estrogens	(+)	Cigarette smoke _	_
I	MFO	25-hydroxycholecalciferol hydroxylase	25-hydroxy-cholecalciferol		_	_

Table A3-2. Partial list of human placental xenobiotic and hormone metabolizing enzymes or isoenzymes (Cont'd)

Phase	Type	Reaction (gene)	Substrate	Constitutive	Inducer	Inhibitor
I	Oxido-reductase	17β-Hydroxydehydrogenase Type 1 Type 2	Estrone to Estradiol Estradiol to Estrone	(+)	_	16-Methylene estradiol
I	Oxido-reductase	11β-Hydroxydehydrogenase	Cortisol/cortisone	(+)	_	_
I	Oxidation	Dehydrogenase	Alcohol/acetaldehyde	(+)	_	_
I	Oxidation	Monoamine	Norepinephrine	(+)	_	MAO inhibitors
II	Sulfatase	Sulfate cleavage	Steroid sulfates	(+)	_	_
II	Conjugation	Glutathione-S-transferase	Epoxides	(+)	_	_
II	Conjugation	Catechol-O-methyl-transferase	Catecholamines, catechol estrogens	(+)	_	_

•

Modified from Slikker and Miller (Slikker Jr and Miller 1994; Juchau 1995; Arcuri et al, 1999; Rasheed et al, 1997; Zusterzeel et al, 1999; Mohrabi et al, 1997)

3.4.3 Placental Biotransformation

This factor may be the most critical in influencing the delivery of chemicals to the developing conceptus. Placental biotransformation of a chemical prior to fetal delivery may dramatically alter the chemical profile in the conceptus from that in the mother (Slikker Jr et al, 1982b). Equilibrium factors, which influence the rate of placental transfer, can result in quantitative differences of exposure; placental metabolism, however, can qualitatively alter the exposure of the conceptus to potentially toxic chemicals. Placental metabolism is less well characterized than hepatic metabolism, but existing data suggest that the placenta has considerably less metabolic capacity than adult liver (Mirkin and Singh 1976; Juchau 1980b). A listing of some of the human placental xenobiotic and hormone metabolizing enzymes or isoenzymes is presented in Table A3-2.

3.4.4 Embryo/Fetal Considerations

As with most organ systems, the various chemical-metabolizing systems undergo quantitative if not qualitative changes during development. Chemical biotransformation by the developing conceptus has been extensively reviewed (Slikker Jr 1987; Juchau and Faustman-Watts 1983; Slikker Jr and Miller 1994; Leakey 1983; Dutton and Leakey 1981; Eltom et al, 1993; Rane and Tomson 1980; Neims et al, 1976; Pelkonen 1977; Dutton 1978; Nau and Neubert 1978; Pelkonen 1980; Dvorchik 1981; Slikker Jr 1994). Despite the fact that data have been collected using a variety of techniques and some "data gaps" exist because of technical or ethical reasons, several general conclusions may be drawn from the literature: (1) during prenatal development, the activities of most enzymes which catalyze Phase I and II reactions are lower than in adults; (2) as in the adult, the conceptus exhibits substrate specificity in its ability to metabolize chemicals, suggesting the existence of several sets of enzymes or isozymes which may or may not be the same as in the adult; (3) these enzyme systems may be inhibited or induced by maternal pretreatment with a variety of chemicals; (4) enzyme activity generally increases with gestational age; (5) the ontogeny of each enzyme may be different and the controlling mechanisms of maturation of enzyme activity are incompletely understood; (6) prenatal human and nonhuman primates exhibit higher levels of many metabolizing enzymes (especially P-450s) than do other commonly used laboratory species; (7) as in the adult, the liver of the conceptus appears to have the greatest capacity for chemical metabolism. The fetal adrenal, kidney, lung and brain also exhibit metabolic capabilities. Table A3-3 summarizes recent data concerning some cytochrome P-450 (CYP) isozymes and their occurrence in human fetal tissues.

3.4.5 Fetal Distribution and Elimination

The majority of chemicals entering the fetal circulation do so via the umbilical vein after passage through the placenta. A portion of the blood flow entering the liver of the conceptus is shunted via the ductus venosus directly to the inferior vena cava and to the heart for total body distribution (Rudolph and Heymann 1967). The remaining umbilical flow enters hepatic tissue and exits to the vena cava via the portal vein (Dawes 1968). Therefore, there is the potential for a sigificant first pass effect from chemical passing through the fetal liver prior to other sites; however, a substantial fraction passes directly to the remaining tissues (Power and Longo 1975).

As in the adult, approximately 16% of the fetal cardiac output is directed toward the fetal brain (Behrman et al, 1970).

Just as placental transfer of chemicals is the predominate pathway from the maternal system to the conceptus, placental transfer is also the predominate route for embryo/fetal elimination of chemicals. The same toxicokinetic rules apply for fetal-to-maternal transport across the placenta: chemicals which are nonionized and lipid soluble will diffuse across the placenta according to the concentration gradient from conceptus to mother. If, however, a chemical has been conjugated by the fetus (e.g., glucuronidation, sulfation, etc.) or otherwise metabolized to a more polar form, the rate of return to the maternal circulation will be slower than that for the parent compound (Dancis et al, 1958; Levitz et al, 1960; Goebelsmann et al, 1968; Goebelsmann et al, 1972). Physiologically-based pharmacokinetic models to predict the fate of toxicants in fetal tissues as a function of development are emerging (Sandberg et al, 1996; Kim et al, 1996).

Table A3-3. Some Cytochrome P-450 (CYP) Isozymes and Their Occurrence in Human Tissues (Hakkola et al, 1994, 1997, 1998; Carpenter et al, 1997; Chen et al, 1999; Farin and Omiecinski 1993; Lacroix et al, 1997; Maenpaa et al, 1993; Murray et al, 1992; Ravindranath and Boyd 1995; Slikker Jr 1997; Yang et al, 1995)

CYP	Adult Liver	Adult Brain	Fetal Liver	Fetal Brain
1A1	+	+	+	
1A2 1B1	+++ +	-	- +	 +
2A6 2A7	++ +		- -	
2B1/2B2	+	+		
2B6/2B7	+		-	
2C 2C8-19	+++ +		± +	 +
2D6	+	+	±	
2E1	+	+	±	
2F1	-		-	
3A4 3A5	+++ ++	+	± +	
3A7	+		+++	
4B1	-		-	

± = possibly present in small quantities or mRNA observed but no protein confirmation;
- = not detected; + = present in low levels; ++ = present in moderate levels;
+++ = present in high levels

3.5 Use of animal TK information to evaluate how development can affect internal dosimetry

Laboratory animals can be useful in a number of respects in providing toxicokinetic data and related information for children's risk assessments of environmental pollutants. The discussion here will be limited primarily to mice and rats, as most toxicokinetic and toxicology studies employ these two rodent species. Some of the major advantages of using immature animals as models, or surrogates for infants and children will be described, as will some of the inherent disadvantages. In several instances relevant examples will be briefly described.

Diversity is one of the more daunting problems anticipated when studying children due to the numerous variability factors described earlier. Intersubject variability is substantially lower in homogenous rodent populations provided by major animal suppliers. Uniform groups of animals of the same established genetic and husbandry backgrounds can be maintained under defined and carefully-controlled conditions. Thereby it is possible with animal studies to control more variables and to better focus on age-dependent differences in chemical metabolism and disposition.

Animals can be utilized to examine the maturation of specific physiological and biochemical systems, and their influence on toxicokinetics. Human data may be sparse or difficult to obtain, but findings in animals can pinpoint specific processes and/or critical developmental periods which significantly impact disposition of particular toxicants. Attention can then be focused on assessing their toxicological relevance in humans (Harroff 1997).

3.5.1 Animal TK and susceptibility to xenobiotics

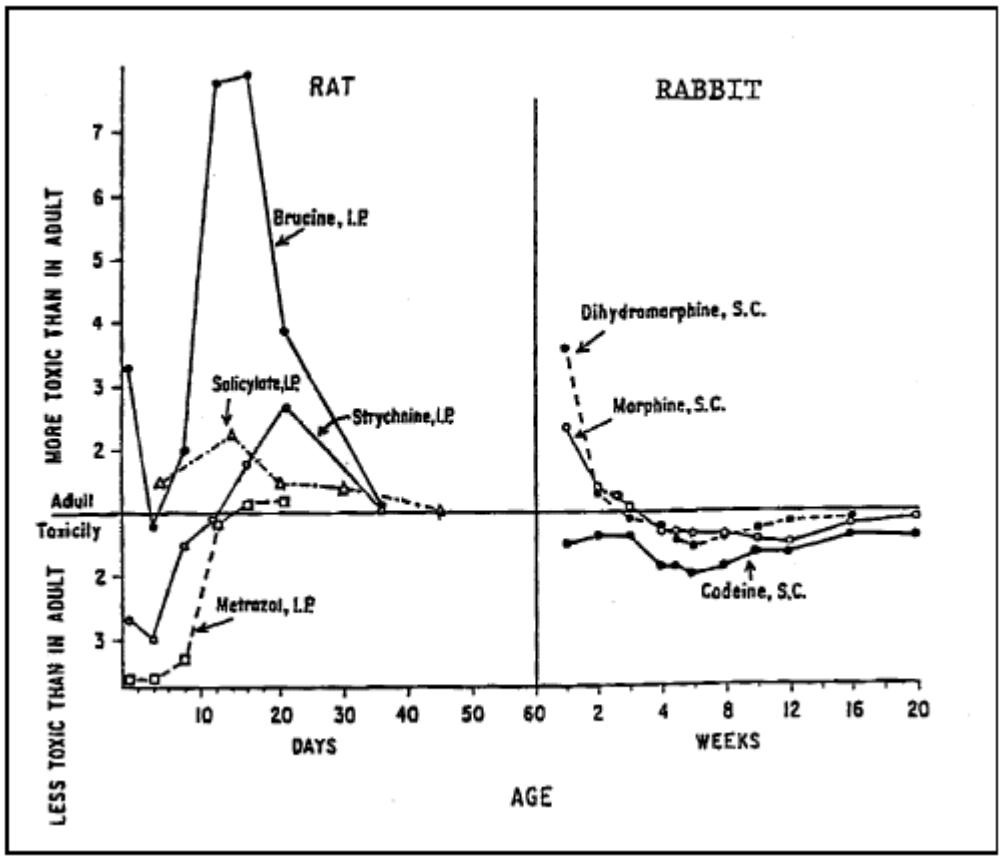
Series of studies of organophosphates (OPs) in rats have clearly shown that the relatively low detoxification capacity of weanlings places them at increased risk of acute, high-dose OP poisoning. Benke and Murphy (1975) concluded that the lower acute toxicity of parathion and methyl parathion in adult rats was due to the adults' greater detoxification capacity. More recent studies have confirmed and extended these findings. The maturational profiles of P450-catalyzed desulfuration (activation) and dearylation (inactivation), as well as carboxylesterase and arylesterase (inactivation) activities have been characterized in the liver and/or blood of rats (Atterberry et al, 1997; Moser et al, 1998). Despite very low carboxylesterase and arylesterase activities, rat fetuses and sucklings appear to be protected from chlorpyrifos, due to limited transfer of the compound from the mother (Mattsson et al, 2000). However, this protective effect would not be expected if neonates were exposed directly (i.e., not via the mother) to environment OPs. Based upon somewhat anecdotal information of groups of people poisoned by these pesticides, the suggestion is that young children (0 - 4 years) are at increased risk (Diggory et al, 1977; Zwiener and Ginsburg 1988). However, the exposure information is not detailed enough in these cases to confirm the sensitivity of any particular person or age group. Ecobichon and Stephens (1973), reported that blood arylesterase activity is relatively low in young children (1-2 yrs old), with this group having pronounced intersubject variability… No more recent information could be located on the time-course of maturation of arylesterase or related enzymes in human blood or liver.

Comparative studies show that neonatal rodents are frequently more susceptible to toxicants than adult animals, but such findings should be interpreted with caution when extrapolating to humans. Done (1964) and Goldenthal (1971) compiled the results of LD_{50} studies of several hundred chemicals in neonatal and mature rodents. The neonatal animals were more sensitive to many, but not all the compounds. Almost all the age-dependent differences in LD_{50}s were less than an order of magnitude, indeed most varied no more than 2- to 3-fold. More pronounced interage differences were seen for a few drugs, some (e.g., chloramphenicol, diazepam) of which are known to be poorly metabolized and to accumulate to toxic levels in human newborns. It is logical that the greatest toxicokinetic and toxicity differences between infants/children and adults should be manifest in the most immature individuals (i.e., premature newborns). As full-term human newborns are more mature than their rodent counterparts with respect to liver metabolism, interage differences might be less pronounced in humans. However, maturation is much more rapid in rodents, such that even a few days of growth can result in marked disparity in chemical metabolism, disposition and effects. Results from the Done (1964) acute toxicity compilation (Figure A3-6) suggest that there are dramatic differences in sensitivity in early postnatal life. These types of examples from the animal literature may be excellent research opportunities for exploring the relative importance of TK vs. toxicodynamic (TD) mechanisms in determining susceptibility in early life. While the animal research may be able to point out mechanisms of TK susceptibility, extrapolation of temporal trends across species is made difficult because organs and their associated functions mature at different rates in different species. Therefore, when the variable of interspecies maturation patterns is introduced, the choice of an appropriate animal model for infants or children becomes complex. This makes cross-species extrapolations more uncertain than what we are accustomed to in adult risk assessments (NAS 1993; Bruckner 2000).

The use of immature animals is obviously necessary where serial blood or tissue sampling is required, and when potentially toxic or carcinogenic chemicals are to be studied. Ethical questions are raised if it is proposed to give even trace doses of environmental contaminants to children. Despite the dissimilarities in maturation mentioned above, rodent studies can provide valuable information on mechanisms and on specific immaturities that may be broadly applicable to infants and children. Heavy metals are a good case in point. It is widely recognized that dietary antigens, maternal antibodies and other macromolecules may be absorbed through the immature gastrointestinal (GI) mucosa. Increased levels of relatively polar molecules in the cerebrospinal fluid of infants suggest that such compounds penetrate the blood-brain, or blood-cerebral spinal fluid barrier more readily in infants than in children and adults (Adinolfi 1985; Dziegielewska et al, 2000). Therefore, it has been hypothesized that equivalent exposures of infants and adults to heavy metals such as mercury and lead will result in greater oral absorption and in greater CNS deposition (and toxicity) in the infants. Such a theory can be tested directly in animal models, and related to more limited observations in humans. Kostial et al. (1978) demonstrated substantially greater GI absorption and whole-body retention of lead, mercury and cadmium by suckling rats than by adults. A similar phenomenon has been reported for lead in juvenile monkeys (Pounds et al, 1978) and human infants (Ziegler et al, 1978). Retention of i.v-injected lead was found to be 8 times higher in the brain of suckling rats than in the brain of adults (Kostial et al, 1978). Brain retention of injected mercury was 19 times higher during the perinatal period. Rodents have been very useful in delineation of the toxicokinetic basis of age-dependent differences in heavy metal toxicity, but sub-human primates are generally the animals of choice to examine toxicodynamic factors (Davis et al, 1990).

Toxicokinetic and metabolic data are lacking in both laboratory animals and humans for many chemicals (Renwick 1998). This is particularly true for immature populations. This data gap is partially compensated for by the extensive *in vivo* database for therapeutic agents in children and *in vitro* human liver bank studies as described above. Relatively few *in vivo* data are available from kinetics studies of drugs or other chemicals in pre-adolescent animals, due to technical difficulties in working with such small subjects, as well as prior lack of interest in immature populations. Similarly, there is a paucity of information on the maturation of many physiological processes in small animals. The maturation of hepatic xenobiotic metabolism in rats, in contrast, has been relatively well characterized (Imaoka et al, 1991; Watanabe et al, 1993; Renwick 1998). While rat liver is immature at birth with respect to many metabolic functions, certain CYP functions, epoxide hydrolase, and glucuronidation function reach adult levels with the first week to 10 days. Other functions, such as glutathione transferase and aryl hydrocarbon hydroxylase, take longer to develop (Renwick 1998).

Figure A3-6. Changes in Chemical Susceptibility with Age (Adapted from Done AK (1964))



In spite of these known metabolic differences during development, intrigueing findings of increased susceptibility in juvenile rodents have not been followed up to determine whether TK mechanisms underly these susceptibility differences. For example, Yoo et al. (1987) observed that P450 2E1-catalyzed metabolic activation of N-nitrosodimethylamine to a mutagen was substantially greater by hepatic microsomes of weanlings (25 days old) than by adult rats.

Carbon tetrachloride, a chemical that also undergoes P450 2E1-catalyzed metabolic activation, was found to be more hepatotoxic in 15- than in 60-day-old male rats (Jahn et al, 1993). However, no accounts were located of animal studies relating susceptibility to injury by these 2E1 substrates or other chemicals to the time-course of maturational changes in the chemical's metabolism.

3.5.2 Data gaps and challenges in applying animal data to children's TK assessments

It is possible, under certain well-defined conditions, to make reasonable predictions of the disposition of drugs in ***adult*** humans on the basis of animal studies (Lin 1995). This author points out that oral absorption of quite lipophilic compounds is relatively species-independent, but that dissimilarities in diet, gastic and intestinal transit, GI blood flow and pH can result in differences in absorption of ionizable agents. The extent of plasma protein binding of drugs differs considerably among mammals, though the volume of distribution of the unbound fraction is often less variable (Fichtl et al, 1991). Extrapolation of results from xenobiotic metabolism studies in rodents to humans if often difficult. There are considerable differences between rats and humans in the expression and catalytic activities of a number of cytochrome P450 isozymes (Nedelcheva and Gut 1994). Stevens et al. (1993) observed higher hepatic P450 levels and greater *in vitro* metabolism of 7 out of 8 phase I and II substrates by rhesus monkeys than by humans. Nevertheless, metabolic clearance of low doses of flow-limited drugs should be less dependent on species-inherent rates of metabolism than on relative liver blood flow rates (Lin 1995). Similarly, reasonable estimates of renal excretion of filtered drugs in people can be made by use of glomerular filtration rate ratios between humans and animals. Thus, data from animal studies can often be quite useful in forecasting toxicokinetics in humans when its limitations are taken into account. However, this conclusion is based upon comparison of adult animals to human adults. The development of toxicokinetic functions in early life is sufficiently different in rodents as compared to humans (see above) to make direct extrapolation from juvenile animal studies difficult.

One of the most promising approaches to quantitative estimation of internal doses of chemicals in children is physiologically-based pharmacokinetic (PBPK) modeling. PBPK modeling has been successfully applied to risk assessments of a number of environmental contaminants in adults, but its use has been extremely limited in protection of infants and children. Models have been developed to describe the disposition of a number of chemicals in pregnant rodents and their fetuses, as well as the lactating rat and nursing pup (Krishnan and Andersen 1998). Luecke et al. (1994) and Welsch et al. (1995) have adapted such models to human pregnancy to forecast potential teratogenic events. O'Flaherty developed PBPK models that accurately predicted time-courses of lead in the blood and its deposition in bones of developing rats (1991) and children (1995). These models incorporated age-dependent changes in body weight, tissue volumes and blood flows, and bone formation and resorption rates. Pelekis et al. (2001) have made assumptions about distributions of physiological and biochemical parameters in children, and estimated their effects on the disposition of VOCs. No other accounts of PBPK models for post-lactational exposures of immature humans or animals to drugs or other chemicals were found in the peer-reviewed literature.

Knowledge gained from PBPK modeling efforts with animals can be of significant benefit in understanding the kinetic behavior of chemicals in humans and in developing PBPK

models for humans. Toxicokinetic studies in animals can yield a number of important parameters, including *in vivo* and *in vitro* partition coefficients, metabolic rate constants, and identity and stoichimetric yield of metabolites. Such chemical- and species-specific data are needed to construct an appropriate model. The model may be used to generate simulations of the time-course of parent compound and/or metabolite(s) in blood, tissues and urine. This information is often useful in design of the *in vivo* kinetics experiments needed to assess the accuracy of the model's predictions. Sensitivity analyses can be carried out with the animal model, in order to determine which parameters are major determinants of the toxicokinetics of a chemical. These parameters would receive special attention in human kinetics studies and modeling efforts. The parameters could be scaled allometrically, or adjusted by an optimization routine to fit human plasma time-course data or urinary or pulmonary elimination data.

Development of PBPK models for children faces at least two major hurdles: determination of accurate physiological and biochemical input parameters for different age groups; and validation of models' predictions of internal dosimetry. Anderson et al. (1997), for example, point out that there are no human data on age-dependent changes in cardiac output. There is reasonably good information for some physiological indices, but data obtained with state-of-the-art methods from healthy subjects are limited. Non-invasive techniques (e.g., Doppler ultrasound, MRI, 3-D CT scans) are now available for measuring many key indices (e.g., cardiac output, tissue blood flows, organ volumes), though the procedures are expensive and generally of limited benefit to healthy children. Characterization of physiological and biochemical maturation in rodents seems to be of limited utility for extrapolation to humans, due to laboratory animals' disparate, species-dependent maturation patterns. Such an effort in sub-human primates would appear more worthwhile, due to their availability for research and their similarity to growing children (Conrad et al, 1995). Research projects with monkeys are expensive, but they offer scientists the opportunity to conduct kinetic studies of toxicants and to use the data to develop PBPK models and to validate their predictions.

3.6 Use of basic physiologic differences across age groups to ascertain how factors such as renal clearance, protein binding, and lipid partitioning may vary with age.

Renal clearance and protein binding capacity are immature at early post-natal stages and develop over the course of the first 6 months to year of life (Table A3-1). Glomerular filtration rate as well as transporter (secretory) systems in the proximal convoluted tubule are deficient at birth (Morselli 1989; Kearns and Reed 1989), leading to relatively slow clearance of a wide array of antibiotics and other renally cleared drugs and metabolites. Compounding the chances for greater chemical effect in the first months of life is a deficient protein binding status due to lower levels of albumin (binds weakly acidic chemicals) and alph-1-glycoprotein (binds basic chemicals). Another issue is the relative deficiency to conjugate and excrete bilirubin early in life, which leads to greater occupation of serum binding sites by bilirubin. These factors may contribute to a greater ratio of free as compared to bound chemical, leading to a higher potential for uptake into tissues and toxic action. Diminished protein binding can also lead to shorter duration of action due to greater availability of chemical for metabolism and elimination. These factors would likely only be important for chemicals with that exhibit a high degree of plasma protein binding (e.g., trichloroacetic acid).

3.7 Summary of Phase II TK Analysis

As the available information in the areas described above is gathered and processed, judgements can be made regarding which age group(s) appear to be most exposed from an internal dose perspective. Age groups which appear to differ most from adults can be prioritzed for more detailed analysis as the process proceeds. To assist in this prioritization, the following questions may be helpful:

3.7.1 Are there natural age break points where the PK profile shifts?

An early step in analyzing children's TK data is deciding whether and how to create bins across ages. The rapid development of children requires that the population be broken into age groups that are relatively small, particularly in the early postnatal period. If larger bins are used, the data may become highly variable with age specificity becoming a casuality of the desire to simplify the assessment. Thus, bins must be constrained to reflect key developmental stages. Nevertheless, it is useful to make them as large as possible since this maximizes the "n" (number of subjects per group) and enhances the power of cross-age comparisons. It should be noted that similar age "binning" may occur in the exposure and TD areas to reflect critical changes in behavior or windows of heightened vulnerability. At some point the children's kineticist may be asked to adjust his bins to match those created in these other areas for the sake of harmony and to allow risk calculations to be done for neatly defined age groups. Age bins were developed to organize the data shown in Table A3-1 and Figures A3-2 and A3-3. These bins are somewhat arbitrary, based upon the overall availabilty of data for a variety of pathways and chemicals. For specific chemicals and pathways, alternative bins can certainly be considered.

3.7.2 Which PK parameters are most important in determining internal dose?

Although PBPK models aim for the simplest approach that yields predictive results, there are inevitably many parameters that need to be estimated to run the model. These parameters include compartment size, partition coefficients, cardiac output, organ blood flows, metabolic rates, lung ventilation rates, and urinary elimination rates. Given that these models contain multiple tissue compartments and that we may be dealing with several different age groups, there are a potentially large number of parameter values that would require elucidation. However, the model may not be equally sensitive to all the inputs, making those parameters that have the greatest influence on model performance the most important to calibrate properly. Efforts to simplify PK modeling in both adults and children have relied upon a series of algebraic equations that highlight key PK parameters while not including others which may be model-insensitive (Pelekis et al, 2001). Such approaches point out the importance of identifying those PK parameters which drive internal dose. Which PK factors are most influential will depend upon the properties of the chemical under investigation. The clearance of chemicals with high hepatic extraction (rapid liver metabolism) will depend primarily upon hepatic blood flow, while chemicals with low hepatic extraction depend most on the rate of liver metabolism relative to other competing rates (urinary elimination or exhalation of parent compound). To determine how to focus a children's TK analysis, it is important to first analyze which TK parameters are most important in a validated adult model. Then, extra effort can be put into identifying how these parameters change as different child age groups are considered. Ultimately, the fate of any

chemical will be determined by the interaction of all PK factors acting simulataneously. This kind of sensitivity analysis will ensure that those parameters with the greatest influence are given the attention they warrant.

3.7.3 How do these parameter values change during development?

Regardless of whether one is conducting a full PBPK assessment across ages or a less rigorous approach, it is important to know how key parameters change with age. As discussed above, various data resources and analytic approaches may be used to determine how key parameter values differ between children and adults. Similar to Table A3-1, it may be helpful to develop a table of key physiologic and metabolic parameters that affect the fate of the chemical in question, and then list what is known about those pathways for the *in utero* and post-natal periods. If the parameter values are not materially different between children and adults, or if it appears that certain factors negate one another, then one might conclude that it is not important to conduct any further TK assessment for that age groups(s). However, for those chemicals and age groups where children appear to receive a greater internal dose of active toxicant, further analytic steps may be considered as follows:

3.7.3.1 Semi-quantitative approaches

Utilize information on the predominant disposition pathways to conclude how much different children are vs. adults. It may be possible to base conclusions on one or more particularly useful surrogates from the drug literature.

3.7.3.2 Quantitative approaches - PBPK analysis

This involves constructing a child age group-specific model and then incorporating chemical-specific parameter values to simulate how children will handle the chemical. It is essential to carefully consider data needs for such an analysis and whether sufficient data exist or can be generated/estimated.

Thus, Phase II of the TK children's risk assessment will hopefully result in the following:

- An understanding of the TK mechanisms that govern the fate of the target chemical(s).
- Data resources that describe how these mechanisms change during development.
- An initial prioritization of age groups for more detailed analysis based upon likelihood of internal dosimetry differences relative to adults.
- An analytic strategy (semi-quantitative or quantitative techniques) for incorporating these data into the risk assessment process.

4.0 Phase III. Risk Characterization

The analytic approaches developed in Phase II would be followed through so that Phase III can attempt to answer the following questions:

- How similar/dissimilar are *in utero* and childhood stages to adults in terms of internal dose received per unit of administered dose?

- How variable are young age groups versus adults?

- Do all age groups fall within the default interindividual variability factor of 3.2?

- Do child:adult differences warrant the development of chemical- and age-specific uncertainty factors;

- Can internal dosimetry estimates for different age groups be developed and used in quantitative risk assessmnt?

In describing the analytical results, the risk characterization will usually try to describe the degree of uncertainty and variability in the analysis. The following questions may be particularly pertinent to such a discussion.

Are children more variable in their PK responses than adults? How do we assess and apply this information? The role of variability in the risk assessment process can range from purely qualitatitive (e.g., people are different from one another so we are not as certain as we'd like to be about the degree of risk), to semi-quantitative (e.g., people vary considerably in metabolizing chemical X so it seems prudent to use a half-log TK uncertainty factor), to fully quantitative (e.g., we have enough individual data to plot the population distribution of effective internal doses per unit of external dose, so let's show the risk manager the cleanup options that protect the 50th, 75th, 90th and 95th percentiles). The fully quantitative mode of variability analysis is used in lead uptake/biokinetic modeling to enable risk assessors to calculate the amount of lead reduction in water, soil, air, or diet needed to bring 95% of the population of exposed children below the blood lead target of 10ug/dl (U.S. EPA 1994). It is logical that children are more toxicokinetically variable than adults due to their variable growth and maturation rates, as well as the genetic, nutritional, disease, body composition, and prescription (and other) drug factors that create TK variability in adults. Children also present the problem of variable growth rates, which can make even small age bins relatively heterogeneous. Greater variability can affect risk conclusions especially if one is concerned about protecting the tails (e.g., 90th percentile) of the distribution. With greater variability, it is also more likely that a substantial fraction of a certain age group will lie outside the half-log TK variability range we normally allocate to the adult defaults. Thus, it is critical that a children's TK assessment characterize in some way the degree of variability present in each age group's dataset, and determine whether that variability is greater than in the adult case.

By addressing these questions, the risk characterization will endeavor to determine whether TK differences are likely between children and adults and whether these differences will be important to the overall risk assessment conclusions for this chemical/scenario. If so, then the risk characterization can describe the advantages and disadvantages of applying qualitative (e.g., professional judgement), semi-quantitative (e.g., modified uncertainty factors) or quantitative approaches (PBPK modeling with distributional analyses). The major caveats and data gaps should be elaborated so that the limitations of the assessment are clear and critical research needs

identified. Finally, the characterization should describe which TK factors appear most influential in creating child/adult differences so that the key risk drivers for children are explained.

References to APPENDIX 3

Adams J, Barone S Jr, LaMantia A, Philen R, Rice DC, Spear L et al. 2000. Workshop to identify critical windows of exposure for children's health: neurobehavioral work group summary. Environ Health Perspect 108(suppl 3):S535-S544.

Adinolfi M. 1985. The development of the human blood-CSF-brain barrier. Dev Med Child Neurol 27:532-537.

Andersen ME, Clewell HJ 3rd, Gargas ML, Smith FA, Reitz RH. 1987. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol 87:185-205.

Andersen ME, Clewell H 3rd, Krishnan K. 1995. Tissue dosimetry, pharmacokinetic modeling, and interspecies scaling factors. Risk Anal 15: 533-537.

Anderson BJ, McKee AD, Holford NH. 1997. Size, myths, and the clinical pharmacokinetics of analgesia in pediatric patients. Clin Pharmacokinet 33:313-327.

Arcuri F, Sestini S, Cintorino M. 1999. Expression of 11ß-hydroxysteroid dehydrogenase in early pregnancy: implications in human trophoblast-endometrial interactions. Semin Reprod Endocrinol 17:53-61.

Atterberry TT, Burnett WT, Chambers JE. 1997. Age-related differences in parathion and chlorpyrifos toxicity in male rats: target and nontarget esterase sensitivity and cytochrome P450-mediated metabolism. Toxicol Appl Pharmacol 147:411-418.

Augustinsson KB, Barr M. 1963. Age variation in plasma arylesterase activity in children. Clin Chim Acta 8:568-573.

Barr M, Brent RL. 1978. Uterine vascular interruption and combined radiation and surgical procedures. In: Handbook of Teratology (Wilson JG, Fraser C, eds). New York:Plenum, 275-304.

Behrman RE, Lees MH, Peterson EN, De Lannoy CW, Seeds AE. 1970. Distribution of the circulation in the normal and asphyxiated fetal primate. Am J Obstet Gynecol. 108:956-969.

Benke GM, Murphy SD. 1975. The influence of age on the toxicity and metabolism of methyl parathion and parathion in male and female rats. Toxicol Appl Pharmacol 31:254-269.

Bertz RJ, Granneman GR. 1997. Use of *in vitro* and *in vivo* data to estimate the likelihood of metabolic pharmacokinetic interactions. Clin Pharmacokinet 32:210-258.

Besunder JB, Reed MD, Blumer JL. 1988. Principles of drug biodisposition in the neonate. A critical evaluation of the pharmacokinetic-pharmacodynamic interface (Part I). Clin Pharmacokinet 14:189-216.

Bogaert MC, Thiery M. 1983. Pharmacokinetics and pregnancy. Eur J Obstet Gynecol Reprod Biol 16:229-235.

Bois FY, Zeise L, Tozer TN. 1990. Precision and sensitivity of pharmacokinetic models for cancer risk assessment: tetrachloroethylene in mice, rats, and humans. Toxicol Appl Pharmacol 102: 300-315.

Brock-Utne JG, Downing JW, Mankowitz E. 1980. Drugs and the fetomaternal unit. S Afr Med J 58:366-369.

Bruckner JV, Weil WB. 1999. Biological factors which may influence an older child's or adolescent's responses to toxic chemicals. Regul Toxicol Pharmacol 29:158-164.

Bruckner JV. 2000. Differences in sensitivity of children and adults to chemical toxicity: the NAS panel report. Regul Toxicol Pharmacol 31:280-285.

Carpenter SP, Savage DD, Schultz ED, Raucy JL. 1997. Ethanol-mediated transplacental induction of CYP2E1 in fetal rat liver. J Pharmacol Exp Ther 282:1028-1036.

Cazeneuves C, Pons G, Rey E, Treluyer JM, Cresteil T, Thiroux G, et al. 1994. Biotransformation of caffeine in human microsomes from foetuses, neonates, infants and adults. Br J Clin Pharmacol 37:405-412.

Chen H, Brzezinski MR, Fantel AG, Juchau MR. 1999. Catalysis of drug oxidation during embryogenesis in human hepatic tissues using imipramine as a model substrate. Drug Metab Dispos 27:1306-1308.

Conrad S, Ha J, Lohr C, Sackett G. 1995. Ultrasound measurement of fetal growth in Macaca nemestrina. Am J Primatol 36:15-35.

Cummings AJ. 1983. A survey of pharmacokinetic data from pregnant women. Clin Pharmacokinet 8:344-354.

Dancis J, Money WL, Condon GP, Motimer L. 1958. The relative transfer of estrogens and their glucuronides across the placenta in the guinea pig. J Clin Invest 37:1373-1378.

Davis JM, Otto DA, Weil DE, Grant LD. 1990. The comparative developmental neurotoxicity of lead in humans and animals. Neurotoxicol Teratol 12:215-229.

Dawes GS. 1968. Umbilical blood flow and vascular resistance: changes with age. In: Foetal and Neonatal Physiology (Dawes GS, ed). Chicago:Year Book Med Publ, 69.

Dempsey D, Jacob P 3rd, Benowitz NL. 2000. Nicotine metabolism and elimination kinetics in newborns. Clin Pharmacol Ther 67:458-465.

Diggory HJ, Landrigan PJ, Latimer KP, Ellington AC, Kimbrough RD, Liddle JA, et al. 1977. Fatal parathion poisoning caused by contamination of flour for international commerce. Am J Epidemiol 106:145-153.

Dollery C. 2000. Therapeutic Drugs. 2nd ed. Edinburg:Churchill Livingstone.

Done AK. 1964. Developmental pharmacology. Clin Pharmacol Ther 5:432-479.

Dorne JL, Walton K, Renwick AG. 2001. Uncertainty factors for chemical risk assessment: human variability in the pharmacokinetics of CYP1A2 probe substrates. Food Chem Toxicol 39:681-696.

Dutton GJ. 1978. Developmental aspects of drug conjugation, with special reference to glucuronidation. Annu Rev Pharmacol Toxicol 18:17-35.

Dutton GJ, Leakey JE. 1981. The perinatal development of drug-metabolizing enzymes: what factors trigger their onset? Prog Drug Res 25:189-273.

Dvorchik BH. 1981. Nonhuman primates as animal models for the study of fetal hepatic drug metabolism. In: Drug Metabolism in the Immature Human. (Soyka LF, Redmond GP, eds). New York:Raven Press, 146-162.

Dziegielewska KM, Knott GW, Saunders NR. 2000. The nature and composition of the internal environment of the developing brain. Cell Mol Neurobiol 20:41-56.

Ecobichon DJ, Stephens DS. 1973. Perinatal development of human blood esterases. Clin Pharmacol Ther 14: 41-47.

Eichelbaum, M., Ekbom, K., Bertilsson, L., Ringberger, V.A., Rane, A. (1975) Plasma kinetics of carbamazepine and its epoxide metabolite in man after single and multiple doses. Eur. J. Clin. Pharmacol. 8, 337-341.

Eichelbaum M, Tomson T, Tybring G, Bertilsson L. 1985. Carbamazepine metabolism in man induction and pharmacogenetic aspects. Clin Pharmacokinet 10:80-90.

Eltom SE, Babish JG, Schwark WS. 1993. The postnatal development of drug-metabolizing enzymes in hepatic, pulmonary and renal tissues of the goat. J Vet Pharmacol Ther 16:152-163.

Farin FM, Omiecinski CJ. 1993. Regiospecific expression of cytochrome P-450s and microsomal epoxide hydrolase in human brain tissue. J Toxicol Environ Health 40:317-335.

Fichtl B, Nieciecki AV, Walter K. 1991. Tissue binding versus plasma binding of drugs: General principles and pharmacokinetic consequences. In: Advances in Drug Research. (Testa B, ed.), Vol. 20. London:Academic Press, 117-166.

Garbis-Berkvens JM, Peters PW. 1987. Comparative morphology and physiology of embryonic and fetal membranes. In: Pharmacokinetics in Teratogenesis (Nau H, Scott WJ, eds), Vol. 1. 14-44.

Gibbs JP, Murray G, Risler L, Chien JY, Dev R, Slattery JT. 1997. Age-dependent tetrahydrothiophenium ion formation in young children and adults receiving high dose busulfan. Cancer Res 57:5509-5516.

Ginsberg G, Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, et al. 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicol Sci 66:185-200.

Goebelsmann U, Wiqvist N, Diczfalusy E. 1968. Placental transfer of estriol glucosiduronates. Acta Endocrinol (Copenh) 59:426-432.

Goebelsmann U, Roberts JM, Jaffe RB. 1972. Placental transfer of ^{3}H-oestriol-3-sulphate-16-glucosiduronate and ^{3}H-oestriol-16-glucosiduronate-^{14}C. Acta Endocrinol (Copenh) 70:132-142.

Goldenthal EI. 1971. A compilation of LD_{50} values in newborn and adult animals. Toxicol Appl Pharmacol 18:185-207.

Greiss FC Jr, Gobble FL Jr. 1967. Effect of sympathetic nerve stimulation on the uterine vascular bed. Am J Obstet Gynecol 97:962-967.

Hakkola J. Pasanen M, Purkunen R, Saarikoski S, Pelkonen O, Maenpaa J, et al. 1994. Expression of xenobiotic-matabolizing cytochrome P450 forms in human adult and fetal liver. Biochem Pharmacol 48:59-64.

Hakkola J, Pasanen M, Pelkonen O, Hukkanen J, Evisalmi S, Anttila S, et al. 1997. Expression of CYP1B1 in human adult and fetal tissues and differential inducibility of CYP1B1 and CYP1A1 by Ah receptor ligands in human placenta and cultured cells. Carcinogenesis 18:391-397.

Hakkola J, Pelkonen O, Pasanen M, Raunio H. 1998. Xenobiotic-metabolizing cytochrome P450 enzymes in the human feto-placental unit: role in intrauterine toxicity. Crit Rev Toxicol 28:35-72.

Hakkola J, Tanaka E, Pelkonen O. 1998. Developmental expression of cytochrome P-450 enzymes in human liver. Pharmacol Toxicol 82:209-217.

Haroff HH Jr. 1997. Use of animal models in pediatric research. Adv Pediatr 44:453-489.

Hattis D, White P, Koch P. 1993. Uncertainties in pharmacokinetic modeling for perchloroethylene: II. Comparison of model predictions with data for a variety of different parameters. Risk Anal 13:599-610.

Hattis D, Russ A, Banati P, Kozlak M, Goble R, Ginsberg GL. 2000. Development of a Comparative Child/Adult Pharmacokinetic Database based upon the Therapeutic Drug Literature. Draft Report of EPA/State of Connecticut Assistance Agreement #827195-0, October, 2000.

Heistad DD. 1984. The Blood-Brain Barrier. Federation Proceeding, 43:185.

Hytten FE, Leitch I. 1971. The product of conception. In: The Physiology of Human Pregnancy. Oxford:Blackwell Scientific Publications, 322.

Idanpaan-Heikkila J, Jouppila, P, Akerblom HK, Isoaho R, Kauppila E, Koivisto M. 1972. Elimination and metabolic effects of ethanol in mother, fetus, and newborn infant. Am J Obstet Gynecol 112:387-393.

Illsley NP, Hall S, Penfold P, Stacey TE. 1985. Diffusional permeability of the human placenta. Contrib Gynecol Obstet 13:92-7.

Imaoka S, Fujita S, Funae Y. 1991. Age-dependent expression of cytochrome P-450s in rat liver. Biochim Biophys Acta 1097:187-192.

Jahn F, Reuter A, Karge E, Danz M, Klinger W. 1993. Age dependent different influence of carbon tetrachloride on biotransformation of xenobiotics, glutathione content, lipid peroxidation and histopathology of rat liver. Exp Toxicol Pathol 45:101-107.

Juchau MR. 1980a. Extrahepatic drug biotransformation in the placenta. In: Extrahepatic Metabolism of Drugs and other Foreign Compounds (Gram TE, ed). New York:S.P. Medical and Scientific Books, New York, 211-238.

Juchau MR. 1980b. Drug biotransformation in the placenta. Pharmacol Ther 8:501-524.

Juchau MR, Faustman-Watts E. 1983. Pharmacokinetic considerations in the maternal-placental-fetal unit. Clin Obstet Gynecol 26:379-390.

Juchau MR. 1995. Placental enzymes: cytochrome P450s and their significance. In: Placental Toxicology (Rama Sastry BV, ed). Boca Raton:CRC Press, 197-212.

Jusko WJ. 1972. Pharmacokinetic principles in pediatric pharmacology. Pediatr Clin North Am 19:81-100.

Kaufmann P, King BF. 1982. Structural and functional organization of the placenta. S. Karger - Basel.

Kearns GL, Reed MD. 1989. Clinical pharmacokinetics in infants and children. A reappraisal. Clin Pharmacokinet 17(suppl 1):S29-S67.

Kim CS, Binienda Z, Sandberg JA. 1996. Construction of a physiologically based pharmacokinetic model for 2,4-dichlorophenoxyacetic acid (2,4-D) dosimetry in the developing rabbit brain. Toxicol Appl Pharmacol 136:250-259.

Kivalo I, Saarikoski S. 1972. Placental transmission and foetal uptake of ^{14}C-dimethyltubocurarine. Br J Anaesth 44:557-561.

Kivalo I, Saarikoski S. 1976. Placental transfer of ^{14}C-dimethyltubocurarine during Caesarean section. Br J Anaesth 48:239-242.

Korinthenberg R, Haug C, Hannak D. 1994. The metabolism of carbamazepine to CBZ-10,11-epoxide in children from the newborn age to adolescence. Neuropediatrics 25:214-216.

Kostial K, Kello D, Jugo S, Rabar I, Maljkovic T. 1978. Influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81-86.

Krauer B, Krauer F, Hytten FE. 1980. Drug disposition and pharmacokinetics in the maternal-placental-fetal-unit. Pharmacol Ther 10:301-328.

Krishnan K, Andersen ME. 1998. Physiological pharmacokinetic models in the risk assessment of developmental neurotoxicants. In: Handbook of Developmental Neurotoxicology (Sikker W, Chang L, eds). New York:Academic Press, New York, 709-725.

Kuhnz W, Jager-Roman E, Rating D, Diechl A, Kunze J, Helge H, et al. 1983. Carbmazepine and carbamazepine-10,11-epoxide during pregnancy and postnatal period in epileptic mothers and their nursed infants: pharmacokinetics and clinical effects. Pediatr Pharmacol (New York) 3:199-208.

Kurata N, Nishimura Y, Iwase M, Fischer NE, Tang BK, Inaba T, et al. 1998. Trimethadione metabolism by human liver cytochrome P450: evidence for the involvement of CYP2E1. Xenobiotica 28:1041-1047.

LaCroix D, Sonnier M, Moncion A, Cheron G, Cresteil T. 1997. Expression of CYP3A in the human liver - evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. Eur J Biochem 247:625-634.

Leakey JEA. 1983. Ontogenesis. In: Biological Basis of Detoxication (Caldwell J, ed). New York:Academic Press, 77-103.

Lefauconnier JM, Tayarani Y, Bernard G. 1986. Blood-brain barrier permeability to excitatory amino acids. Adv Exp Med Biol 203:191-198.

Lehmann H, Cook J, Ryan E. 1957. Pseudocholinesterase in early infancy. Proc R Soc Med 50:147-150.

Levine RR. 1973. Pharmacology Drug Actions and Reactions. Boston:Little, Brown, 103.

Levitz M, Condon GP, Money WL, Dancis J. 1960. The relative transfer of estrogens and their sulfates across the guinea pig placenta: sulfurylation of estrogens by the placenta. J Biol Chem 235:973-977.

Levy G, Khanna NN, Soda DM, Tsuzuki O, Stern L. 1975. Pharmacokinetics of acetaminophen in the human neonate: formation of acetaminophen glucuronide and sulfate in raltion to plasma bilirubin concentration and D-glucaric acid excretion. Pediatrics 55:818-825.

Levy G. 1981. Pharmacokinetics of fetal and neonatal exposure to drugs. Obstet Gynecol 58(suppl 5):S9-S16.

Lin JH. 1995. Species similarities and differences in pharmacokinetics. Drug Metab Dispos 23:1081-1021.

Luecke RH, Wosilait WD, Pearce BA, Young JF. 1994. A physiologically based pharmacokinetic computer model for human pregnancy. Teratology 49:90-103.

Maenpaa J, Rane A, Raunio H, Honkakoski P, Pelkonen O. 1993. Cytochrome P450 isoforms in human fetal tissues related to phenobarbital-inducible forms in the mouse. Biochem Pharmacol 45:899-907.

Mattson JL, Maurissen JP, Nolan RJ, Brzak KA. 2000. Lack of differential sensitivity to cholinesterase inhibition in fetuses and neonates compared to dams pretreated perinatally with chlorpyrifos. Toxicol Sci 53:438-446.

Mihaly GW, Morgan DJ. 1983. Placental drug transfer: effects of gestational age and species. Pharmacol Ther 23:253-266.

Miller RK, Koszalka TR, Brent RL. 1976. The transport of molecules across placental membranes. In: The Cell Surface in Animal Embryogenesis and Development (Post G, Nicolson GL, eds). New York:Elsevier/North-Holland Biomedical Press, 145-223.

Millicovsky G, DeSesso JM. 1980. Differential embryonic cardiovascular responses to acute maternal uterine ischemia: an *in vivo* microscopic study of rabbit embryos with either intact or clamped umbilical cords. Teratology 22:335-343.

Mirkin BL, Singh S. 1976. Placental transfer of pharmacologically active molecules. In: Perinatal Pharmacology and Therapeutics (Mirkin, BL, ed) New York:Academic Press, 1-69.

Moghrabi N, Head JR, Andersson S. 1997. Cell type-specific expression of 17ß-hydroxysteroid dehydrogenase type 2 in human placenta and fetal liver. J Clin Endocrinol Metab 82:3872-3878.

Morselli PL. 1989. Clinical pharmacology of the perinatal period and early infancy. Clin Pharmacokinet 17(suppl 1):13-28.

Moser VC, Chanda SM, Mortensen SR, Padilla S. 1998. Age- and gender-related differences in sensitivity to chlorpyrifos in the rat reflect developmental profiles of esterase activities. Toxicol Sci 46:211-222.

Mulhall A, de Louvois J, Hurley R. 1983. Chloramphenicol toxicity in neonates: its incidence and prevention. Br Med J (Clin Res Ed) 287:1424-1427.

Murray GI, Foster CO, Barnes TS, Weaver RJ, Snyder CP, Ewen SW, et al. 1992. Cytochrome P4501A expression in adult and fetal human liver. Carcinogenesis 13:165-169.

Nakamura H, Tanaka E, Ishikawa A, Fukao K, Tsuji K, Ohkawa H. 1998. Age-related changes in hepatic drug-oxidizing activity using trimethadione as a probe drug in humans. Hepatol Res 12:85-92.

National Research Council (NRC). 2000. Scientific frontiers in developmental toxicology and risk assessment. Committee on Developmental Toxicology. Washington, DC:National Academy Press.

NAS 1993. Pesticides in the Diets of Infants and Children. Washington, DC:National Academy of Sciences.

Nau H, Neubert D. 1978. Placental transfer of drugs during early human pregnancy. In: Role of Pharmacokinetics in Prenatal and Perinatal Toxicology (Neubert D, Merker H-J, Nau H, Langman J, eds). West Germany:Theime, Stuttgart, 13-44.

Nau H, Liddiard C. 1978. Placental transfer of drugs during early human pregnancy. In: Role of Pharmacokinetics in Prenatal and Perinatal Toxicology (Neubert D, Merker H-J, Nau H, Langman J, eds). West Germany:Theime, Stuttgart, 465-481.

Nedelcheva V, Gut I. 1994. P450 in the rat and man: methods of investigation, substrate specificities and relevance to cancer. Xenobiotica 24:1151-1175.

Neims AH, Warner W, Loughnan PM, Aranda JV. 1976. Developmental aspects of the hepatic cytochrome P-450 monooxygenase system. Annu Rev Pharmacol Toxicol 16:427-445.

Noschel H, Peiker G, Voigt R, Meinhold P, Muller B, Schroder S, et al. 1980. Research on pharmacokinetics during pregnancy. Arch Toxicol Suppl 4:S380-S384.

O'Flaherty EJ. 1991. Physiologically based models for bone-seeking elements. II. Kinetics of lead disposition in rats. Toxicol Appl Pharmacol 111:313-331.

O'Flaherty EJ. 1995. Physiologically based models for bone-seeking elements. V. Lead absorption and disposition in childhood. Toxicol Appl Pharmacol 131:297-308.

Ohmori S, Nakasa H, Asanome K, Kurose Y, Ishii I, Hosokawa M, et al. 1998. Differential catalytic properties in metabolism of endogenous and exogenous substrates among CYP3A enzymes expressed in COS-7 cells. Biochim Biophys Acta 1380:297-304.

Pariente-Khayat A, Pons G, Rey E, Richard MO, D'Athis P, Moran C, et al. 1991. Caffeine acetylator phenotyping during maturation in infants. Pediatr Res 29:492-495.

Parsons WD, Aranda JV, Neims AH. 1976. Elimination of transplacentally acquired caffeine in fullterm neonates. Pediatr Res 10:333.

Parsons WD, Neims AH. 1978. Effect of smoking on caffeine clearance. Clin Pharmacol Ther 24:40-45.

Pelekis M, Gephart LA, Lerman SE. 2001. Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. Regul Toxicol Pharmacol 33:12-20.

Pelkonen O. 1977. Transplacental transfer of foreign compounds and their metabolism by the foetus. Prog Drug Metab 2:119-161.

Pelkonen O. 1980. Biotransformation of xenobiotics in the fetus. Pharmacol Ther 10:261-281.

Pikkarainen PH, Raiha NC. 1967. Development of alcohol dehydrogenase activity in the human liver. Pediatr Res 1:165-168.

Pons G, Rey E, Carrier O, Richard MO, Moran C, Badoual J, et al. 1989. Maturation of AFMU excretion in infants. Fundam Clin Pharmacol 3:589-595.

Pounds JG, Marlar RJ, Allen JR. 1978. Metabolism of lead-210 in juvenile and adult rhesus monkeys (Macaca mulatta). Bull Environ Contam Toxicol 19:684-691.

Power GG, Longo LD. 1975. Fetal circulation times and their implications for tissue oxygenation. Gynecol Invest 6:342-355.

Pyonnonen S, Sillanpaa M, Frey H, Iisalo E. 1977. Carbamazepine and its 10,11-epoxide in children and adults with epilepsy. Eur J Clin Pharmacol 11:129-133.

Rane A, Tomson G. 1980. Prenatal and neonatal drug metabolism in man. Eur J Clin Pharmacol 18:9-15.

Rapoport MD. 1976. Blood-Brain Barrier In Physiology and Medicine. New York:Raven Press, 1-86.

Ratanasavanh D, Beaune P, Morel F, Flinois JP, Guengerich FP, Guillouzo A. 1991, Intralobular distribution and quantitation of cytochrome P-450 enzymes in human liver as a function of age. Hepatology 13:1142-1151.

Rao HV and Ginsberg GL. 1997. A physiologically-based pharmacokinetic model assessment of methyl t-butyl ether in groundwater for a bathing and showering determination. Risk Anal 17:583-597.

Ravindranath V, Boyd MR. 1995. Xenobiotic metabolism in brain. Drug Metab Rev 27:419-448.

Renwick AG. 1998. Toxicokinetics in infants and children in relation to the ADI and TDI. Food Addit Contam 15(suppl):S17-S35.

Renwick AG, Dorne JL, Walton K. 2000. An analysis of the need for an additional uncertainty factor for infants and children. Regul Toxicol Pharmacol 31:286-296.

Rudolph AM, Hymann MA. 1967. The circulation of the fetus *in utero*. Circ Res 21:163-184.

Sandberg JA, Binienda Z, Lipe G, Rose LM, Parker WB Ali SF, et al. 1995. Placental transfer and fetal disposition of 2',3'-dideoxycytidine (ddC) and 2',3'-dideoxyinosine (ddI) in the rhesus monkey. Drug Metab Dispos 23:881-884.

Sandberg JA, Duhart HM, Lipe G, Binienda Z, Slikker W Jr, Kim CS. 1996. Disposition of 2,4-dichlorophenoxyacetic acid (2,4-D) in maternal and fetal rabbits. J Toxicol Environ Health 49:497-509.

Slikker W Jr, Hill DE, Young JF. 1982b. Comparison of the transplacental pharmacokinetics of 17ß-estradiol and diethylstilbestrol in the subhuman primate. J Pharmacol Exp Ther 221:173-182.

Slikker W Jr. 1987. The role of metabolism in the testing of developmental toxicants. Regul Toxicol Pharmacol 7:390-413.

Slikker W Jr, Miller RK. 1994. Placental metabolism and transfer role in developmental toxicology. In: Developmental Toxicology (Kimmel CA, Buelke-Sam J, eds). Raven Press, 245-283.

Slikker W Jr. 1994. Placental transfer and pharmacokinetics of developmental neurotoxicants. In: Principles of Neurotoxicology (Chang LW, ed). New York:Marcel Dekker, 659-680.

Slikker W Jr. 1997. The Developing Nervous System. In: Comprehensive Toxicology (Sipes IG, McQueen CA, Gandolfi AJ, eds). Pergamon, 295-307.

Sonnier M, Cresteil T. 1998. Delayed ontogenesis of CYP1A2 in the human liver. Eur J Biochem 251:893-898.

Stevens JC, Shipley LA, Cashman JR, Vandenbranden, Wrighton SA. 1993. Comparison of human and rhesus monkey *in vitro* phase I and phase II hepatic drug metabolism activities. Drug Metab Dispos 21:753-760.

Strange RC, Howie AF, Hume R, Matharoo B, Bell J, Hiley C, et al. 1989. The developmental expression of alpha-, mu- and pi-class glutathione S-tranferases in human liver. Biochim Biophys Acta 993:186-190.

Szorady I, Santa A, Veress I. 1987. Drug acetylator phenotypes in newborn infants. Biol Res Pregnancy Perinatol 8:23-25.

Tanaka E. 1998. *In vivo* age-related changes in hepatic drug-oxidizing capacity in humans. J Clin Pharm Ther 23:247-255.

Treyluyer JM, Jacqz-Aigrain E, Alvarez F, Cresteil T. 1991. Expression of CYP2D6 in developing human liver. Eur J Biochem 202:583-588.

Treyluyer JM, Cheron G, Sonnier M, Cresteil T. 1996. Cytochrome P450 expression in sudden infant death syndrome. Biochem Pharmacol 52:497-504.

U.S. EPA. 1989. Risk Assessment Guidance for Superfund. Vol. I. Human Health Evaluation Manual, Part A. United States Environmental Protection Agency. EPA/540/1-89/002.

U.S. EPA. 1992. Draft Report: A Cross-Species Scaling Factor for Carcinogen Risk Assessment Based on Equivalence of mg/kg $^{3/4}$ /day. Federal Register 57:24152-24173. United States Environmental Protection Agency.

U.S. EPA. 1994. Guidance Manual for the Integrated Exposure Uptake Biokinetic Model for Lead in Children. United States Environmental Protection Agency. EPA 540-R-93-081; PB93-963510.

U.S. EPA. 2000. IRIS File for Vinyl Chloride and Toxicology Review of Vinyl Chloride in Support of Summary Information on IRIS. United States Environmental Protection Agency. EPA/635R-00/004.

Vest MF. 1965. The development of conjugation mechanisms and drug toxicity in the newborn. Biol Neonat 8:258-266.

Vieira I, Sonnier M, Cresteil T. 1996. Development expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. Eur J Biochem 238:476-483.

Waddell WJ, Marlowe C. 1981. Transfer of drugs across the placenta. Pharmacol Ther 14:375-390.

Watanabe J, Asaka Y, Kanamura S. 1993. Postnatal development and sublobular distribution of cytochrome P-450 in rat liver: a microphotometric study. J Histochem Cytochem 41:397-400.

Welfare MR, Bassendine MF, Daly AK. 2000. The effect of NAT2 genotype and gender on the metabolism of caffeine in nonsmoking subjects. Br J Clin Pharmacol 49:240-243.

Welsch F. 1982. Placental and fetal uptake of drugs. J Vet Pharmacol Ther 5:91-104.

Welsch F, Blumenthal GM, Conolly RB. 1995. Physiologically based pharmacokinetic models applicable to organogenesis: extrapolation between species and potential use in prenatal toxicity risk assessments. Toxicol Lett 82-83:539-547.

Williams RT. 1959. Detoxication Mechanisms. 2nd ed. London:Chapman & Hall.

Wilson JG, Ritter EJ, Scott WJ, Fradkin R. 1977. Comparative distribution and embryotoxicity of acetylsalicylic acid in pregnant rats and rhesus monkeys. Toxicol Appl Pharmacol 41:67-78.

Wilson JG. 1964. Embryological considerations in teratology. In: Principles and Techniques, (Wilson JG, Warkany J, eds). Chicago and London:The University of Chicago Press, 251-261.

Yang HL, Namkung MJ, Juchau MR. 1995. Expression of functional cytochrome P-450A1 in human embryonic hepatic tissues during organogenesis. Biochem Pharmacol 49:717-726.

Yoo J-SH, Ning SM, Patten CJ, Yang CS. 1987. Metabolism and activation of N-nitrosodimethylamine by hamster and rat microsomes: comparative study with weanling and adult animals. Cancer Res 47:992-998.

Ziegler EE, Edwards BB, Jensen RL, Mahaffey KR, Fomon SJ. 1978. Absorption and retention of lead by infants. Pediatr Res 12:29-34.

Zsigmond EK, Downs JR. 1971. Plasma cholinesterase activity in newborns and infants. Can Anesth Soc J 18:278-285.

Zusterzeel PL, Peters WH, De Bruyn MA, Knapen MF, Merkus HM, Steegers EA. 1999. Gluthathione S-transferase isoenyzmes in decidua and placenta of preeclamptic pregnancies. Obstet Gynecol 94:1033-1038.

Zwiener RJ, Ginsburg CM 1988. Organophosphate and carbamate poisoning in infants and children. Pediatrics 81:121-126.

APPENDIX 4: FOCUS QUESTIONS

WORKSHOP TO DEVELOP A FRAMEWORK FOR ASSESSING RISKS TO CHILDREN FROM EXPOSURE TO ENVIRONMENTAL AGENTS

The Workshop Planning Committee has suggested a number of questions that may help to focus your preparation for the workshop and to guide our discussions during the workshop. They are grouped below as (1) questions to be addressed by all breakout groups, focusing primarily on the Framework, (2) generic questions that raise some broad issues, and (3) potential questions for the individual breakout groups.

All Breakout Groups

The Planning Committee has suggested that the Framework should lead the risk assessor through a process that addresses questions like:

- What kinds of generic information do we have related to children's risks?
- What kinds of chemical-specific information do we have/need?
- How do we link and interpret this information to assess risks at critical life stages from conception through adolescence?

The Committee proposed that, given the complexity of the matrix of developmental life stages and potential adverse effects, the Framework should help the risk assessor focus on the most important (critical) effects for the chemical/exposures in question, identifying the resources and data available to make this determination. It was agreed that the unique exposure patterns during development are key factors that need to be integrated somehow into the risk assessment process and recognized in the Framework (building on the EPA/RAF July 2000 workshop). It was also suggested that the Framework might point to options that may be considered in the absence of chemical-specific data.

The Framework is not intended to be as detailed or prescriptive as Guidelines, but EPA (and perhaps other organizations) intends to use the Framework that we are developing as a starting point for the preparation of a guidance document for assessing children's risks.

Questions for all of the workshop breakout groups, therefore, are:

1. Does the draft Framework capture the major components of risk assessment for effects resulting from exposures occurring from conception through adolescence?

2. What modifications to the Framework and the associated Framework Diagram does your breakout group recommend? Can more detail be added to the Diagram?

3. How can the Framework be modified/expanded to incorporate concepts from your breakout group? How can your responses to the focus questions be reflected in the Framework?

4. How do the conclusions/recommendations from the EPA Risk Assessment Forum Workshop (July 2000) affect the Framework and your breakout group's responses to the focus questions?

5. How could the Framework be tested, following the workshop? Are there specific case studies that you could recommend?

6. What are the critical research needs and data gaps for the assessment of children's risks from exposure to environmental agents?

Generic Questions

1. What are the unique life stages from conception through adolescence?

2. Why/how are they important in risk assessment?

3. What outcomes/endpoints are likely to be the most important for each life stage?

4. How can animal models/studies be used to predict potential health consequences for humans?

Kinetics Breakout Group

1. What are the critical PK determinants of internal dose (e.g., those to which PBPK models are most sensitive)?

2. How do these PK determinants differ across the age spectrum from *in utero* through adolescence, and how do they compare in adults?

3. Which analytical methods and risk assessment practices can be applied to child/adult PK differences? Under what conditions would you use each method? Which methods are currently feasible and what new data are needed to improve the use of these techniques for children?

4. Is it appropriate to create age groupings that represent specific PK developmental phases? If so, what might these look like?

5. Are children more or less variable than adults in terms of PK handling of xenobiotics?

6. Is it possible to lump together groups of xenobiotics handled via similar PK mechanisms so that generalizations regarding groups of chemicals can be made for various developmental stages?

7. How can animal data from *in utero* and early life stage studies be used to inform children's PK assessments?

Dynamics Breakout Group

1. What do we know about the dynamics of developmental processes that may make them uniquely sensitive (qualitatively and/or quantitatively) to environmental responses?

2. Are there common characteristics of these processes that can help us in identifying or focusing our assessments?

   i.e., Does the presence of active proliferation automatically target those times, tissues for further investigation for unique sensitivity? What other process might we "target" for consideration?

3. Define the types of dynamic information that we need for our Framework.

4. Can we use the overall developmental process timeline to target key tissues, systems and processes for evaluation of developmental dynamics for unique sensitivity at specific times in development? As was asked for Kinetics, is it appropriate to create age groupings that represent specific developmental phases for dynamics? If so, what might these look like?

5. Are the animal tests robust enough to provide the types of dynamic information that we need? Again, what are our qualitative and quantitative needs for these assessments?

6. Presuming that we can answer some of the above questions, can the breakout group provide some indications of level and type of impact that might result from altering these developmental processes? How would these compare with adult dynamic processes? How would we go about answering this question?

   For example, if we use the revised WHO/Renwick approach for evaluating kinetic and dynamic factors in reference dose calculations, do we have support for specific values for the dynamic component? How might we evaluate proposed factors for evaluating dynamic processes?

7. How can dynamic information support or refute proposed modes of action?

8. How might this differ from support for modes of action for that chemical in adults?

Risk Characterization Breakout Group

What are the critical components to consider in the context of a comprehensive risk assessment that explicitly addresses risks to children? In particular:

1. Have unique susceptibilities been identified associated with one or more stages of prenatal development or childhood?

2. Are there mechanisms of toxicity unique to children, or are just the outcomes different?

3. Are there behaviors that are peculiar to children that make exposure by certain routes or media more of concern?

4. In the context of risk assessment, how should we address responses in children that are different from those in adults? For example, is an additional uncertainty factor warranted? If so, what is the nature of the uncertainty? Could there be multiple sources of child-specific uncertainty? Is the use of an uncertainty factor dependent on whether the difference between child and adult response is qualitative or quantitative? For qualitative differences in outcome, does it matter if these are mechanistically different? Does it matter whether there is likely to be a cumulative/chronic effect from exposure? What magnitude (or range) should the uncertainty factor be? What data would be necessary to alleviate the uncertainty represented by the UF?

5. What assumptions can we make about childhood susceptibility or childhood exposure when the data set is incomplete?

APPENDIX 5: WORKSHOP PARTICIPANTS AND OBSERVERS CONTACT INFORMATION

ILSI RISK SCIENCE INSTITUTE

WORKSHOP TO DEVELOP A FRAMEWORK FOR ASSESSING RISKS TO CHILDREN FROM EXPOSURE TO ENVIRONMENTAL AGENTS

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APPENDIX 6: WORKSHOP PROGRAM

WORKSHOP TO DEVELOP A FRAMEWORK FOR ASSESSING RISKS TO CHILDREN FROM EXPOSURE TO ENVIRONMENTAL AGENTS

July 30 – August 2, 2001
Topnotch at Stowe Resort and Conference Center
Stowe, VT

Monday, July 30

DINNER

6:30-9:00 p.m.
"Child-Protective Risk Assessment: Challenge and Opportunity *Phil Landrigan*

Tuesday, July 31

8:30-10:30

- Welcome *Steve Olin & Bob Sonawane*
- Setting the Stage *Steve Olin*
  - Workshop Objective and Scope
  - Process and Procedures
- Background Presentations (+ Q&A)
  - Conclusions from EPA/RAF July 2000 Workshop *Steven Knott*
  - Brief overviews of the draft framework and the background papers *Lead Authors*

BREAK

10:45-11:00

- Charge to the Breakout Groups *Steve Olin*

11:00-12:00

- Breakout Group Session I – Kinetics, Dynamics, Risk Characterization

LUNCH

1:00-3:00

- Breakout Group Session I (Cont'd)

BREAK

3:30-5:00

- Breakout Group Session I (Cont'd) – Risk Characterization BOG meets briefly with Kinetics and Dynamics BOGs to present its questions for these BOGs

5:00-5:30

- Coordination Meeting: Chairs & Rapporteurs of the Breakout Groups

6:00 DINNER

Wednesday, August 1

8:00-8:30

- Status Reports from Breakout Groups *Chairs/Rapporteurs*

8:30-10:00

- Case Studies: Presentation and discussion of examples of risk assessments in which differential internal dose or sensitivity of the developing human was specifically considered and impacted the risk characterization
  - Atrazine *Vicki Dellarco*
  - Vinyl chloride *Harvey Clewell*
  - Pediatric drugs *Karen Davis-Bruno*

BREAK

10:30-12:00

- Breakout Group Session II

LUNCH

1:00-3:30

- Breakout Group Session II (Cont'd)

BREAK

4:00-5:30

- Reports from Breakout Groups *Chairs/Rapporteurs*

5:30-6:00

- Coordination Meeting: Chairs & Rapporteurs of the Breakout Groups

6:30 DINNER

Thursday, August 2

8:00-10:30

- Breakout Group Session III (internal review and further work on draft reports)

BREAK

11:00- 12:30

- Presentation and Discussion of Drafts *Chairs/Rapporteurs*

LUNCH

1:30-3:00

- Presentation and Discussion of Critical Data Gaps and Research Needs *Chairs/Rapporteurs*

3:00 ADJOURN