WORLD HEALTH ORGANIZATION

INTERNATIONAL LABOUR ORGANIZATION

UNITED NATIONS ENVIRONMENT PROGRAMME

WHO

ILO

UNEP

WORLD HEALTH ORGANIZATION

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY (IPCS)

ENVIRONMENTAL HEALTH CRITERIA DOCUMENT ON

PRINCIPLES FOR EVALUATING HEALTH RISKS IN CHILDREN ASSOCIATED WITH EXPOSURE TO CHEMICALS

Draft Prepared by:

Dr Germaine BUCK-LOUIS, Division of Epidemiology, Statistics and Preventive Research, NIH, National Institute of Child Health Development, USA; Dr Terri DAMSTRA, World Health Organization, International Programme on Chemical Safety, Interregional Research Unit, USA; Dr Fernando DIAZ-BARRIGA, Universidad Autonoma de San Luis Potosi, Facultad de Medicina, Mexico; Dr Elaine FAUSTMAN, Institute for Risk Analysis and Risk Communication, University of Washington, USA; Dr Ulla HASS, Danish Danish Institute of Food & Veterinary Research (FDIR), Department of Toxicology & Risk Assessment, Denmark; Dr Robert KAVLOCK, US Environmental Protection Agency, Reproduction Toxicology Facility, USA; Dr Carole KIMMEL, US Environmental Protection Agency, National Center for Environmental Assessment, USA; Dr Gary KIMMEL, Silver Spring, Maryland, USA; Dr Kannan KRISHNAN, University of Montreal, Canada; Dr Ulrike LUDERER, Center for Occupational and Environmental Health, University of California at Irvine, USA; Dr Linda SHELDON, US Environmental Protection Agency, Human Exposure and Atmospheric Sciences Division, USA.
TABLE OF CONTENTS

CHAPTER 1 – Executive Summary, Conclusions, and Recommendations
  1.1 Introduction
  1.2 Conclusions

CHAPTER 2 – Introduction and Background
  2.1 Introduction
  2.2 Purpose and Scope of Document
  2.3 Global Burden of Disease in Children
  2.4 Major Environmental Threats to Children
    2.4.1 Economic and nutritional factors
    2.4.2 Social, cultural, demographic, and lifestyle factors
    2.4.3 Chemical hazards
  2.5 Intrinsic Factors
  2.6 The Significance of a Developmental Stage Approach

CHAPTER 3 – Unique Biological Characteristics of Children
  3.1 Growth and Development
    3.1.1 Body weight and height
    3.1.2 Organ weights/volumes
    3.1.3 Skin
  3.2 Anatomical and Functional Characteristics
  3.3 Physiological Characteristics
    3.3.1 Breathing rate
    3.3.2 Cardiac output
    3.3.3 Blood flow to organs
    3.3.4 Body composition
    3.3.5 Tissue composition
    3.3.6 Bone growth and composition
  3.4 Metabolic Characteristics
  3.5 Toxicokinetics
    3.5.1 Absorption, distribution, metabolism and elimination
    3.5.2 Physiological changes in mother and their influences on toxicokinetics
    3.5.3 Dose to target
  3.6 Normal Development
    3.6.1 Basic principles of normal development
    3.6.2 Nervous system
    3.6.3 Reproductive system
    3.6.4 Endocrine system
    3.6.5 Cardiovascular system
    3.6.6 Immune system
CHAPTER 4 – Developmental Stage Specific Susceptibilities and Outcomes in Children

4.1 Introduction
4.2 Mortality, Growth Restriction, and Birth Defects
4.2.1 Mortality
4.2.2 Growth restriction
4.2.3 Birth defects (structural malformations)
4.2.3.1 Etiology
4.2.3.2 Functional developmental toxicity
4.3 Specific Organ Systems
4.3.1 Nervous system
4.3.1.1 Periods of vulnerability and consequences of exposure
4.3.1.2 Specific Examples
4.3.2 Reproductive system
4.3.2.1 Periods of vulnerability
4.3.2.2 Consequences of exposure to chemicals
4.3.3 Endocrine and metabolic disorders
4.3.3.1 Periods of vulnerability
4.3.3.2 Consequences of exposures
4.3.4 Cardiovascular system
4.3.5 Immune system
4.3.5.1 Periods of vulnerability
4.3.5.2 Consequences of early exposure
4.3.6 Normal development respiratory system
4.3.6.1 Periods of vulnerability
4.3.6.2 Consequences of exposures
4.3.7 Kidney
4.3.7.1 Periods of vulnerability
4.3.7.2 Consequences of exposure
4.4 Cancer
4.4.1 Childhood cancers that may have environmental causes
4.4.2 Adult cancers related to childhood exposures
4.4.3 Chemical exposures of special concern
4.5 Conclusions

CHAPTER 5 – Exposure Assessment of Children
5.1 Introduction
5.2 General Principles of Exposure Assessments
5.3 Methods for Conducting Exposure Assessments
5.3.1 Direct methods
5.3.2 Biomarkers of exposure
5.3.3 Modeling
5.4 Unique Characteristics of Children that Affect Exposure
5.5 Exposure as It Relates to Children Around the World
   5.5.1 Sources/geographical location
   5.5.2 Pathways of exposure
      5.5.2.1 Ambient air exposure pathway
      5.5.2.2 Indoor exposure pathways
      5.5.2.3 Water exposure pathway
      5.5.2.4 Soil exposure pathway
      5.5.2.5 Food-chain exposure pathway
      5.5.2.6 Human exposure pathways
   5.5.3 Settings/microenvironments
      5.5.3.1 Residential
      5.5.3.2 School
      5.5.3.3 Child care centers
      5.5.3.4 Recreational
      5.5.3.5 Special settings
   5.5.4 Environmental equity factors (vulnerable communities)
5.6 Special Considerations for Children’s Exposure: Case Studies
   5.6.1 Influence of activities
   5.6.2 Environmental equity
   5.6.3 Aggregate exposure
   5.6.4 Cumulative exposure
5.7 Conclusions

CHAPTER 6 – Methodologies to Assess Health Outcomes in Children
   6.1 Introduction
      6.1.1 Methodologic approaches for children’s health
      6.1.2 Methodologic approaches for animal studies
   6.2 Growth and Development
      6.2.1 Human studies
      6.2.2 Animal studies
   6.3 Reproductive Development and Function
      6.3.1 Human studies
      6.3.2 Animal studies
   6.4 Neurological and Behavioral Effects
      6.4.1 Human studies
      6.4.2 Animal studies
   6.5 Cancer
      6.5.1 Human studies
      6.5.2 Animal studies
   6.6 Immune
      6.6.1 Human studies
      6.6.2 Animal studies
   6.7 Respiratory
      6.7.1 Human studies
      6.7.2 Animal studies
   6.8 Hemopoeitic/Cardiovascular, Hepatic/Renal, Skin/Musculoskeletal,
CHAPTER 7 – Implications and Strategies for Risk Assessment for Children

7.1 Introduction

7.2 Problem Formulation

7.3 Hazard Identification

7.3.1 Endpoints and critical periods of exposure

7.3.2 Human studies

7.3.3 Relevance of animal studies for assessing potential hazards to children

7.3.4 Reversibility and latency

7.3.5 Characterization of the health related data base

7.4 Dose Response Assessment

7.4.1 Application of health outcome data

7.4.2 Quantitative evaluation

7.4.2.1 Tolerable daily intake (TDI) and reference dose

7.4.2.2 Benchmark dose (BMD) – benchmark concentration

7.4.2.3 Biologically-based dose-response models

7.4.2.4 Duration adjustment

7.4.2.5 Toxicokinetics

7.5 Exposure assessment

7.5.1 Age-specific exposures

7.5.2 Assessment methods

7.6 Risk Characterization

7.7 Conclusions
CHAPTER 1 - EXECUTIVE SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

1.1 Introduction

Environmental factors play a major role in determining the health and well-being of children.\(^*\) Accumulating evidence indicates that children (who comprise over one-third of the world’s population) are among the most vulnerable and that environmental factors can affect children’s health quite differently than adults. Poor, neglected, and malnourished children suffer the most. These children often live in unhealthy housing, lack clean water and sanitation services, and have limited access to health care and education. One in five children in the poorest parts of the world will not live to their fifth birthday, mainly because of environment-related diseases. The World Health Organization (WHO) estimates that over 30% of the global burden of disease in children can be attributed to environmental factors.

Health is determined by a variety of factors. In addition to the physical environment, genetics, and biology, social, economic, and cultural factors play a major role. It is critical to understand the various driving forces during childhood that shape health and behaviour throughout life. The purpose of this document is to evaluate the scientific principles to be considered in assessing health risks in children from exposures to environmental agents during distinct developmental stages and provide information that can be used to protect children at the individual, family and community levels. The central focus of this document is on the developmental stage rather than on a specific environmental chemical or a specific disease or outcome. Developmental stage-specific periods of susceptibility have been referred to as “critical windows for exposure” or “critical windows of development.” These distinct life stages are defined by relevant dynamic processes occurring at the molecular, cellular, organ system, and organism level. It is the differences in these life stages along with exposures that will define the nature and severity of environmental impacts.

\(^*\) The term “children” as used in this document includes the stages of development from conception through adolescence.
Children have different susceptibilities during different life-stages, due to their dynamic growth and developmental processes as well as physiological, metabolic and behavioural differences. From conception through adolescence, rapid growth and developmental processes occur which can be disrupted by exposures to toxic chemicals. These include anatomical, physiological, metabolic, functional, toxicokinetic, and toxicodynamic processes. Exposure pathways and exposure patterns may also be different in different stages of childhood. Exposure can occur in utero through transplacental transfer of chemicals from mother to foetus, or via breast milk in nursing infants. Children consume more food and drink per kg of body weight than adults, and their dietary patterns are different and often less variable during different developmental stages. They have a higher inhalation rate and a high body surface-to-body weight ratio which may lead to increased dermal exposure. Children’s normal behaviours such as crawling on the ground and putting their hands in their mouths can result in exposures not faced by adults. Children’s metabolic pathways may differ from adults. Children have more years of future life and thus more time to develop chronic diseases that take decades to appear and which may be triggered by early environmental exposures. They are often unaware of environmental risks and generally have no voice in decision-making.

The accumulating knowledge that children may be at increased risk at different developmental stages, with respect to both toxicology and exposure, has raised the awareness that new risk assessment approaches may be necessary in order to adequately protect children. Traditional risk assessment approaches and environmental health policies have focused mainly on adults and adult exposure patterns, utilizing data from adult humans or adult animals. There is a need to expand risk assessment paradigms to evaluate exposures relevant to children from pre-conception to adolescence, taking into account the specific susceptibilities at each developmental stage. The full spectrum of effects from childhood exposures cannot be predicted from adult data. Risk assessment approaches for exposures in children must be linked to life stages.

A broad spectrum of diseases in children are known (or suspected) to be associated with unhealthy environments. For much of the world, traditional environmental health hazards continue to remain the primary source of ill-health. These include lack of adequate nutrition,
poor sanitation, contaminated water, rampant disease vectors (e.g., mosquitoes and malaria), and
unsafe waste disposal. In addition, rapid globalization and industrialization coupled with
unsustainable patterns of production and consumption have released large quantities of toxic
substances into the environment. Most of these substances have not been assessed for potential
toxicity to children, nor have the most vulnerable subpopulations of children been identified.
The incidence of a number of important paediatric diseases and disorders (e.g., asthma,
neurobehavioural impairment) is increasing, and this may be due, at least in part, to the quality of
the environment in which children live, grow, and play.

However, establishing causal links between specific environmental exposures and complex,
multifactorial health outcomes is difficult and challenging, particularly in children. For children,
the stage in their development when the exposure occurs may be just as important as the
magnitude of exposure. Very few studies have characterized exposures during different
developmental stages. Examples have shown that exposures to the same chemical result in very
different health outcomes in children than in adults. Some of these outcomes have been shown
to be irreversible and persist throughout life. Furthermore, different organ systems mature at
different rates, and the same dose of a chemical during different periods of development can
have very different consequences. There may also be a long latency period between exposure
and effects, with some outcomes not apparent until later in life. Some examples of health effects
resulting from developmental exposures include those observed prenatally and at birth (e.g.,
miscarriage, stillbirth, low birth weight, birth defects), in young children (e.g., infant mortality,
asthma, neurobehavioural and immune impairment), and in adolescents (e.g., precocious or
delayed puberty). Emerging evidence suggests that certain diseases in adults (e.g., cancer, heart
disease) result from exposure during childhood.

While research has successfully assessed the impact of environmental factors on children’s
health, typically investigators have focused on a particular exposure such as heavy metals or
endocrine disruptors and a particular organ system or endpoint. Noticeably absent are
prospective longitudinal studies capturing exposures over key developmental windows or life
stages. Virtually no studies have captured peri-conceptional exposures either alone or in addition
to other life stage exposures. Advancing technology and new methodologies now offer promise
for capturing exposures during these critical windows. This will enable investigators to capture
early conceptions and estimate the potential competing risk of early embryonic mortality when
considering children’s health outcomes that are conditional upon survival during the embryonic
and fetal periods.

The special vulnerability of children should form the basis for development of child-protective
policies and risk assessment approaches. A lack of full proof for causal associations should not
prevent efforts to reduce exposures or implement intervention and prevention strategies.

1.2 Conclusions

While substantial knowledge has been gained on the effects of environmental agents on
children’s health, much remains to be learned. Child-protective risk assessment approaches must
be based on a better understanding of the interactions of exposures, biological susceptibility,
socio-economic, and cultural (including nutritional) factors at each stage of development. In
order to gain a better understanding, further research is needed in the following areas:

- Design and implement prospective cohort studies of pregnant women, infants and
  children with longitudinal capture of exposures at critical windows and sensitive
  health endpoints along the continuum of human development. Efforts to recruit
  couples prior to conception are needed to address critical data regarding peri-
  conceptional exposures and children’s health.

- Continue to develop and enhance population based surveillance systems for the
  real time capture of sentinel health endpoints. This includes current surveillance
  systems such as vital registration for birth size and gestation or birth defects
  registries for capture of major malformations. Consideration of emerging sentinel
  endpoints such as fecundability as measured by time-to-pregnancy and sex ratios
  should receive added research consideration.

- Strengthen exposure monitoring efforts in children during different developmental
  stages, including efforts to assess aggregate and cumulative exposures.

- Strengthen exposure monitoring efforts in developing countries.

- Identify subpopulations with the highest exposure levels.
• Develop validated, sensitive and cost-effective biomarkers of exposure, susceptibility, and effects, particularly during early developmental stages.
• Improve characterization of the differences in toxicokinetic and toxicodynamic properties of xenobiotics at different developmental stages. Develop databases of developmental-stage specific physiological and pharmacokinetic parameters in both human and animal studies.
• Conduct studies focusing on mechanisms of action during different developmental stages by which exposures may cause adverse outcomes.
• Develop endpoints for assessing organ system functions that can be used in both humans and animal species; and that can be used to identify analogous periods of development across species.
• Examine the utility of newer molecular and imaging technologies to assess causal associations between exposure and effect at different developmental stages.
• Improve characterization of the windows of susceptibility of different organ systems in relation to structural and functional endpoints.
• Develop and validate biological models and animal testing guidelines that can address health outcomes at different developmental stages.
• Determine which exposure reductions will have the greatest overall impact on children’s health.

The development of risk assessment strategies that address the developmental life stages through which all future generations must pass is essential to any public health strategy. Protection of children is at the core of the sustainability of the human species. It should be a priority of all countries, international and national organizations, to provide safe environments for all children and reduce exposure through promotion of healthy behaviours, education, and awareness raising at all levels, including the community, family, and child him/herself. In order to better accomplish this, research on the effectiveness of risk-reduction and intervention practices, including the most effective means to educate and communicate the need for child-protective public health policies, legislation, and safety standards, is needed. The active participation of all sectors of society, including children and young people, plays an important role in promoting safe and healthy environments for all.
CHAPTER 2 – INTRODUCTION AND BACKGROUND

2.1. Introduction

Although the last three decades have witnessed a significant decline in childhood mortality and morbidity, these gains have not been apparent everywhere, and, in some countries, mortality and morbidity are increasing (WHO, 2005a). Exposure to environmental risks is a major reason for ill health in children, primarily in children that are impoverished and malnourished. Yet, because of their increased susceptibility, these children are the very group that can least afford to be exposed to environmental hazards. The heightened susceptibility of children derives primarily from the unique biological and physiological features that characterize the various stages of development from conception through adolescence (see Chapter 3), as well as certain behavioral characteristics and external factors that may result in increased exposure levels (see Chapter 5).

The increased awareness about the special vulnerability of children has led to a number of new research programs, international agreements, and international alliances which specifically address and promote healthy environments for children (UNICEF, 1990, WHO, 1997, UNICEF, 2001a, Suk, 2002, WHO, 2002a, Suk, et al., 2003). A few key international activities are cited below:

• In 1989, the UN Convention on the Rights of the Child laid down the basic standards for the protection of children, taking into consideration the dangers of environmental pollution, and declared that children are entitled to special care and assistance.

• In 1990, the World Summit for Children adopted a declaration on the survival, protection, and development of children in which the signatories agreed to work together to protect the environment so that all children could enjoy a safer and healthier future.

• In 1992, the United Nations Conference on Environment and Development (the Earth Summit) declared that the health of children is affected more severely than any other

* The term “children” or “child” as used in this document includes the stages of development from conception through adolescence.
population group by unhealthy environments, that children are highly vulnerable to the
effects of environmental degradation, and that their special susceptibilities need to be
taken fully into account.

- In 1997, the Declaration of the Environmental Leaders of the Eight on Children’s
  Environmental Health acknowledged the special vulnerability of children and committed
  their countries to take action on several specific environmental health issues such as
  chronic lead poisoning, microbiologically contaminated drinking-water, endocrine
  disrupting chemicals, environmental tobacco smoke, and poor air quality.

- In 2002, the WHO launched the Healthy Environments for Children Alliance which seeks
to mobilize support and intensify global action to provide healthy environments for
  children.

- In 2002, the Bangkok Statement (adopted by over 400 participants) at the WHO
  International Conference on Environmental Threats to Children: Hazards and
  Vulnerability, in Bangkok, Thailand, identified the need for improved risk assessment
  methodologies in children.

- In 2005, the Second International Conference of Environmental Threats to Children, in
  Buenos Aires, Argentina, assessed major environmental threats to children in Central and
  Latin American (LAC) countries and identified priority areas for research collaboration.

Many countries have also established specific regulations to protect children from exposure to
certain environmental risks, including toxic chemicals. Examples include: banning of heavy
metals in toys, strict limit setting for persistent toxic substances in baby foods, and the setting of
environmental limit values derived on the basis of infant’s sensitivities (e.g., nitrates in drinking
water). In the U.S., concerns about children’s special vulnerabilities resulted in the Food Quality
Protection Act (FQPA, 1996), which directs the U.S. EPA to use an additional 10-fold safety
factor in assessing the risks of exposure of infants and children to pesticides, particularly when
there is limited toxicology and exposure data. In Europe, an action plan is being developed to
evaluate risks through the SCALE (Science for Children through Awareness, Legislation and
Continuous Evaluation; EU, 2004).
Approaches to assessing risks from chemicals, in the past, were based largely on adult exposures, toxicities, and default factors. The publication of the 1993 NAS Report on Pesticides in the Diet of Infants and Children (NRC, 1993) was critical in raising awareness of the importance of considering the vulnerable life stages of children when conducting risk assessments of exposure in children. In 2001, the International Life Sciences Institute (ILSI) convened a number of scientific experts to develop a conceptual framework for conducting risk assessments from chemical exposure in children which takes into consideration their unique characteristics and special vulnerabilities (Olin and Sonawane, 2003; Daston et al., 2004). This document builds on these previous activities, and takes into account the availability of updated test guidelines, new technologies, and revised models for exposure assessment in order to evaluate the scientific knowledge base that underlies a “child-centered” risk assessment strategy (see Chapters 6 and 7).

2.2. Purpose and Scope of Document

The primary purpose of this document is to provide a systematic analysis of the scientific principles to be considered in assessing health risks in children from exposures to environmental agents during distinct stages of development. The developmental stages used throughout this document are defined in Table 2.1 and are considered temporal intervals with distinct anatomical, physiological, behavioural, or functional characteristics that contribute to potential differences in vulnerability to environmental exposures. Exposure before conception (either maternal, paternal, or parental) may also affect health outcomes during later stages of development. Adverse health effects may be detected during the same life stage as when the exposure occurred or they may not be expressed until later in life. The central focus of this document is on the child rather than on a specific environmental agent, target organ, or disease. Thus, it addresses the difficult task of integrating all that is known about exposure, toxicity, and health outcomes at different life stages, which is especially challenging when data are limited for particular life stages (e.g., exposure levels during pregnancy).
# TABLE 2.1

## WORKING DEFINITIONS FOR STAGES IN HUMAN DEVELOPMENT

<table>
<thead>
<tr>
<th>DEVELOPMENTAL STAGE/EVENT</th>
<th>TIME PERIOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE-CONCEPTION</td>
<td>Pre-fertilization</td>
</tr>
<tr>
<td>PREGNANCY</td>
<td></td>
</tr>
<tr>
<td>Preimplantation embryo</td>
<td>Conception – implantation</td>
</tr>
<tr>
<td>Postimplantation embryo</td>
<td>Implantation to 8 week of pregnancy</td>
</tr>
<tr>
<td>Foetus</td>
<td>8 weeks of pregnancy – birth</td>
</tr>
<tr>
<td>PRE-TERM BIRTH</td>
<td>24 – 37 weeks of pregnancy</td>
</tr>
<tr>
<td>NORMAL TERM BIRTH</td>
<td>40± 2 weeks of pregnancy</td>
</tr>
<tr>
<td>PERINATAL STAGE birth</td>
<td>29 weeks of pregnancy – 7 days after birth</td>
</tr>
<tr>
<td>NEONATE</td>
<td>birth – 28 days</td>
</tr>
<tr>
<td>INFANT</td>
<td>28 days – 1 year</td>
</tr>
<tr>
<td>CHILD</td>
<td></td>
</tr>
<tr>
<td>Young child</td>
<td>1 – 4 years</td>
</tr>
<tr>
<td>Toddler</td>
<td>2 – 3 years</td>
</tr>
<tr>
<td>Older child</td>
<td>5 – 12 years</td>
</tr>
<tr>
<td>ADOLESCENT</td>
<td>beginning with the appearance of secondary sexual characteristics – achievement of full maturity (usually 12 – 18 years)</td>
</tr>
<tr>
<td>ADULT</td>
<td>usually 18 years (certain systems continue to develop, e.g., skeleton, brain)</td>
</tr>
</tbody>
</table>

The term “environmental exposures” as used in this document generally refers to specific environmental pollutants. Although health threats due to dietary factors, behavioural and
lifestyle factors, use of pharmaceuticals, etc., are also considered environmentally related, they
fall beyond the scope of this document, except when they interact with environmental exposures.

Similarly, the document is not intended to be a comprehensive review of the literature on the
effects of exposures of all environmental pollutants on the health of children. Rather, the effects
of illustrative pollutants are described to demonstrate how exposure patterns, susceptibilities, and
mechanisms of toxicity change at different life-stages and how these changes can impact risk
assessment. References are provided throughout the document for more detailed information on
environmental threats to children. A list of WHO websites relevant to children’s health is
provided in Table 2.2. These WHO websites also provide links to other relevant sites.

**TABLE 2.2**

<table>
<thead>
<tr>
<th>WHO WEBSITE RESOURCES RELEVANT TO CHILDREN’S HEALTH *</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO Child and Adolescent Health and Development: <a href="http://www.who.int/child-adolescent-health/">www.who.int/child-adolescent-health/</a></td>
</tr>
<tr>
<td>WHO Children’s Environmental Health: <a href="http://www.who.int/ceh">www.who.int/ceh</a></td>
</tr>
<tr>
<td>WHO Database on Child Growth and Malnutrition: <a href="http://www.who.int/nutgrowthdb">www.who.int/nutgrowthdb</a></td>
</tr>
<tr>
<td>WHO Data on Global Burden of Disease by Country, Age, Sex. Available from: <a href="http://www.who.int/evidence">www.who.int/evidence</a></td>
</tr>
<tr>
<td>WHO Food Safety: <a href="http://www.who.int/foodsafety/en/">www.who.int/foodsafety/en/</a></td>
</tr>
<tr>
<td>WHO Global Environmental Change: <a href="http://www.who.int/globalchange/en/">www.who.int/globalchange/en/</a></td>
</tr>
<tr>
<td>WHO International Programme on Chemical Safety: <a href="http://www.who.int/ipcs">www.who.int/ipcs</a></td>
</tr>
<tr>
<td>WHO Maternal and Newborn Health: <a href="http://www.who.int/reproductive-health/mbnh/index.htm">www.who.int/reproductive-health/mbnh/index.htm</a></td>
</tr>
<tr>
<td>WHO Millennium Development Goals for Health: <a href="http://www.who.int/mdg/publications">www.who.int/mdg/publications</a></td>
</tr>
<tr>
<td>WHO Nutrition: <a href="http://www.who.int/nut/">www.who.int/nut/</a></td>
</tr>
<tr>
<td>WHO Quantifying Environmental Health Impact Assessments: <a href="http://www.who.int/quantifying_ehimpacts/en/">www.who.int/quantifying_ehimpacts/en/</a></td>
</tr>
<tr>
<td>WHO School Health and Youth Health Promotion: <a href="http://www.who.int/school_youth_health/en/">www.who.int/school_youth_health/en/</a></td>
</tr>
<tr>
<td>WHO Water, Sanitation &amp; Health: <a href="http://www.who.int/water_sanitation_health/en/">www.who.int/water_sanitation_health/en/</a></td>
</tr>
<tr>
<td>WHO World Health Reports: <a href="http://www.who.int/whr">www.who.int/whr</a></td>
</tr>
<tr>
<td>WHO World Health Statistics: <a href="http://www.who.int/healthinfo/statistics">www.who.int/healthinfo/statistics</a></td>
</tr>
</tbody>
</table>

* These websites also provide links to other sites relevant to children’s health.
This document is intended to be used as a tool for use by public health officials, research and regulatory scientists, and risk assessors. It does not provide practical advice, guidelines or protocols for the conduct of specific tests and studies. In addition to the documents cited in the Introduction to this chapter, it builds on two previous EHCs addressing methodologies for assessing risks in children: EHC 30, “Principles for Evaluating Health Risks to Progeny Associated with Exposure to Chemicals During Pregnancy” (IPCS, 1984) and EHC 59, “Principles for Evaluating Health Risks from Chemicals During Infancy and Early Childhood: The Need for a Special Approach” (IPCS, 1986a). EHC 30 focused on the use of short-term tests and in vivo animal tests to assess prenatal toxicity and postnatal alterations in reproduction, development, and behaviour following chemical exposure during gestation, and EHC 59 focused on methods to detect impaired reproductive and neurobehavioural development in infants and children who were exposed during the prenatal and early postnatal periods.

2.3. Global Burden of Disease in Children

Scientific, medical, and public health advances, expanded access and receipt of primary healthcare and basic social services have significantly improved the health and well-being of children. Yet, at the beginning of the 21st century, nearly 11 million children (29,000/day) under five years of age will die from causes that are largely preventable. Among these are 4 million babies who will not survive the first month of life. A similar number will be stillborn (WHO, 2005a). Most of these deaths will occur in developing countries, particularly in the African and Southeast Asian regions of the world.

At the global level, an analysis of the WHO database on burden of disease data (http://www.who.int/evidence) shows that most of these deaths result from a handful of causes (WHO, 2005a). Figure 2.1 shows the major causes of death in children under five years of age. Estimates from the 2000-2003 database attribute 37% to neonatal causes, 19% to pneumonia, 17% to diarrhoea, 20% to “other” (including injuries, measles, and HIV/AIDS), and 8% to malaria.
Figure 2.1 also shows the major causes of neonatal deaths (birth to 28 days). The largest fraction of deaths (28%) is attributed to pre-term births, which may also result in long-term adverse health consequences (see Chapter 4). Thus, the perinatal and neonatal developmental stages can be considered particularly vulnerable periods.

The estimates in Figure 2.1 are at the global level and will differ significantly among various regions of the world and among countries within a given region. For example, in a number of
African countries, the surge of HIV/AIDS in recent years is now becoming one of the top killers of children (WHO, 2003). Many are also, largely, diseases of the developing world rather than diseases of the developed world.

Numerous risk factors contribute to the global burden of disease. Genetics, economics, social, lifestyle, and nutritional factors, as well as environmental exposures, play a large role and are discussed in the following sections.

2.4. Major Environmental Threats to Children

In its broadest sense, the environment is all that is external to the human host. As shown in Figure 2.2, children are exposed to numerous environmental hazards from multiple sources and in a variety of settings.

WHO estimates that over 30% of the global burden of disease can be attributed to environmental factors and that 40% of this burden falls on children under five years of age who account for only 10% of the world’s population (WHO, 2004a). At least 3 million children under five years of age die annually due to environmentally related illnesses. Environmental risk factors act in concert and are exacerbated by adverse social and economic conditions, particularly poverty and malnutrition.

It should also be noted that millions of children suffer from unsafe environments, abuse and neglect due to armed conflict, natural disasters (e.g., hurricanes and earthquakes), and man-made disasters. Many of these children become refugees and/or orphans and are engaged in forced, hazardous, and exploitative labour. These "marginalized" children suffer from the very beginning of their lives. Many are "invisible", and over 36% of all births go unregistered, mainly in developing countries (UNICEF 2006).
In 2003, there were an estimated 143 million orphans under the age of 18 in 93 developing countries (UNICEF 2005a). The precise number of refugee children, street children, and children caught up in armed conflict is difficult to quantify, but estimates are in the millions (UNICEF 2006). The estimated number of children affected by natural disasters is in the hundreds of thousands, including several thousand children orphaned following the December, 2004, Tsunami (UNICEF 2005a).

These vulnerable groups of children are also the ones that suffer from extreme poverty, malnutrition, under-nutrition and lack of health care and, thus, live in the most hazardous environments with often devastating, irreversible health consequences.
For the majority of the world’s population the primary environmental threats continue to be the following “traditional” risks: (1) unsafe drinking water, (2) poor sanitation, (3) indoor air pollution from household solid fuel use, (4) diarrhoeal, infectious and vector born diseases, and (5) contaminated food supplies. However, in both developing and developed countries, “emerging” and “modern” risks pose an increasing threat to children’s health. These include: exposure to natural or man-made toxic substances in air, water, soil, and the food chain, inadequate toxic waste disposal, injuries and poisonings, urbanization, and environmental degradation associated with unsustainable patterns of consumption and development. More recently, emerging environmental hazards, such as trans-boundary contamination by persistent toxic substances, ozone depletion, global climate change, and exposure to chemicals that disrupt endocrine function have been identified as potential risks to children’s health globally. In both developing countries and in countries in transition, “emerging” and “modern” hazards can compound the effects of the “traditional” hazards, and children from all socio-economic backgrounds are vulnerable to all these hazards. WHO projects that the burden of chronic diseases (e.g., cancer, cardiovascular) in developing countries is becoming relatively more important and will outweigh the burden of infectious disease by 2025 (WHO, 2003).

2.4.1. Economic and nutritional factors

Poverty is one of the major driving forces for unhealthy environmental conditions and ill health in children. Over 1.2 billion people struggle to survive on less than $1 US dollar per day; at least half of these are children (UN, 2001a). Even in the world’s richest countries, one in six children lives below the poverty line, mainly in urban centers (UN, 2001a). Patterns of unsustainable development, globalization, and urbanization are major driving forces influencing poverty and directly impacting children.

Poverty is also intricately linked to malnutrition, which in turn, is a major contributor to children’s mortality and morbidity. It is estimated that over 50% of all under-five deaths globally are associated with malnutrition (see Figure 2.1). The devastating effects of poverty and malnutrition on health, particularly children’s health, were recognized by representatives from 189 countries at the Millennium Summit in 2000 (http://www.un.org/millenniumgoals) where
Eight Millennium Development Goals were established. The first goal agreed upon by all UN Member States was to reduce poverty and hunger by 50% by 2015.

The effects of general under-nutrition (reduced caloric intake and protein deficiency) are frequently compounded by deficiencies in important micronutrients, such as iodine, vitamin A, iron, zinc and folate. Protein malnutrition in pregnant women results in anemia which can severely impact foetal growth and development resulting in diminished birth size or infant and child morbidity. Low birth weight infants are more likely to have developmental and learning deficits throughout childhood (see Chapter 4). Chronic under-nutrition during the first two to three years of life may result in similarly delayed growth and learning disabilities. Underweight children may also have impaired immune systems and thus be more prone to infections. Iron deficiency is a major cause of anemia and affects over 2 billion people worldwide (WHO, 2002b). About one-fifth of perinatal mortality is attributed to iron deficiency, and there is a growing body of evidence that iron deficiency anemia in early childhood reduces intelligence in mid-childhood (Stolzczus et al., 2001). Iron deficiency has also been associated with increased susceptibility to lead exposure (Gulson et al., 1999). Other examples of micronutrient deficiencies that have a major impact on the global health of children include vitamin A, vitamin B, and folic acid. Vitamin A deficiency is the leading cause of preventable blindness in children (WHO, 2002b). Inadequate folic acid prior to conception and during early pregnancy has been associated with birth defects including neural tube defects (see Chapter 4.2.3.1). The incidence of these serious birth defects varies from country to country, but a large percentage of them can be prevented by periconceptional folate supplementation. Unfortunately, to date, fewer than 40 countries have initiated such supplementation programs (Oakley et al., 2004). WHO maintains a data base (http://www3.who.int/whosis/micronutrient/) which indicates the status of micronutrient deficiencies in a number of countries.

At the other end of the nutrition scale, obesity in children is becoming a health threat, mainly in developed countries but increasingly in developing countries (de Onis et al., 2004; Koplan et al., 2005). Poor maternal nutrition has been linked to adverse health outcomes in affected offspring later in life (see Chapter 4.3.3).
2.4.2. Social, cultural, demographic, and lifestyle factors

Social, cultural, demographic, and lifestyle factors also play significant roles in influencing the exposure of children to environmental threats and consequently their health (see Chapter 5). For example, these factors can determine dietary habits, and thus the nature and extent of exposures of children to chemicals via the food chain. They also impact whether and for how long infants are breast fed. Other examples determined largely by cultural factors include the use of toys and medicines (e.g., folk medicines and herbs). Lifestyle factors will influence the extent of concomitant exposures such as alcohol and tobacco smoke, and demographic factors (including climate) will determine certain exposures such as indoor air pollutants from wood burning stoves. Rural versus urban settings may determine the extent and nature of children’s exposure to pesticides. Another example of a particularly sensitive subpopulation of children is those whose families (e.g., indigenous peoples) that rely on marine mammals and fish heavily contaminated with persistent organic pollutants (POPs), or heavy metals for food (Damstra, 2002; Damstra et al., 2002).

WHO considers social, cultural, and economic factors to be major determinants of ill health, the “causes behind the causes” of morbidity and mortality. In 2004, WHO established a high-level commission to develop plans that address key social determinants of health including the health of children.

2.4.3. Chemical hazards

The production and use of toxic chemicals poses another major environmental threat to the health of children and is the major focus of this document. Global industrialization, urbanization, and intensified agriculture, along with increasing patterns of unsustainable consumption and environmental degradation have released large amounts of toxic substances into the air, water, and soil. In addition, children may be exposed to naturally occurring dangerous chemicals, such as arsenic and fluoride in groundwater (WHO, 2001a,b,c). An estimated 50,000 children die annually as a result of accidental or intentional ingestion of toxic substances (Pronczuk de Garbino, 2002). The global burden of disease in children attributed to
environmental chemical exposures is largely unknown and has only recently begun to be investigated (see www.who.int/quantifying_ehimpacts/global/en/). The WHO European Region has published estimates of the global burden of disease due to environmental risks for children in European countries (Valent et al., 2004; EEA 2005).

Although estimates of the burden of disease in children due to environmental exposures are generally not available, there is clear scientific evidence that exposure to environmental chemicals during different developmental stages can result in a number of adverse outcomes in children and have resulted in an increased incidence of certain childhood diseases (see Chapter 4). A wide range of chemicals can affect children’s health but a few chemical classes are of particular concern. These include heavy metals, persistent organic pollutants (POPs), pesticides and air pollutants. Heavy metals and lipophilic POPs cross the placenta and also favor transfer into breast milk, usually the primary source of food for neonates. Heavy metals and POPs are known to interfere with the normal growth and development of children (Damstra et al., 2002). Because of the persistence and toxicity of these chemicals, an international global treaty (Stockholm Convention) was ratified in 2004 which called for the elimination or phase-out of initial POPs (UNEP, 2004).

Neonates and infants are also exposed to toxic chemicals (e.g., organochlorine pesticides, heavy metals) through breast milk. As infants are weaned from breast milk, they become exposed to a greater range of toxic chemicals via formula, drinking water, and solid foods. They may also be heavily exposed to air pollutants, particularly indoor air pollutants such as carbon monoxide (CO) and polycyclic aromatic hydrocarbons (PAHs). In households dependent on biomass fuel for cooking and heating (2.5 billion people worldwide), infants are at particular risk while resting on the backs of mothers as they tend fires. In addition, mouthing or play behavior of infants can lead to ingestion of toxic chemicals that accumulate on surfaces (e.g., toys) or in soil.

The younger child and toddler are susceptible to exposure from chemicals in solid food (e.g., pesticides) and air (e.g., particulate matter) and through dermal exposure (e.g., heavy metals in soil). As children are introduced to day care and schools, potential new sources of exposure to certain chemicals (e.g., cleaning agents) may occur. Older children continue to be exposed to
chemicals present in school and/or day care environments. In addition, exploratory behaviors
may result in exposure to chemicals present in outdoor environments and dangerous settings
(e.g., hazardous waste sites).

Exposure to organophosphate pesticides (OPs) typically occurs in older children and adolescents
in rural areas through agricultural work or as bystanders during agricultural pest control.
Adolescents may also be exposed occupationally to other chemicals such as solvents. Puberty is
associated with growth spurts and hormone fluctuations, and the effects of chemical exposure are
of unknown impact. A more comprehensive list of chemical hazards and their effects can be
found in a number of reviews (American Academy of Pediatrics, 2003; EEA, 2002; WHO,
2002c; WHO, 2004b).

2.5. Intrinsic Factors

In addition to some of the extrinsic factors addressed in section 2.3, intrinsic factors (e.g.,
genetic) which control the dynamics of development play a key role in determining the
susceptibility of children to environmental exposures at different life-stages (see also section
3.6).

The outcome of developmental exposure is influenced significantly by the genetics of the
organism. The basis for these differences is due to complex and multiple mechanisms, which
have been discussed in a number of recent reviews (NRC, 2000a; Faustman et al., 2000). Recent
advances in genomics have also provided valuable information on gene-environment interaction,
which is generally defined as the disease risk of an environmental exposure in persons with
different genotypes (Ottman, 1996). Most studies of gene-environment interactions have
focused on adults, but studies are becoming available demonstrating the existence of genetic
polymorphisms for developmentally important genes that may enhance the susceptibility of
children. The most frequent genetic polymorphisms which have been examined involve
differences in the capacity to metabolize toxic agents over the course of various developmental
stages.
One example of a gene-environment interaction affecting children is the association between heavy maternal cigarette smoking (> 10 cigarettes/day) and cleft lip and/or palate in the offspring. This association is only marginally significant unless an allelic variant for TGF-alpha is present. The combination of smoking and the uncommon variant of the gene raises the odds ratio to a highly significant level (Hwang et al., 1995; Shaw et al., 1996). Another example highly relevant to children’s health is illustrated by the gene-environment interactions that mediate the effects of organophosphate pesticides (Costa et al., 2005). Two genes are known to increase susceptibility to OPs: acetylcholinesterase and paraoxonase. About 4% of the population carries a gene that results in lower levels of acetylcholinesterase, the target enzyme of organophosphates (which in turn, increases the vulnerability of the developing brain to OPs (Costa et al., 2003)). Another family of genetically determined enzymes, paraoxonase, further modifies an individual’s susceptibility to OP toxicity. For example, individuals can vary 11-fold in the ability to inactivate the OP pesticide, parathion, depending on which gene they carry (Brophy et al., 2001).

2.6. The Significance of a Developmental Stage Approach

The stages of “childhood” can be viewed from a variety of perspectives (e.g., chronological, developmental, legal, educational). This document focuses on age-specific developmental stages (see Table 2.1) that may exhibit unique susceptibility and sensitivity to environmental influences. Susceptibility is determined by intrinsic factors (e.g., genetics) that can modify the effect of a specific exposure whereas, sensitivity is used to describe the capacity for higher risk due to the combined effects of susceptibility and differences in exposure. Age-specific periods of susceptibility have been termed “critical windows of exposure.” Data on various developmental stages and their corresponding critical windows of exposure have been summarized in a number of recent reviews (Selevan et al., 2000; Rice and Barone, 2000; Dietert et al., 2000; Zoetis and Hurtt, 2003a; Daston et al., 2004; Landrigan et al., 2004).

Adverse effects in children may result from exposure prior to conception (paternal or maternal), during prenatal development, or postnatally to the time of full maturity. Even within a given developmental stage, shorter intervals of exposure may determine susceptibility for particular
outcomes. Different organ systems develop at different rates, but it has been shown that for each developmental stage there are both broad windows of susceptibility and more specific periods of susceptibility (Selevan et al., 2000; Faustman et al., 2000). This has been worked out in some detail for certain systems and agents (e.g. CNS development and radiation exposure), but in most cases, the exact time when organ systems are susceptible to the actions of toxic chemicals is unknown. Limited data are available on susceptibility during the adolescent period, but with the current greater interest in the effects of hormonally active agents, more information is becoming available.

As indicated previously, adverse health outcomes from early exposures may become apparent at any point in the life-span. In some instances, they may only be apparent after long latency periods. Chapter 6 addresses the various methodologies that can be used to assess health outcomes. Studies have shown that the effects of toxic exposures on developmental processes may result from different mechanisms of action and produce different health outcomes than the same exposures in adults. Some examples of health effects resulting from developmental exposures include those observed prenatally and at birth (e.g., miscarriage, stillbirth, low birth weight, birth defects), in young children (e.g., asthma, neurobehavioural and immune impairment), in adolescents (e.g., precocious or delayed puberty), and in adults (e.g., diabetes and heart disease).

2.7. Conclusions

Environmental threats to the health of today’s children result from a complex interaction of influences in children’s biological, social, behavioral, physical, and economic environments. Attempts to partition the global burden of disease by causative risk factors are admittedly oversimplified and difficult due to the inadequacies of the data base and the multifactorial nature of most diseases. Notwithstanding these difficulties, it is clear that: 1) environmental factors play a significant role in adverse health outcomes in children and that this is a serious public health concern, 2) due to health, wealth, or opportunity many children are disadvantaged from the time of conception, 3) the health of the “child” at each stage of development will set the stage
for and affect future health, and 4) current risk assessment paradigms do not address the dynamic interactions of intrinsic and extrinsic factors over different developmental stages of childhood.
CHAPTER 3. UNIQUE BIOLOGICAL CHARACTERISTICS OF CHILDREN

This chapter summarizes the distinct characteristics of children across life-stages that contribute to unique differences in vulnerability to environmental exposures. These include anatomical, physiological, metabolic, functional, toxicokinetic and toxicodynamic characteristics. The normal development of various organ systems is also addressed. Differences in exposure pathways and behavioral characteristics will be addressed in Chapter 5. Different organ systems develop at different rates and comparison across life stages will allow for identification of specific systems that are at risk during specific stages of development.

3.1. Growth and Development

3.1.1. Body weight and height

Human beings grow more slowly than many other species. Birth weight is increased by about 18 times over the first 20 years. Infants gain weight more rapidly during the first 4 to 6 months than during the rest of the life. At the age of 2 years, a boy is about half his adult height whereas a girl is slightly more than half her adult height (Lowrey, 1973). By 6 years of age, children in general are about 70% of their adult height. The height and weight of children of any given age group are highly variable reflecting the complex influence of genetic, cultural, dietary/nutritional and environmental factors.

The records of physical change over time (i.e., growth curves) show how far and how fast the child has grown. In utero, specific periods of growth spurts usually coincide with the last two trimesters. Post-natally, six growth spurts have been named according to the developmental periods where peak velocity of growth is reached (e.g., neonatal, infantile, early-childhood, middle-childhood and late childhood) (Walker and Walker, 2000). Such growth curves have been developed using growth data from a variety of international studies. Empirical mathematical models describing these developmental growth data have also been developed (USEPA, 2000). Most of these growth charts have been developed using data from
industrialized countries. Less information is available on the growth curves of children (by
developmental stages) from developing countries.

For children under 5 years old, WHO maintains the WHO Global Database on Child Growth and
Malnutrition (http://www.who.int/nutgrowthd) which provides anthropometric data on 90% of
the world’s children. The anthropometric indices in the WHO database for assessing child
growth status includes height-for-age which can reveal characteristics of linear growth, and age
and height specific growth for assessing children’s proportionality.

An analysis of the WHO database indicates that childhood malnutrition resulting in stunted
growth remains a global problem. The prevalence of underweight children (de Onis and
Blossnner, 2003) continues to be a major contributor of ill health in children, particularly in
developing countries (WHO, 2005a). By contrast, in developed countries, there is concern about
the increasing prevalence of obesity in children (NAS 2005). A person is considered obese if he
or she has a body mass index of 30 kg/m² or greater. Little information is available about the
prevalence of obesity in children in developing countries, although some developing countries
(particularly Latin American countries) are reporting increasing numbers of obese children (de
Onis and Blossner, 2000).

3.1.2. Organ weights/volumes

From birth to adulthood, physical changes including the size of body parts and organs occur at an
uneven rate. At any time during infancy and early childhood, one part of the body or organ
grows faster than another. This asynchrony is typical of how humans grow before and after
birth. Vital organs grow at different rates because their cells divide and grow at different rates.
The absolute brain weight, for example, does not change much with age, but the relative brain
weight decreases with age. In contrast, the absolute weights of kidney and liver increase with
age, whereas the relative kidney and liver weights show minimal change. Even though there is a
general increase in muscle and adipose tissue volumes as a function of age, the rate of their
relative increase is somewhat different at the early ages. For example, between 2 and 6 months
of age the increase in volume of adipose tissue is more than twice as great as increase in the
volume of muscle. But, between 6 and 12 months of age, the increase in muscle volume is
slightly more rapid than that of adipose tissue. Quantitative relationships for computing organ
weights from age, body weight and height have been developed (ICRP, 1975; Haddad et al.,
2001; Price et al., 2003a,b).

Figure 3.1 Organ weight from birth to adolescence in boys.
Based on Haddad et al., (2001)
3.1.3. Skin

A baby at full-term has a mature skin with barrier properties similar to those of older children and adults. However, the hydration state of the epidermis is greater in neonates than in older children, suggesting the potential for some chemicals to be absorbed more efficiently. In preterm infants, the epidermal barrier is poorly developed resulting in increased percutaneous absorption of chemical agents. The pre-term infant represents a special case, since studies of development of the stratum corneum in fetal life have suggested that the permeability barrier is incomplete until just before term (Singer et al., 1971). There have been reports of greater blood concentrations of chemicals in preterm newborns than full-term newborns when both are bathed in the same solution (Greaves et al., 1975). Neonates and infants in general have larger surface area relative to body weight than adults.

3.2. Anatomical and Functional Characteristics

Most organs and organ systems lack structural or functional maturity at birth. The blood-brain-barrier is immature at birth and the development of this barrier and the nervous system in general continues in postnatal life. Much of the myelination of the brain takes place after birth and continues until adolescence (Hoar and Monie, 1981). The structural development of the lung also continues postnatally in terms of the alveolar surface area (Langston, 1983). Components of the immune system are not fully developed at birth, resulting in enhanced susceptibility of newborns to certain bacterial infections (Andersson et al., 1981). The gastrointestinal, endocrine and reproductive systems are all immature at birth. A number of factors influencing gastro-intestinal absorption of drugs and chemicals undergo maturational changes during the first 2 years of age (gastric acidity, gastro-intestinal motility, enzymic activity, bacterial flora), and less is known about these changes in such parameters between 2 and 18 years of age. These factors contribute to higher gastric pH in children and increased gastric and intestinal motility compared to adults.
Although the full-term neonate is born with kidneys containing essentially the same number of nephrons as the adult, overall renal function is reduced compared to older children or adults (Stewart and Hampton, 1987). Therefore, neonates are less able to eliminate xenobiotics and endogenous chemicals than older children and adults. The function of the renal tubules is less mature at birth than in adulthood and this persists until 6 months of age (Schwartz et al., 1976). The glomerular filtration rate increases after birth in humans (Gomez et al. 1999). These changes are attributed to vascular factors such as blood pressure and vascular resistance within the kidney. In rats, the glomerular filtration barrier and proximal tubule resorption become mature about seven days after birth (Gomez et al. 1999). It takes about a week after birth for the infant to start making concentrated urine; concentrating ability in largely matures by one year of age (Gomez et al. 1999). In the rat, the concentrating ability is not mature until 2-3 weeks after birth (Kavlock and Gray 1982; Zoetis and Hurtt 2003b).

Another difference between adults and infants is that most of the cells are smaller in infants than adults. Small cells have greater surface area in relation to mass than larger cells. This may have important implications for chemicals that come in contact with the cells in target organs (NRC, 1993).

3.3. Physiological Characteristics

3.3.1. Breathing rate

Neonates have fewer alveoli and greater breathing rate compared to adults. Emery and Mithal (1960) suggested that the number of alveoli in the terminal respiratory branches of infants shows a rapid rise during the first year and a gradual increase up to age 12, by which time there are nine times as many alveoli as were present at birth. The breathing rate relative to body weight is greater in infants than in adults. For example, the volume of air passing through the lungs of a resting infant is twice that of a resting adult per unit body weight, which implies that twice the amount of chemical reaches the infant than the adult under identical exposures. The breathing rate decreases steadily during growth in both boys and girls.
3.3.2. Cardiac output

The heart rate and cardiac output in newborns are greater in older children than in adults (Cayler et al., 1963; Sholler et al., 1987). This is in line with the general notion that animals with smaller body size have greater heart rate. The circulation time (from any two points of the body) is shorter in infants and children than in adults, due to small body size coupled with greater heart rate. Heart rate falls gradually as a function of age between birth and adolescence (Shock, 1944; Illiff and Lee, 1952), with no apparent sex difference until the age of 10. Quantitative descriptions of the relationship between cardiac output and body surface and body height have been established for infants, children and adults (Cayler et al., 1963; Krovetz, 1969).

3.3.3. Blood flow to organs

The rate of blood flow to organs changes with age and it is not always proportional to changes in organ weights. For example, liver blood flow rates measured in children of 4 – 8 years, 9 – 12 years and 13 – 15 years, corresponded to 325, 665 and 915 ml/min, respectively (Szantay et al., 1974). These values are smaller compared to the reference adult value (1612 ml/min) and it remains so even when expressed on the basis of liver weight (Arms and Travis, 1988). Regional cerebral blood flow is lower in neonates compared to adults; it increases until 5 – 6 years of age to values 50 – 85 % higher than those for adults and thereafter decreases, reaching adult levels in the late teen years. Ogawa et al. (1989) reported that there was a marked decrease in blood flow with age in grey matter where neurons are located but did not observe any age-related change in white matter. In general, cognitive development of children seems to be related to changes in blood flow to corresponding brain regions (Chiron et al., 1992).

Renal blood flow increases with age as a result of the reduction of peripheral vascular resistance. The kidneys of neonates receive only 5-6% of total cardiac output compared to 15 – 25% for adults (Hook and Baillie, 1979). The blood flow to kidney normalized to tissue weight, however, remains fairly constant after about the age of one year (Grunert et al., 1990). This is somewhat consistent with reports that renal maturity is attained during the second year of life. No significant difference in muscle blood flow rate, compared to adults, is reported for children.
aged 5 to 17 years of age (Goetzova, 1977). However, blood flow normalized to muscle weight decreases as a function of age (Lindbjerg, 1966; Amery et al., 1969).

3.3.4. Body composition

The body composition changes from birth to adulthood. Water, expressed as percentage of body weight, decreases rapidly between birth and 6 months of age, and then remains fairly constant (Figure 3.2). Body water content is about 80-90% in neonates and decreases to 55 – 60% in adulthood, with most of the excess water in neonates being extra-cellular (Widdowson and Dickerson, 1964). The percent fat in the body, however, increases rapidly up to 6 months and then decreases, accounting for similar percentages of body weight at ages 4 and 12 months. Whole body analysis suggests that fat comprises about 0.5% at five months of gestation, increasing to 11 – 16% at birth and over 20% body weight in adults. Protein content of the body also increases from birth to adulthood mainly due to the growth of muscular tissue which is much higher in proportion to the adult body (Figure 3.2).

3.3.5. Tissue composition

The lipid, water and protein contents of certain tissues vary markedly as a function of age (White et al., 1991). For example, the adipose tissues of neonates contain about 55% water and 35% lipids whereas the corresponding figures for the adult are about 25% and 70% respectively (Friis-Hensen, 1971). The proportion of water in skin falls as a function of age, due to an increase in collagen. The water content of liver, brain and kidneys decrease from birth to adulthood by 5 – 15%. The decrease in water content of liver and kidneys is primarily due to an increase in protein whereas in the brain this change is due to an increase in myelin. The overall composition of muscle in terms of lipid and water does not seem to vary with age (Dickerson and Widdowson, 1960).
Bone formation begins during fetal life with cartilage, which then calcifies into bone. Whole body mineral content triples between weeks 32-33 and weeks 40-41 of gestation in humans (Salle et al, 2000). Despite this dramatic mineralization, the bones of infants contain more water and less fat, protein, and minerals than adult bones. The chemical maturation of bone, as evidenced by a decrease in percent water and increase in calcification takes place after 1 to 2 years. Linear growth occurs at the metaphyses, the body of cartilage that separates the diaphysis (body of bone) and epiphysis (the end of the bone). Linear growth ceases around age 16 years in
girls and 18 years in boys, but epiphyseal closure of some bones (e.g. posterior spinous
processes) does not occur until age 25 years of age.

3.4. Metabolic Characteristics

Metabolism, in the present context, refers to the elimination or transformation of specific
functional groups of chemicals (Phase I) and conjugation of chemicals and their metabolites with
endogenous co-factors (e.g., UDP-glucuronic acid, sulphate, glutathione; Phase II). Neonates
and young children may be better or less able to deal with toxic substances than adults, due to
differences in metabolic capacity (Spielberg, 1992; NRC, 1993; Dorne et al., 2005). Studies
have indicated that the sometimes increased sensitivity of neonates may be related to their very
low, or at times, unmeasurable metabolizing capacity.

Phase I reactions are predominantly catalyzed by cytochrome P450 (CYP) dependent
monooxygenases that exist in more than 20 isoforms as well as flavin-dependent
monooxygenases. Even though the liver is the major site of phase I reactions, the P450 isozymes
are also present in all other tissues except red blood cells albeit at lower levels. The total P450
content of human liver microsomes remains fairly stable at about one-third of the adult value
during fetal life (second and third trimester of gestation) and the first year following birth
(Treluyer et al., 1996). It is suggested that P450 isoforms develop independently and are
regulated during the perinatal period by multiple mechanisms and elements (Cresteil, 1998).
Altogether three groups of P450s could be described: a first group expressed in the fetal liver
including CYP3A7 and CYP4A1, and a second group including CYP2D6 and CYP2E1 that
surge within hours after birth although the protein levels associated with these isozymes cannot
always be detected in all fetal samples. Finally, there is a group that develops during the months
following birth (CYP3A4, CYP2C and CYP1A2) (Figure 3.3).
Figure 3.3 Hepatic cytochrome P450 1A2 and P450 2E1 in children of various age groups as percentage of adult weights.

Limited data are available regarding the ontogeny of phase II enzymes in human tissues. Epoxide hydrolase is active in the fetal liver and accounts for 50% of the adult activity, but is extremely low in the fetal lung (Cresteil, 1998). Glutathione S-transferases exist as multiple isoforms, of which GSTπ has been reported to be responsible for 50% of glutathione conjugation in fetal liver but regresses at birth and is not expressed in adults. Other classes of GST, including GSTμ and α, are present in the fetal liver at low levels and increase after birth (Cresteil, 1982; Strange et al., 1989). Conjugation with glucuronic acid is significantly lower at birth than in adults, although the capability for conjugation with sulphate is well developed in neonates (Levy et al., 1975). The levels of conjugation to glycine in newborns are comparable to those of adults (Dutton, 1978).
In general, most of the metabolizing enzyme systems would appear to develop from the middle of gestation until a few months after birth. Enzyme activities related to oxidation/hydroxylation and reduction are developed early after birth and reach adult levels at approximately 6 months of age. Oxidative demethylation, on the other hand, is not expressed until several months after birth and the adult capacity will not be reached until 1-2 years after birth.

3.5. Toxicokinetics

3.5.1. Absorption, distribution, metabolism and elimination

Absorption of chemicals may occur by a variety of routes such as oral, dermal and pulmonary. Since the functional determinants of these uptake processes vary as a function of age, the uptake of chemicals is also likely to vary between children and adults. For example, the respiratory ventilation rate in infants is significantly larger relative to lung surface (133 ml/min/kg bw/m²) compared with adults (2 ml/min/kg bw/m²). Therefore, infants potentially receive a greater exposure of lung surface to airborne compounds on a body weight basis (Bennett et al., 1996). Ginsberg et al. (2005) indicated that the particle dose in the pulmonary region is also likely to be 2 – 4 times higher in 3-month old children compared to adults, particularly for submicron size particles. The skin surface area relative to body weight is greater in children compared to adults, such that the potential dose received following dermal exposure is likely to be about 3 times greater in infants compared to adults (Clewell et al., 2002). The permeability of the epidermal barrier is poorly developed in the preterm infant, resulting in greater percutaneous absorption of chemical agents.

Gastric pH is alkaline in newborns (pH 6 – 8) compared to adults (pH 1 – 3), thus causing differences in ionization and absorption of certain chemicals (Radde, 1985). The adult levels of gastric acid production are reached at about 2 years of age. The alkaline gastric pH in newborns and infants may lead to enhanced bioavailability of weakly basic compounds but reduced bioavailability of weakly acidic compounds.
Physiological distribution volume for chemicals may vary between children and adults because of the differences in water and lipid content as a function of age. For example, the relatively larger extra-cellular fluid volume of the infant means somewhat greater dilution of water soluble chemicals. However, the lipid soluble substances would be more concentrated in the fat of young children because of the lower amount of fat per kg body weight. The tissue and volume distribution of chemicals is determined by plasma protein concentrations. Even though the total plasma protein concentrations do not seem to change dramatically with age, the specific binding proteins such as steroid hormone binding protein, albumin $\alpha$1-acid glycoprotein and serum lipoproteins do vary with age. The concentration of albumin in the plasma of neonates, for example, is low and hence the number of binding sites is low. Moreover, these binding sites are occupied by endogenous substances such as fatty acids, steroids and bilirubin. Therefore, newborn infants have a lower capacity for binding exogenous chemicals to plasma albumin. The binding affinity is also different between neonates and adults. The neonate-adult difference in albumin binding affinity for many drugs is likely related to the differences in the form of protein as well as the amino acid content of albumin (Alcorn and McNamara, 2003).

Metabolism and elimination rates are generally lower in neonates compared to adults. The elimination half-lives of substances used as indicators of liver function (e.g., bromosulphthalein, bilirubin), for example, are longer in newborns than in adults. Renal clearance has been shown to be lower in neonates than older children and adults, for all chemical classes; lipophilic, hydrophilic, and organic ions (Clewell et al., 2002). Glomerular filtration rate at normal term birth is about one-third of the adult value when expressed on the basis of body surface and matures in about six months. On the other hand, the tubular reabsorption process reaches adult levels within a few days after birth.

A systematic comparison of the available information on age-dependent maturation of metabolic and elimination processes as well as the toxicokinetics of chemicals in developing neonatal and young animals with infants and children can be found elsewhere (Gladtke, 1973; Stewart and Hampton, 1987; Crom, 1994; Renwick, 1998; Clewell et al., 2002; Alcorn and McNamara, 2003; Kearns et al., 2003; Ginsberg et al., 2004; and de Zwart et al., 2004). Table 3.1 summarizes the age-dependency of toxicokinetic processes and determinants in children vs. adults.
Table 3.1 Summary of the age-dependency of the determinants of toxicokinetics in children in comparison with normal adults (↑ = increased, ↓ = decreased, ↔ = unaltered). Based on Rylance (1988)

<table>
<thead>
<tr>
<th>Age of Child</th>
<th>Newborn Preterm</th>
<th>Term (0-4 weeks)</th>
<th>Infancy</th>
<th>1-4 years</th>
<th>5-12 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Absorption</td>
<td>↓</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Distribution:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body water</td>
<td>↑↑↑</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Body fat</td>
<td>↓↓</td>
<td>↓</td>
<td>↓ Slight</td>
<td>? ↔</td>
<td>? ↔</td>
</tr>
<tr>
<td>Plasma albumin</td>
<td>↓</td>
<td>↓</td>
<td>↓ Slight</td>
<td>↔ ↔</td>
<td>↔ ↔</td>
</tr>
<tr>
<td>Biotransformation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidation/hydrolysis</td>
<td>↓↓↓</td>
<td>↓</td>
<td>↑↑</td>
<td>↑</td>
<td>↑ Slight</td>
</tr>
<tr>
<td>N-demethylation</td>
<td>↓↓↓</td>
<td>↓</td>
<td>↑↑</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Acetylation</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Conjugation-glucouronidation</td>
<td>↓↓</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Renal excretion:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerular filtration</td>
<td>↓↓</td>
<td>↓</td>
<td>↓</td>
<td>} Slight to 6</td>
<td>↔ ↔</td>
</tr>
<tr>
<td>Tubular secretion</td>
<td>↓↓↓</td>
<td>↓</td>
<td>↓</td>
<td>↓ months</td>
<td>↔ ↔</td>
</tr>
</tbody>
</table>

3.5.2. Physiological changes in mother and their influence on toxicokinetics

Pregnancy. During pregnancy many physiological changes occur in the maternal organ system as a consequence of, and in order to support, the growing fetus. These changes may influence the uptake, absorption, distribution, metabolism and excretion of xenobiotics not only in the mother but also in the developing fetus (Hytten, 1984; Krauer, 1987; Mattison et al., 1991). For example, the gastric emptying time increases and the intestinal motility decreases during...
pregnancy, which may result in longer retention of ingested xenobiotics in the upper intestinal
tract resulting in the possibility for increased absorption of xenobiotics and increased exposure of
the fetus (Klaassen, 1996). Cardiac output and peripheral blood flow increase by approximately
30% during the first trimester of gestation. The net respiratory volume is raised by about 50%
due to increased tidal volume, while the pulmonary ventilation rate is not changed. Thus,
volatile and airborne chemicals tend to be absorbed more readily. However, faster elimination of
volatile compounds from the body may also occur. Thus, depending upon the characteristics of
chemicals, the change in net respiratory volume of the mother could result in either higher or
lower exposure of the fetus. The renal blood flow and glomerular filtration rate are increased
during pregnancy, which may result in enhanced renal clearance of certain xenobiotics, thus
protecting the fetus from exposure to chemicals in the systemic circulation.

Changes in the blood concentrations of lipids, free fatty acids, and hormones during pregnancy
may also influence the distribution of xenobiotics in the body. By the end of pregnancy, the total
body water content has increased by up to 30%, and often fat-deposits have been formed. The
albumin concentration in plasma decreases to about two thirds of the normal level. This may
lead to a higher amount of free unbound xenobiotics in the blood, and thus increase the risk for
transfer via the placenta. These conditions may influence the distribution of xenobiotics between
the mother and the conceptus. In addition to maternal physiology, the limited evidence available
suggests that relative rates of drug metabolizing enzymes also change during pregnancy (Juchau,
1981; Juchau and Faustman-Watts, 1983). In experimental animals, the hepatic metabolism of
xenobiotics is altered during pregnancy. In the rat, decreases in hepatic monooxygenase and
glucuronidation activities have been observed, and there seems to be an overall decrease in
hepatic xenobiotic biotransformation during pregnancy. However, this decrease is accompanied
by an increase in liver weight of 40% during pregnancy. A similar change in liver weight has
not been reported in humans.

Lactation and breast milk. Breast-feeding plays a critical role in human infant development
since it provides not only essential nutrition but also protection against infection and other
immunological disorders (Lawrence, 1989). Unfortunately, the nursing mother can also serve as
a source of infant exposure to drugs and chemicals. Unlike drug therapy, which can be
terminated in most cases, environmental exposures may be continuous and chronic in nature. However, based on the numerous advantages of breast-feeding, the benefit in the majority of cases by far exceed the potential risk (Kacew, 1992; Pronczuk et al., 2004).

Milk composition is not constant and is influenced by the timing of feeding and duration of nursing postpartum. Human milk contains about 2 – 3 % fat and a large number of proteins. The pH of milk tends be lower than that of plasma. Most water-soluble substances are excreted into the milk by simple diffusion and lipid- soluble compounds are transported along with lipid molecules from plasma into the mammary gland. The amount of a certain substance transferred to the milk depends on the physico-chemical factors of the substance, the milk composition, and maternal factors such as dose, frequency and route of exposure and infant-related factors such as the amount, duration and frequency of feeding. A number of mathematical models for predicting transfer of chemicals and drugs into human milk have been developed (Corley et al., 2003; Fleishaker, 2003).

3.5.3. Dose to target

The dose to target tissues, a key determinant of the ensuing toxicity, is determined by toxicokinetics or the rate and extent of the absorption, distribution, metabolism and excretion of chemicals. When neonates and adults are exposed to the same concentration of atmospheric contaminants, the initial uptake rate is greater in neonates. This behavior has been reported with several anesthetics such as halothane, cyclopropane and nitrous oxide (Gregory et al., 1969; Salanitre and Rackow, 1969; Gallagher and Black, 1985). The greater rate of uptake of these chemicals is the net result of a larger alveolar ventilation rate relative to body weight, greater perfusion rates, and lower fat content in neonates and infants (Cook, 1976). The steady-state blood concentration, however, is comparable in children of all age groups and adults, when the only critical determinant is the blood:air partition coefficient which is fairly similar between adults and children (Price et al., 2003b).

Following inhalation exposure to the same ambient concentrations, the peak blood concentrations of highly metabolized chemicals (e.g., furan) in children of age groups 6, 10 and
14 years has been computed to be greater than adults by a factor of 1.5 using a physiological
modeling approach (Price et al., 2003b). In the case of such highly extracted chemicals, the
blood concentration is determined by the age-dependent tissue volumes, blood flow rates,
breathing rate and cardiac output. Based on such modeling studies, Sarangapani et al. (2003)
concluded that adult to children differences in blood concentration would be <2 in the case of a
number of inhaled gases and vapors (vinyl chloride, isopropanol, styrene, ozone,
perchloroethylene) as well. Recently, Nong et al. (2006) using subject-specific data on
physiology and CYP 2E1 protein content in PBTK models, reported that the inter-individual
differences in internal dose would be greater in neonates than in older children or adults. These
authors reported that the magnitude of difference in internal dose between adults and neonates
was about 3.9 for inhaled toluene. The greater variability of internal dose in neonates compared
to adults was attributed to: (i) interchild differences in the expression and maturation of CYP
2E1, and (ii) the adult-children difference in the limiting factor of hepatic metabolism (i.e.,
enzyme capacity in neonates vs liver blood flow rate in adults) (Nong et al. 2006).

The toxicokinetic difference between children and adults may also be evaluated by comparing
certain parameters such as clearance (volume of blood from which chemical is eliminated per
unit time) and half-life (time taken to reduce the initial concentration by 50%). Ginsberg et al.
(2002) compiled these toxicokinetic parameters for children and adults for 45 drugs. Analyzing
these data, Hattis et al. (2003) reported that the half-lives of orally administered drugs in children
of 2 months – 18 years were within a factor of 3.2 of the adult half-lives. However, 27% of the
0-1 week age group and 19% of the 1 week – 2 month age group had half-lives that exceeded the
adult values by more than a factor of 3.2 (Figure 3.4). It should be noted that most of the drugs
evaluated by these authors have short half-lives (<1 day).

The toxicokinetic parameters and models, when available, would be useful in evaluating the half-
lives and target tissue dose in infants and children following exposures. In the absence of such
chemical-specific toxicokinetic data or models, dose adjustment based on body surface appears
to be a reasonable alternative. Crom (1994) suggested that for drugs such as antipyrine and
indocyanine green (which are metabolized by microsomal enzymes and eliminated quickly), the
dosages based on body weight will yield lower serum concentrations in younger children, while
dosages based on body surface are more likely to result in similar concentrations in children and adults. These authors also showed that the age-dependent differences in clearance correlate better with body surface rather than body weight.

Adjustment based on body surface area signifies that the oral dose for children (mg/kg/d) is likely to be greater than for adults by a factor of two or so (Renwick, 1998). Such a body surface based dose adjustment should result in blood or tissue concentrations of parent chemicals that are comparable between children and adults, provided the toxicokinetic determinants are all related to body surface area. When this is not the case, and when the toxic moiety is not the parent chemical (as is the case with a number of environmental contaminants), the default approaches of adult-child dose extrapolation are unlikely to be accurate. In such cases, physiological modeling approaches that take into account the quantitative change in physiological and biochemical determinants may be used to evaluate the age-dependent change in target tissue dose of chemicals.

The adult-child difference in susceptibility may not only be related to differences in target tissue dose but also due to toxicodynamics or the interaction between the toxic moiety and biological macromolecules in target tissues resulting in the onset and progression of injury (Ginsberg et al., 2004). The following section provides an overview of the functional and structural development of various organ systems as well as the molecular determinants associated with these processes.

3.6. Normal Development

3.6.1. Basic principles of normal development

Following fertilization and replication to the blastocyst stages, the body axes are determined and the three germ layers of the embryo are formed. Early organ development is largely defined by the complex orchestration of the critical processes: cell proliferation, differentiation, migration, and apoptosis (programmed cell death). Cell differentiation is the process by which undifferentiated cells increasingly take on characteristics of differentiated adult cells. These characterizations include stage-specific gene and protein expression, altered functional activities
such as cell cycling and altered physical characteristics such as shape or location. In the neocortex, for example, 11 cell cycles occur during neurogenesis before neuronal differentiation and migration from the pseudostratified ventricular epithelium to the periventricular zone (Gohlke et al., 2002; Olney et al., 2000).

In other brain regions such as the cerebellum, there are cells simultaneously undergoing proliferation, differentiation, migration and apoptosis. Thus, the contribution of these basic cellular processes to organ development can vary temporally across organ regions and in relationship to each other.
Apopotosis in development is extremely important for controlling physical development as indicated by its role in limb and digit development where cells programmed to die allow for digit separation. It is also important in neurodevelopment where apoptosis plays a critical role in culling neurons that have not properly formed functioning synapses. Cell proliferation plays an important role in development by providing critical numbers and types of cells to differentiate or migrate. In general, less differentiated cells proliferate more and, as differentiation proceeds, cells lose their ability to actively cycle.

The interactions between cells involved in these critical processes for organ development is of great importance and makes them vulnerable to perturbations by outside influences. Characteristics of these interactions include the need for sufficient populations of cells in proximity with each other and the need for sufficient functional capabilities of the cell populations to produce sufficient messages of developmental importance (e.g., inducers) at specific times and locations to influence other populations of cells (responders). The responding cells must then be functionally competent to be able to receive such signals (e.g., already expressing a receptor for the inducer substance to influence) (Daston et al., 2004).

A report by the US National Academy of Sciences (NAS) reviewed 17 cell signaling pathways that are evolutionarily conserved and which can explain how cells and cell populations can accomplish the complex and timed interactions outlined above (NRC, 2000a). As highlighted in this report, it has been postulated that these signaling pathways appear to be able to explain most, if not all, relevant signaling pathways necessary for development. A review of the data shows that disrupting these processes (either by using genetically sensitized animal models or by using agents that cause developmental toxicity) can have devastating impacts on development (NRC 2000a). Interesting examples are the vinca alkaloids such as cyclopamine that can inhibit the sonic hedgehog signaling pathway (NRC 2000a). Many of the signaling pathways can exert overlapping functions, especially in mammalian species. This redundancy has contributed both to the plasticity of developing organs to develop normally if challenged, and has also emphasized the need to evaluate toxicological impacts in the broader context of in vivo development. For example, isolated reports that particular toxicants may impact apoptosis must be viewed in a
temporal and toxicokinetic and dynamic context to interpret the potential for overall impacts on in vivo development.

3.6.2. Nervous System

The central nervous system arises from a thickened area of the ectoderm called the neural plate on Day 19 in the human embryo. This process is referred to as induction. The neural plate then differentiates into the neural tube (providing the origins for the brain and spinal cord) and the neural crest (forming the basis of the peripheral nervous system). The process by which the neural tube arises from the neural plate is referred to as neurulation. To form the neural tube, the neural plate changes shape and forms a pronounced groove, closing from the cranial end to the caudal end. The neural tube has openings on both ends that close on about day 25 and day 27, respectively.

The neural tube provides the basis for the entire central nervous system. The spinal cord, a tubular structure in the mature nervous system, retains the basic original shape of the neural tube. Early in histogenesis, the walls of the neural tube are made up of neuroepithelial cells that constitute the ventricular zone. A zone known as the marginal zone develops into the white matter of the spinal cord. In the ventricular zone, some cells differentiate into neurons called neuroblasts. Primitive supporting cells called glioblasts also differentiate from the neuroepithelial cells of the ventricular zone. Some glioblasts become astrocytes and other glioblasts become oligodendrocytes. Neuroepithelial cells will ultimately give rise to all the neurons and microglial cells of the CNS. Many other important processes such as the formation of the spinal ganglia, spinal meninges and myelin sheaths take place in the developing spinal cord over time. Myelination begins between the fifth and sixth month of fetal development in the cervical portion of the spinal cord and continues until well into adolescence and young adulthood. Corticospinal tracts begin to myelinate immediately prior to birth and are not fully myelinated until the second or third year of life.

In contrast to the spinal cord, which retains the original shape of the neural tube, the brain undergoes a series of transformations that take place well into adolescence to reach the adult
form of the human brain. Starting at the cranial end, the neural tube begins to close during the 
fourth week of embryonic life and three brain primary vesicles are formed, the prosencephalon, 
the mesencephalon and the rhombencephalon by day 26. During the fifth week, the 
prosencephalon (forebrain) divides into the telencephalon (cerebral hemispheres) and the 
diencephalon (thalam, posterior lobe of the pituitary gland, pineal body, optic vesicles). At the 
same time, the rhombencephalon divides into the metencephalon (pons and cerebellum) and the 
myelencephalon (medulla oblongata). The end result of this developmental stage is the creation 
of five secondary brain vesicles that form the structural backdrop of the human brain.

During the fourth week of development, the brain is growing quickly and bends ventrally with 
the head fold to produce the midbrain flexure and the cervical flexure. The formation of the 
flexures allows for significant changes in the shape of the developing brain and the distribution 
of the gray and white matter. By day 30, rudimentary cerebral hemispheres are apparent in the 
embryo. Individual cerebral hemispheres grow in the shape of horseshoes, remaining in 
communication with the third ventricle in the diencephalon. As they expand, the hemispheres 
gradually cover the diencephalon, midbrain and the hindbrain and eventually meet in the 
midline. The corpus callosum, the largest cerebral commissure that connects the neocortical 
areas, is apparent by the 12th week and has reached its structural maturity by the 20th week of 
fetal development. Brain sulci (fissures) are present by the fifth month of prenatal development 
and are firmly in place at birth in human infants. In most animal species, with the exception of 
monkeys and the great apes, the brain hemispheres are smooth at birth and lack the sulci 
observed in newborn human infants. Because CNS development begins during the embryonic 
period, continues during the fetal period and post-natally, there are numerous periods of 
vulnerability, which are discussed in Section 4.3.1.

3.6.3. Reproductive System

The gonads are derived from the urogenital ridges, which are derivatives of the intermediate 
mesoderm. Also arising from the urogenital ridges are the Wolffian (male) and Mullerian 
(female) ducts, which are contained in the mesonephros. The embryonic germ cells migrate from 
the hindgut to the primitive, undifferentiated gonads. Male sex is determined by a Y 
chromosome genetically. Gonadal sex determination, i.e., decision whether a primordial gonad
differentiates into a testis or an ovary, is initiated by the activation of the SRY (sex determining region of the Y chromosome) gene located in the pseudoautosomal region of the short arm of the Y-chromosome. SRY gene expression starts at 41-44 days after ovulation, peaks at day 44 and continues at low levels thereafter (Hanley et al., 2000). SRY activation initiates the testis-determining cascade by the transformation of pre-Sertoli cells to Sertoli cells, and SOX9 (SRY related HMG BOX gene 9) is proposed to be a specific target of SRY (Bishop et al., 2000). The genetic pathways involved in gonadal development, include the roles of SRY, the transcription factor WT-1, the orphan nuclear receptors SF-1 and DAX-1, and SOX-9 (Park and Jameson, 2005). In the absence of SRY in females, the SOX9 gene remains silent and Sertoli cells are not formed. However, this lack of testicular differentiation is not enough for ovarian development. Active WNT-4 signaling is needed for normal ovarian development (Biason-Lauber et al., 2004; Vainio et al., 1999).

Hormones produced by the developing testis control differentiation of male genitalia. Ovaries remain hormonally inactive during development, and in the absence of male reproductive hormones female inner and outer genitalia are formed. Sertoli cells secrete anti-Müllerian hormone (AMH) during weeks 8-10 of gestation, resulting in the regression of Müllerian ducts. In the absence of AMH (i.e. absence of the testis), Müllerian structures differentiate to oviducts, uterus and the upper part of the vagina. The testicular hormone, testosterone is needed to stimulate Wolffian ducts to differentiate into the vas deferens, epididymis, and seminal vesicle. Leydig cells in the testis secrete testosterone and insulin-like hormone 3 (INSL3), that are needed for testicular descent. In the absence of androgens and INSL3, Wolffian ducts regress in the female and the ovaries remain in the abdomen.

Testosterone is converted to dihydrottestosterone (DHT) by 5α-reductase type II enzyme in the prostate and outer genitalia. DHT is necessary for normal development of the scrotum, penis and prostate (Wilson et al., 1993). In the absence of DHT, female-type external genitalia develop and the prostate remains rudimentary. Testicular testosterone production is dependent first on placental secretion of human chorionic gonadotropin (hCG) and increasingly on pituitary secretion of luteinizing hormone (LH). Another gonadotropin, follicle stimulating hormone
(FSH) stimulates Sertoli cell proliferation in the testis and folliculogenesis in the ovary. Both
gonadotropins are stimulated by hypothalamic gonadotropin releasing hormone (GnRH).
Estrogens do not seem to play an essential role in sexual differentiation, since normal
development is possible in the absence of estrogen receptors (Couse and Korach 1999; Smith et
al., 1994) and the aromatase enzyme that is needed for conversion of androgens to estrogens
(Morishima et al., 1995). However, excess estrogens can inhibit INSL3 activity and thereby
contribute to cryptorchidism (Emmen et al., 2000; Nef et al., 2000). Imbalance in the
androgen/estrogen ratio has been suggested to be a reason for testicular disruption during
development (Sharpe, 2003).

Although the precise mechanisms for initiating puberty remain unclear, it is under CNS control
and involves complex neuroendocrine interactions. In infancy, serum concentrations of
gonadotropins and sex hormones are similar to those of children with normal puberty, but in the
months and years after birth, hypothalamic-pituitary-gonadal activity decreases; this period is
known as the juvenile pause (Styne, 2003). In primates, when the time is right (probably due to
size and internal clock), the KISS-1 gene activates production of the peptide hormone kisspeptin,
which initiates the release of the puberty-inducing hormone gonadotropin releasing hormone
(GnRH). GnRH in turn stimulates the pituitary to release the gonadotropin hormones, triggering
changes in the ovaries and testes (Shahab et al., 2005). Windows of vulnerability in
reproductive system development is discussed in Section 4.3.2.

3.6.4. Endocrine System

The primary purpose of the endocrine system is to maintain homeostasis, that is, to maintain a
relatively constant internal environment in the face of a constantly changing external
environment. The endocrine system consists of hormones and the glands and tissues that
produce the hormones. A hormone is a chemical substance released by certain cells to affect the
function of other distant cells (endocrine function). Many compounds act as endocrine hormones
as well as having paracrine and autocrine functions. Paracrine and autocrine describe actions on
nearby cells and on other cells that produces the substance, respectively. There is considerable
overlap between substances classified as hormones and other chemical messengers such as
neurotransmitters and cytokines. Many substances function in more than one of these categories. For example, epinephrine and norepinephrine function as both neurotransmitters and as adrenal medullary hormones.

Hypothalamic-pituitary axis: The endocrine system can be broadly divided into the hormones of the hypothalamic-pituitary axes and the glands and target organs they regulate and other endocrine hormones and glands that are not part of these axes (Figure 3.4). The hypothalamus regulates the hormones of the anterior pituitary gland by secreting releasing hormones (gonadotropin releasing hormone, thyrotropin releasing hormone, corticotropin releasing hormone, somatotropin/growth hormone releasing hormone) or inhibiting hormones (dopamine, somatostatin) into the portal circulation. These hormones act on specialized groups of cells in the anterior pituitary gland to stimulate or inhibit the secretion of other hormones. Figure 3.4 also illustrates the central concept of negative feedback, whereby a hormone regulates the secretion of another hormone, which in turn feeds back to inhibit the secretion of the first hormone. This maintains the levels of both hormones within a narrow range.

The hypothalamus regulates the hormones of the posterior pituitary gland via direct neural connections. Thus, hypothalamic neuroendocrine magnicellular neurons terminate in the posterior pituitary where they secrete oxytocin and vasopressin into the general circulation. Other endocrine organs that are not part of the hypothalamic-pituitary system include the islet cells of the pancreas, which secrete insulin, glucagon, and somatostatin; the parathyroid glands, which secrete parathyroid hormone; the parafollicular cells of the thyroid, which secrete calcitonin; the pineal gland, which secretes melatonin; and the gut, which produces several hormones including gastrin, secretin, and cholecystokinin.

The anterior and posterior lobes of the pituitary gland have different embryonic origins, with the anterior pituitary being of oral ectodermal origin and the posterior pituitary of neural ectodermal origin. The anterior pituitary develops from an upgrowth of the roof of the primitive mouth, called Rathke’s pouch. The posterior pituitary develops from a downward growth of the neural ectoderm of the floor of the diencephalon or forebrain. As these two outgrowths converge, the stalk joining Rathke’s pouch with the mouth regresses, while the stalk from the diencephalon
remains to form part of the infundibulum or pituitary stalk. The other part of the infundibulum arises from the upgrowth of Rathke’s pouch.

The pituitary gland begins to synthesize and secrete hormones during weeks 8 to 12 of gestation in humans (Porterfield and Hendry, 1998; Marty et al., 2003; Sadler, 2000). The target organs of the pituitary gland hormones are depicted in Figure 3.5. The hypothalamic-pituitary thyroid and hypothalamic-pituitary-gonadal axes begin to function during fetal life around week 12; gestation, however, and complete maturation of some of these target organs does not occur until after birth (e.g., gonads, adrenals). The anterior pituitary hormone prolactin begins to be secreted at 11 weeks gestation in humans, The most well-known function of prolactin is its indispensability for milk production by the mammary glands; however, prolactin also plays important roles in modulating immune function and in the development of the dopaminergic tuberoinfundibular neurons (Shyr et al., 1986; Ben-Jonathan et al., 1996).

Thyroid Gland: The genetic pathways involved in thyroid development have been recently reviewed (Jahnke et al., 2004). Thyroid hormone is critical for normal CNS development (Porterfield and Hendry, 1998; Sher et al., 1998). Thyroid hormone regulates cellular proliferation within the developing CNS. It also regulates cytoskeletal and microtubular assembly and stability, which are important for cellular migration and neuronal outgrowth. It regulates the expression of genes that are critical for synaptic development, neuronal growth, and myelination. During the embryonic period and the early fetal period, the developing human is entirely dependent on maternal thyroid hormone. The fetal thyroid begins to function during week 12 of pregnancy, but the maternal thyroid gland contributes thyroid hormone throughout gestation. In both humans and rodents full maturation of thyroid system function does not occur until 4 weeks after birth (Jahnke et al., 2004). Thus, the effects of congenital hypothyroidism are partially counteracted in utero by maternal thyroid hormone; however, after birth it is critical to identify these infants so that thyroid hormone replacement can begin as quickly as possible. Thyroid hormones are also involved in development of the male reproductive system in humans and rodents by promoting Sertoli cell differentiation (Jahnke et al., 2003).
Figure 3.5  Half-life results for 40 substrates in children relative to adults. A ratio of 1 indicates that the half-lives in adults and children are the same. Asterisks mean that data are significantly different from adults. Reprinted from Ginsberg et al., (2002) with permission from Oxford University Press.

Adrenal glands: Histologically, the human adrenals comprise an outer cortex, the site of steroid hormone synthesis, and an inner medulla, the site of catecholamine synthesis. The steroidogenic tissue arises from coelomic mesoderm in the genital ridge of the embryo. In both humans and mice, the fetal adrenal cortex contains a definitive or adult outer zone that surrounds a fetal zone. The apoptotic degeneration of the fetal zone occurs at puberty in male mice and after the first pregnancy in female mice. The definitive cortex is itself composed of four zones that synthesize different hormones. The outer zona glomerulosa synthesizes the mineralocorticoid aldosterone.
Next comes the zona intermedia, which does not appear to synthesize hormones, followed by the zona fasciculata and zona reticularis which synthesize glucocorticoids (primarily cortisol in humans). The catecholaminergic cells arise from the neural crest and migrate into the developing cortex, forming the medulla. The nuclear receptor/transcription factor steroidogenic factor 1 (SF-1), which was discussed above as being critical for gonadal development, is also necessary for adrenal gland development (Bland et al., 2003).

The adrenal glands play an important role in pubertal development. Termed adrenarche, the maturation of a prominent zona reticularis, the innermost layer of the cortex, begins around age 6 to 8 in girls, resulting in increased secretion of the adrenal androgens, dehydroepiandrosterone (DHEA) and DHEA sulfate (Beckman and Feuston, 2003). The rise in these hormones leads to the development of pubic and axillary hair. Recent evidence suggests that premature adrenarche may be associated with subsequent development of polycystic ovarian syndrome and the related Syndrome X/ Metabolic Syndrome, characterized by obesity, insulin resistance, dyslipidemia, and high blood pressure (Ibanez et al. 2000).

Gonads: Gonadal development and the hormones of the reproductive system are discussed in in Section 3.6.3.

Somatotropin (Growth Hormone), Calcium Homeostasis and Bone Development: During the first two months of embryonic life, there is extensive differentiation of progenitor cells, without rapid cell replication. Thereafter, in the fetal period, the highest growth rates of these cells are observed. Growth slows in late gestation and continues to decline in childhood. The high growth rate of the fetus compared with the child is mostly the result of cell replication; the proportion of cells which are dividing becomes progressively less as the fetus becomes older. Insulin-like growth factors (IGFs, also called somatomedins) are the primary factors that drive intrauterine growth. During gestation, growth hormone receptors are expressed at very low levels, and growth hormone is not the primary regulator of IGFs. Instead, fetus insulin, which in turn is regulated by fetus glucose levels, is the primary regulator of IGF-1 (Gluckman and Pinal, 2003). This pattern of regulation of IGF-1 continues until about 6 months after birth, by which time growth hormone receptor populations have increased and growth hormone regulation of
IGF-1 takes over (Gluckman and Pinal, 2003). IGFs cause growth of the epiphysial regions of the long bones by stimulating the proliferation of chondrocytes, the cartilage producing cells.

Pancreas: The endocrine islet cells comprise only 1 to 2% of the pancreatic tissue. They synthesize the hormones insulin, glucagon, somatostatin, and pancreatic polypeptide. Insulin and glucagon maintain glucose homeostasis via their actions on lipid, carbohydrate, and protein metabolism. The pancreas originates from two patches of epithelium in the duodenum during the fifth week of gestation in humans. The endocrine pancreatic cells begin to differentiate very soon after the pancreas begins to bud (Murtaugh and Melton, 2003). These endocrine cells then delaminate from the epithelium and aggregate into islets. The transcription factors, Sox17, Pdx1, Hlxb9, and Ptf1a are known to be essential for normal pancreatic development based on knockout mouse models (Murtaugh and Melton, 2003). It is during the second half of gestation that the endocrine cells begin to differentiate into the specialized cell types containing a single hormone (Hellerstrom and Swenne, 1991). By term, the islets have the appearance of adult tissue, but there are still considerable changes in size and arrangement of the islets for four or more years in humans. Specific vulnerable windows of development for the various endocrine components are discussed in section 4.3.3.

3.6.5. Cardiovascular System

The formation of the heart is one of the earliest events in development, as it is essential for the delivery of oxygen and nutrients to the rapidly developing cells of the embryo. The molecular decision to form cardiac cells is made at the time of gastrulation. The heart begins to beat at 3 weeks of embryonic age. Important elements of cardiac formation include formation of the heart forming fields as cells migrate out of the primitive streak, the segregation of cell lineage (myocardial and endocardial) within the fields; the elongation and segmentation of the tubular heart, the internal differentiation/septation of first the atria, and later the ventricle, and development of the conducting system. The heart also descends as it is developing, starting cephalic to the somites and winding up at the mid-thoracic level. All this development takes place while the heart is performing a critical function to the rest of the developing embryo (O’Rahilly and Muller, 1992; Markwald et al., 1997). Molecular control factors for cardiac
morphogenesis are being elucidated, including T-box transcription factors (Stennard and Harvey, 2005), homeobox transcription factors (Akawaza and Komoro, 2005); and growth factors and extracellular remodeling (Corda et al., 2000).

3.6.6. Immune System

The immune and hematopoietic systems arise from pleuripotent stem cells (West, 2002; Holsapple et al., 2003). As gestational development proceeds, these give rise to hematopoietic stem cells that produce the array of hematopoietic and immune cell populations. In humans, many of the critical steps in formation of specific lineages within immune development occur during the first and second trimesters of pregnancy. Lymphocyte progenitors are present in the liver by weeks 7-8 of gestation (Migliaccio et al., 1986). Holsapple et al. (2003) discuss the evidence indicating that lineage specific progenitors are at potential risk by 7-10 weeks post conception. Around this same window, the thymus stroma forms and T cell progenitors migrate from the liver to the thymus. Also, in the first trimester, B-cell progenitors appear in the blood, and the gastrointestinal tract associated lymphoid tissues emerge. This might be considered an early window of vulnerability. West (2002) discusses lymphogenesis within the human bone marrow that emerges within this same window of vulnerability. In general, early B and T cell differentiation seem to occur in parallel (Holsapple et al, 2003). The thymic medulla and cortex areas begin to differentiate during the first trimester, and the areas are fully formed with maturational cell migration from the cortex to the medulla in the subsequent trimester (West, 2002).

In rodents, immune development is relatively delayed compared with that of humans; some rodent postnatal events occur prenatally in humans (West, 2002 and Landreth, 2002). Human T cells can respond to several mitogenic and allogeneic stimulatory challenges prenatally while significant responses in rodents usually appear after birth. However, despite the extent of prenatal human immune development, West (2002) has identified several areas of continued immune development that occur during the early neonatal periods. The author points to the fact that, at birth, the human has a relatively low proportion of T cells, reduced myeloid and natural killer (NK) lineages both in numbers of cells and cytokine activation potential, and reduced
development of plasma cells in the bone marrow. Increased susceptibility to some infectious agents may be linked to the relatively incomplete maturation and/or expansion of some immune cell lineages and the lack of a complete cytokine network (West, 2002). Further discussion of periods of vulnerability during immune system development can be found in Section 4.3.4.

3.6.7. Respiratory System

Development of the human lung begins in the embryo and continues until the age of 18-20 years. Cellular differentiation and formation of the primary lung structures occur in stages during fetus development, but the majority of growth and maturation of the lung occurs postnatally through the processes of branching morphogenesis and alveolarisation. The major antenatal and postnatal developmental milestones are summarized in Table 3.2.

The lung originates in the embryo as an out pouching of ventral foregut endoderm that grows into the surrounding mesenchyme tissue. The pseudo glandular period (week 5-17 of gestation) is a critical stage of cellular differentiation and is characterized by the formation of the bronchial tree and pulmonary vasculature. The development of the pre-acinar conducting airways is regulated by interactions between epithelium and mesenchyme tissue at the site of bronchial buds, and by 16 weeks of gestation the branching pattern of the bronchial tree is complete. During the canalicular phase (week 16-24 of gestation) the gaseous exchange regions evolve with adjacent capillary beds. Surfactant synthesis begins in the latter stages. Extracellular matrix components and acini form in the saccular phase, with alveolar formation beginning around 26 weeks gestation and continuing postnatally until approximately 2 years of age. The precise age at which alveolarisation ceases is not known due to the small number of normal lungs studied and the wide variability in the reported adult number of alveoli. Branching morphogenesis is characterized by enlargement of the airways and alveoli and continues well into adolescence. Up to 80% percent of alveoli develop postnatally in parallel with the growth of the lungs and with the increasing metabolic demands of the growing child.
Table 3.2: Normal development of the lungs in humans

Prenatal

- Airway branching to terminal bronchioles complete by 16w
- Functional smooth muscle by 8-10w, to respiratory bronchioles by 26w
- Cartilage complete by 28w
- Blood vessels complete by 17w
- Lamellar bodies in Type II cells by 24w
- 30-50% of alveoli present by term

Postnatal

- Increase in airway size parallels somatic growth
- Rapid increase in smooth muscle early
- Alveolarisation continues until at least 2y
- Maturation of microvasculature during the postnatal phase of alveolar development
- Lung volume approximately doubles from birth to 18 months and again to 5 y
- Lung growth continues until approximately 18 years in females and 20-23 y in males.

Many of the studies on the effects of chemical exposures on the growth and development of the lungs have been performed in animals, especially rodents. However, the pattern of lung development differs between animals and humans. For example, alveolarisation occurs exclusively postnatally in rats (Massaro et al., 1984; 1986) and almost completely prenatally in sheep (Davies et al., 1988). Early lung development in rabbits is similar to humans (Kovar et al., 2002) but alveolarisation continues until adult life. Because of these differences, extreme care must be taken when extrapolating the results from animal studies to human situations. The molecular development of the lung has been reviewed (Pongracz and Stockley, 2006; Bridges and Weaver, 2006; Kumar et al., 2005). Periods of vulnerability during respiratory system development are discussed further in section 4.3.5.

3.6.8. Kidney

Development of the metanephric kidney begins with an outgrowth of the ureteric bud from the distal region of the mesonephric (Wolfian) duct. The ureteric bud must grow into the mesenchyme of the nephrogenic cord. Upon contact the mesenchyme epithelializes to form a nephron, and this process is repeated over and over as the ureteric bud branches. Ultimately, the
branching of the ureteric bud results in the formation of the major and minor calyces (the large
ducts that empty into the renal pelvis) and the system of collecting tubules. The two major
calyces form from the first branching of the ureteric bud, around the end of the sixth week in
humans. Secondary branches arise from these, which in turn give rise to tertiary branches.
About twelve generations of branching occur by the end of the fifth month, which essentially
completes the formation of the collecting duct system.

Although the general pattern of nephron formation is similar across mammals, there are marked
species differences in the timing of development. The onset of nephron development starts at
approximately the same stage of embryogenesis in all species that have been evaluated, but
because of differences in the length of the embryonic period, the day of gestation differs. In
humans, metanephric kidney developments starts around gestation day 35 while in the rat it starts
on gestation day 12, and in the mouse, gestation day 11. Induction and differentiation of
nephrons occurs continuously through the 38th week of gestation in humans and for 10-12 days
postnatally in rats and mice. There are approximately 1.5 million nephrons per kidney in
humans, and 1000-2000 in mice. Zoetis and Hurtt (2003) have published a recent review on
comparative aspects of kidney development. The molecular development of the kidney and
subsequent implications for altered renal development has been the subject of a recent review
(Ruan et al, 2005).

The renin-angiotensin system is essential for normal renal development (Guron and Friberg,
2000). In late gestation, rat expression of angiotensinogen is higher than in the adult and is
localised in the proximal tubules (Niimura et al. 1997). Renin is also expressed at a higher level
during late gestation and again the localisation is different from the adult animal; instead of
being expressed in the juxtaglomerular cells as in adults, it is found in renal arteries in the fetus.
The distribution and type of angiotensin II (AT₂) receptors is altered in the fetus and shows a
characteristic spatio-temporal pattern, with AT₂ receptors showing dominance. This reverses
after birth. The AT₂ receptors are primarily located in the undifferentiated mesenchyme within the
nephrogenic zone. Blockade of this system either pharmacologically or by using gene knockouts
leads to extensive renal vascular abnormalities (Tufro-McReddie et al. 1994). Nephrogenesis is
complete prior to birth in the human although there is still substantial growth and maturation of
function that occurs post-natally. The periods of vulnerability of the kidney during development can be found in section 4.3.6.

3.7. Conclusions

The information presented in this chapter illustrates the complex role and interplay of molecular and physiological factors in the functional and structural development of the various organ systems. These factors ultimately influence the toxicokinetics and toxicodynamics of chemicals. The developing organs are particularly vulnerable to toxic insult, given the increased rate of cell division and immaturity of functional systems. The age at which specific organs undergo their most rapid rate of development and the age at which development is completed have major implications for the susceptibility of growing animals. For example, toxicity that is dependent on rates of cell proliferation (DNA/RNA replication and protein synthesis) might affect different tissues to different degrees at various stages of development. Additionally, the fact that a number of factors determining absorption, distribution, metabolism, and excretion of chemicals change as a function of age signifies that the target tissue dose in children may differ from adults for a given exposure situation. Furthermore, the individual toxicokinetic processes and determinants may change in different ways within each developmental stage, and this information must be considered in toto to understand the net impact on the internal dose in children. In this regard, physiology-based models are likely to be useful tools. For developing physiology-based toxicokinetic models in neonates, however, liver blood flow rate data are lacking. Such information is critical to the better understanding of the metabolism and clearance of xenobiotics by the liver. Additionally, in neonates, the data on cofactors involved in phase II reactions (e.g., glucuronide, sulfate) are required.

Limited data is available on male-female differences in physiological data for the various age groups of children. Most of the current data were obtained in boys with the exception of a few physiological parameters (e.g., cardiac output, breathing rate, fat volumes). Such gender-specific data should facilitate the construction of biologically-based models for simulating uptake and deposition of inhaled gases and particulates in children. There is also a need for data on the expression, development and maturation of various transporters that play a critical role in the
cellular flux of chemicals in children of various age groups. Finally, the availability of data on age- and tissue-specific rates of cell proliferation and molecular events in organ development would be useful for constructing biologically-based models for the conduct of child-specific risk assessments.
CHAPTER 4 DEVELOPMENTAL STAGE SPECIFIC SUSCEPTIBILITIES AND
OUTCOMES IN CHILDREN

4.1 Introduction

In Chapter 3, the structural and functional features that potentially convey special susceptibility
of children to environmental exposures were detailed for a number of key organ systems. In this
chapter, we highlight the impacts of exposure to environmental agents on those organ systems as
a function of life stage. The overview is not meant to be an exhaustive review of the literature,
but rather illustrative of the potential outcomes. Each of the organ systems discussed here
presents different periods of vulnerability during critical windows in their development. It is
clear that the timing of exposure to chemicals or other insults is critical in determining the
consequences to children’s health. Because of the differing windows of vulnerability, the same
dose of the same chemical during different periods of development can have very different
consequences. Structural malformations often arise as a result of exposures during the
embryonic period when organs are beginning to differentiate. Hence, historically the focus of
concern has been on environmental factors that result in adverse pregnancy outcomes such as
foetal loss, intrauterine growth restriction, or birth defects. However, even after the basic
structure of an organ has been established, disruption of processes like growth and cell migration
can have lifelong consequences on the function of key organ systems. It is also important to bear
in mind when assessing the vulnerabilities of children that there are similarities and differences
in the timing of these periods between humans and commonly used laboratory animal species.
For example, as documented in Chapter 3, some developmental events that occur pre-natally in
humans occur after birth in rodents. Table 4.1 provides examples of the types of outcomes and
critical life stages for organ systems that are influenced by environmental factors. Details of the
susceptibilities identified in Table 4.1 are covered in greater detail throughout this chapter. To a
large extent, the review has focused on what is known about the effects of chemicals on human
development, but where there was good supporting evidence from animal models, or where
particularly illustrative information was obtained solely from animal models; such information
was included in the chapter.
Table 4.1  Examples of adverse effects of developmental stage-specific exposures on various organ systems*:

<table>
<thead>
<tr>
<th>Period of vulnerability for exposure</th>
<th>Neuro</th>
<th>Repro</th>
<th>Renal</th>
<th>Endocrine</th>
<th>Cardiac</th>
<th>Immune</th>
<th>Respiratory</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-conception</td>
<td>Periconceptional use of folate acid supplements decreases rate of neural tube defects (Bailey et al, 2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preimplantation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Exposure of male mice to x-rays or urethane causes cancer in their offspring (Anderson et al, 2000)</td>
</tr>
</tbody>
</table>

* This table is not intended as a comprehensive review. Only selected examples are provided.
<table>
<thead>
<tr>
<th>Foetus</th>
<th>Decreased intelligence, increased behavioral problems with lead (Rice, 1996; Bellinger et al., 1994)</th>
<th>Exposure to several phthalates induces reduced AGD and malformations in male rats (Mylcheest et al, 1999; Gray et al, 2000)</th>
<th>ACE fetopathy with neonatal renal failure from maternal exposure to angiotensin inhibitors (Tabacova et al, 2003)</th>
<th>Maternal smoking causes decreased birth weight and increased risk for later diabetes (Montgomery and Ekborn, 2002) and osteoporosis (Cooper et al, 2002). Decreased T3/T4 levels in infant and juvenile rats (Brouwer et al, 1998) exposed to PCBs</th>
<th>Decreased cell mediated immunity in children exposed prenatally to MeHG (Grandjean et al, 2004)</th>
<th>Decreased HR variability in children exposed prenatally to MeHG (Grandjean et al, 2004)</th>
<th>Altered airway growth with increased collagen deposition if airway walls with exposure to maternal smoking (Sekhon et al, 2001). Altered control of breathing with diminished hypoxic response postnatally with maternal smoking in utero (Ueda et al, 1999)</th>
<th>Inorganic arsenic in drinking water caused adrenal tumors in male offspring and ovarian and lung in female offspring as adults (Waalkes et al, 2003).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td></td>
<td>Hydronephrosis with dioxin exposure during neonatal and infantile periods in rats (Birnbaum, 1995)</td>
<td></td>
<td></td>
<td></td>
<td>Increased incidence of respiratory mortality following exposure to particulates in the air [Glinianaia et al., 2004 #882]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant</td>
<td>Exposure of juvenile mice to pesticides caused Parkinson-like declines in dopaminergic neurons in adulthood (Cory-Slecta et al, 2005)</td>
<td>Maternal grooming affects ability to respond to stress in adulthood in rats (Gilbert 2005)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Exposures to chemicals during early life stages can result in adverse effects during the stage when exposure occurred or may not manifest themselves until later stages. Depending on the dose of the chemical and the susceptibility during that life stage to the mode of action of the chemical, effects can range in severity from functional deficits to growth restriction to malformations to mortality. Mortality, growth restriction, and birth defects will be discussed in this section, and functional deficits in particular organ systems will be discussed in subsequent sections.

4.2 Mortality, Growth Restriction, and Birth Defects

4.2.1. Mortality

Because there is currently no sensitive and specific biomarker of conception in humans, the prevalence of preimplantation embryonic mortality is not accurately known; however, studies of preimplantation pregnancy losses after in vitro fertilization suggest that as many as 50% of conceptions are followed by death of the embryo prior to implantation (Mesrogli and Dieterle, 1993). It is often stated that exposures occurring between conception and implantation result in either death of the embryo or result in no effects. However, experiments in rats have shown that exposures to mutagens like ethyl methanesulfonate, 5-azacytidine, and methyl nitrosourea during the preimplantation period can also cause malformations (Rutledge and Generoso, 1998). At higher doses these agents cause death prior to or around the time of implantation (Rutledge and Generoso, 1998).

In contrast to preimplantation mortality, more exact estimates of the incidence of spontaneous abortions (miscarriages occurring prior to 20 weeks gestation) have been possible with the development of sensitive assays for human chorionic gonadotropin (hCG), which begins to be secreted by the conceptus at implantation. These studies show that about one-third of post-implantation pregnancies end in spontaneous abortion. Of these, about two-thirds occur prior to the recognition of pregnancy (Wilcox et al., 1988). Occupational exposure of the mother to a variety of agents during pregnancy has been associated with spontaneous abortion in
epidemiological studies. For example, maternal exposure to organic solvents has been associated with spontaneous abortion in several studies (Pastides et al., 1988; Taskinen et al., 1989; Lindbohm et al., 1990; Windham et al., 1991; Lipscomb et al., 1991; Schenker et al., 1995). However, a meta-analysis of five of these studies resulted in a non-significant odds ratio (McMartin et al., 1998). Two recently published prospective pregnancy studies reported an increased risk of pregnancy loss associated with serum DDT/DDE levels. Risk of having had a prior pregnancy loss (or loss occurring prior to the index pregnancy) was higher for women with higher serum DDE concentrations upon enrollment into a prospective pregnancy study and among women recruited preconceptionally who were prospectively followed through pregnancy (Longnecker et al., 2005 and Venners et al., 2005). Longnecker et al. (2005) reported a significant increased risk of prior foetal loss associated with a 60 microg/L increase in serum DDE (OR=1.4; 95% CI 1.1-1.6). An increase of 10-ng/g serum DDT significantly increased the risk of early pregnancy loss as measured by hCG assays (Venners et al., 2005). In fact, a positive monotonic exposure response association was observed between preconception serum total DDT and risk of early pregnancy loss. However, this pattern was not seen among women with later clinically recognized pregnancy losses underscoring the importance of capturing early pregnancies confirmed by hCG assays.

Stillbirths (death of the foetus after 20 weeks gestation) are much less common than spontaneous abortions, occurring in about 7 per 1000 pregnancies in the US (NCHS, 1997). About 5 of 1000 live-born babies die annually during the neonatal period and about 3 per 1000 die annually during the post-neonatal period in the US. Rates of neonatal and infant mortality vary widely between and within countries with some countries reporting rates in excess of 100 per 1000 (Kramer, 2003). Maternal smoking during pregnancy increases the risks of pregnancy loss, stillbirth, and infant mortality (Platt et al., 2004).

The potential for exposure to pollutants to increase mortality in children has been recognized since the 1950s (Ministry of Public Health, London, 1952). Several studies have investigated the impact of air pollution on neonatal (birth to 28 days) and infant (28 days to one year) mortality (Bobak and Leon, 1999a; Dejmek et al., 2000; Ha et al., 2003; Glinianaia et al., 2004). While exposure to particulate air pollution is not consistently associated with increased neonatal or
infant mortality in general, it is associated with increased mortality from respiratory causes, such as pneumonia and Sudden Infant Death Syndrome (Glinianaia et al., 2004). There is also sufficient evidence for a causal relationship between environmental tobacco smoke (ETS) exposure and Sudden Infant Death Syndrome (Anderson and Cook, 1997), with the risk of death from SIDS increased by up to 150% by exposure to both pre and post-natal ETS (NHMRC, 1997). Increased respiratory mortality in children under 5 years of age associated with NO₂ levels originating from diesel exhaust emissions has been suggested, but little attention was paid to potential confounders (Salvada et al., 1994).

Embryonic or foetal mortality can also lead to altered sex ratio at birth if one sex is more susceptible to the exposure than the other (Taylor et al., 2006). Sex ratios (ratio of male to female live births) for angler populations have received some study given their higher potential for exposure stemming from consumption of contaminated sport fish (Faulk et al., 1999). A reduced sex ratio was reported among Swedish anglers’ wives who lived on the Baltic Sea when compared with the wives who lived near the less contaminated Swedish west coast (Rylander et al., 1995). Equivocal results exist in relation to parental serum PCB concentrations among angler populations. Among mothers in the highest PCB quintile compared to the lowest quintile, a reduced sex ratio (fewer males) was observed, though no relation was observed for paternal exposure, suggesting a maternally mediated effect (Weisskopf et al., 2003). Conversely, a higher sex ratio or male excess was observed in a sample of Michigan anglers and their spouses for paternal but not maternal PCB exposure (Karmaus et al., 2002). Paternal consumption of cooking oil contaminated with PCBs, polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-p-dioxins (PCDDs) was reported to be associated with a significantly lower odds of having a male infant in Taiwan (del Rio Gomez et al., 2002). After an accidental release of dioxin in Seveso, Italy, exposed fathers under the age of 19 sired significantly more girls than boys compared to those fathers who were unexposed (Mocarelli et al., 2000). No significant change in the sex ratio was observed in Fukuoka and Nagasaki, Japan, following accidental contamination of rice oil with dioxin-like compounds (Yoshimura et al., 2001). Using a causality algorithm, Jarrell (2002) concluded that dioxin, dibromochloropropane and hexachlorobenzene reduce the number of male births.
The possibility that early life exposures can lead to increased mortality rates in later childhood and during adulthood, will be discussed in subsequent sections that deal with the influence of early life exposures on the risk for developing diseases later in life (e.g., diabetes and heart disease).

4.2.2. Growth restriction

Effects of pre-natal chemical exposure on growth have most commonly been measured as changes in birth weight. Low birth weight is defined as less than 2500 g at birth; however, birth weight is a continuous variable and many exposures that affect birth weight do not necessarily result in low birth weight. Therefore, recent studies have emphasized the concept of diminished birth size, which can be assessed via several endpoints (weight, length, head and abdominal circumference). Preterm birth is defined as birth prior to 37 weeks gestation. Because birth weight is highly dependent on gestational age, another measure, intrauterine growth restriction (IUGR, also known as small for gestational age) has been defined as weighing less than the 10th percentile of weight-for-gestational age standards for a given population. Maternal smoking is clearly associated with about a two-fold increase in low birth weight and IUGR in multiple studies (Wang et al., 2002; Bosley et al., 1981). Wang et al. (2002) showed that there is an interaction of maternal smoking with maternal polymorphisms in glutathione-S-transferase T1 and cytochrome P450 1A1. Women who smoke during pregnancy and who have one or both of these polymorphisms are at an even greater risk of having a low birth weight baby than women who smoke and do not have these specific polymorphisms. Maternal exposure to environmental tobacco smoke (ETS) is associated with smaller decreases in birth weight than is maternal smoking. In a meta-analysis of 19 studies (Windham et al., 1999). It was estimated that exposure to ETS lowers birth weight an average of 31 grams. Maternal smoking is thought to be the single most important factor for determining birth weight in developed countries (DiFranza et al., 2004). Smoking may induce growth restriction via at least two mechanisms: 1) by lowering maternal uterine blood flow from the uterus to the placenta, or 2) by raising maternal and foetal carboxyhemoglobin levels (DiFranza et al., 2004).
Epidemiological studies have also linked exposure to various components of air pollution to growth restriction. An overview and analysis of these studies was provided by Sram et al., 2005. Exposure to particulate matter less than 10 microns in diameter or less than 2.5 microns in diameter during pregnancy was significantly associated with low birth rate at term and IUGR (Dejmek et al., 1999; Bobak and Leon, 1999a; Bobak et al., 2001; Jedrychowski et al., 2004). Other components of air pollution, including carbon monoxide (Ritz et al., 2000; 2002), sulfur dioxide (Bobak and Leon, 1999b), and polycyclic aromatic hydrocarbons (Perera et al., 1998; Djemek et al., 2005) have also been associated with low birth weight or IUGR. At this time it is not clear which, if any, of the components of air pollution cause the observed decreases in embryonic and/or foetal growth.

Reduced birth weight has also been associated with pre-natal exposure to persistent organochlorine compounds (Rylander et al., 1995). In a cohort of girls exposed pre-natally to polychlorinated biphenyls (PCBs) and polybromined biphenyls (PBBs), PCB exposure, but not PBB exposure, above 5 parts per billion was associated with reduced weight adjusted for height at 5 to 24 years of age. Mothers with PCB levels above the median value had daughters whose current adjusted weights were 11 pounds lower than for daughters whose mothers had levels below the median. This study provides evidence that pre-natal exposure to PCBs may affect child growth (Blanck et al, 2002).

4.2.3 Birth Defects (Structural Malformations)

Development of the child during pregnancy is a complex biological process, as organs and tissues develop from the union of an egg and a sperm that must differentiate in a perfectly organized and well-timed sequence. During that process there may be divergences from the normal development that result in a high rate of spontaneous abortions and stillbirths as were discussed above.

Of liveborn children, an estimated 8 million children are born with major birth defects annually. Additional anomalies will be diagnosed in about 3% of the children up to the age of 7 years. These anomalies will include mental retardation, neurological impairment and a variety of morphologic and functional defects in different organs. Although the prevalence of major malformations detected at birth
is about 3% of live birth, these malformation are responsible for about 20% of the infant mortality rate and the majority of pediatric hospitalizations. Birth defects therefore have a tremendous impact on society and are the major cause of mortality (Lynberg and Khoury, 1990).

A birth defect, a synonym for the clinical term "congenital anomaly," is defined as an anatomical and/or functional defect resulting from disturbance of normal developmental processes. Since this definition includes a wide range of defects, from a visualized structural defect such as spina-bifida to microscopic and metabolic defects such as phenylketonuria. Terms such as malformation, disruption, deformation and sequence have been utilized to describe various manifestations (Jones, 1988).

Distinguishing between the above-mentioned categories is of clinical importance for the prognosis and risk evaluation of the pregnancy outcome and of the newborn. In view of the fact that most of the birth defects are considered to be a “malformation” (Martinez-Frias et al., 2000) the more specific clinical terms "major" and "minor" malformations were established. Major congenital malformations represent a status of a newborn that requires significant medical or surgical care due to an abnormality in an essential anatomical structure; minor malformations are less threatening to health and need less medical interference. Examples for major malformations include congenital heart disease, neural tube defects and cleft lip/cleft palate. Nail hypoplasia, auricular deformities and broad nasal bridge are examples of minor malformations. Umbilical and inguinal hernia are examples of anomalies which may be defined into each of these categories depending on the severity. Most surveillance programs focus on major malformations, thus limited data is available on the incidence of minor malformations.

4.2.3.1 Etiology

Research suggests that about 15-25% of all birth defects can be attributed to genetic background, 4% to maternal conditions, 3 % to maternal infections, 1-2% to deformations, <1% to chemicals and other environmental influences and 65% to unknown etiologies (Brent and Beckman, 1990). Yet, knowing that embryonic development is an outcome of the combination of intrinsic hereditary factors and the surrounding environmental influence, the etiology of most birth defects is likely to be the consequence of both independent or synergistic environmental and genetic factors. Since the exposure to
environmental factors (as opposed to genetic factors) can be altered or prevented, studies on the role of environmental factors is important in spite of the low direct attributable risk.

In humans and in experimental animal models, different environmental factors and chemical agents have been show to affect every-stage of embryonic and foetal development and therefore a wide range of defects have been observed. For example, excess amounts of retinoic acid may affect several developmental processes via interaction with retinoic acid receptors; the outcomes resulting from excess amounts of retinoic acid in experimental animals have been shown to highly stage specific (Shenefelt, 1972; Matt et al., 2003). In humans, inadequate periconceptual folic acid intake was shown to be responsible for a large percentage of neural tube defects. Subsequent studies showed a 70-80% reduction in these defects was possible with periconceptional folic acid supplementation (Bailey et al., 2003). Public health and food fortification efforts to ensure all women of child-bearing age have adequate folic acid intake have been accompanied by reductions in neural tube and other birth defects (Botto et al., 2004). Recent work in mice suggests that the beneficial effects of folate to prevent birth defects may be mediated by increasing methylation of transposable elements within DNA (Gilbert, 2005). These studies have shown that supplementing the diet of pregnant mice with methyl donors like folate significantly increases DNA methylation in a dose-dependent manner.

Given that practically every chemical may have a harmful influence at some dosage and stage of embryonic development, depending on the species studied, understanding the mechanism of action and the ways of disturbing embryogenesis for each agent has a tremendous importance. There are several reference materials that review the evidence for the embryotoxic teratogenic potential of drugs, chemicals and infections. These include Drugs in Pregnancy and Lactation (Briggs et al., 2002), Maternal-Foetal Toxicology (Koren, 2001), Shepard's Catalog of Teratogenic Agents (Shepherd, 2004) and the data base TERIS: Teratogenic Effects of Drugs: A Resource for Clinicians (Friedman and Polifka, 2004). The TERIS data base is also available via the internet as a subscription based teratogen information service to assist physicians and health care professionals (http://depts.washington.edu/terisweb/teris/). "REPROTOX" is another internet based information system that provides information on environmental hazards to human reproduction and development (http://reprotox.org/).
Examples of chemicals that cause birth defects in various organ systems will be discussed in subsequent sections. Here we will discuss periods of vulnerability to various chemicals, particularly those affecting the nervous, respiratory, endocrine, hepatic, reproductive and immunologic systems, as well as periods of vulnerability relevant for the indication of cancers at various life stages.

4.2.3.2 Functional Developmental Toxicity

Spurred on by the observations in children whose mothers were exposed to methyl mercury or PCBs (see Section 4.3.1.3.), have made it increasingly obvious that many manifestations of developmental defects occur in the absence of gross morphological changes. Early studies focused largely on alterations in the function of the CNS prompting the use of the term behavioral teratology; later the term developmental neurotoxicity was used to cover this research area (Rodier et al., 1994). However, other organ systems are also at risk due to developmental toxicity (Holladay and Luster, 1994; Lau and Kavlock, 1994; IPCS, 2002; USEPA, 1991). The critical periods for such effects extend well beyond the period of major organogenesis and include post-natal stages as well. In extrapolating findings between species, it is important to consider comparative rates of development, since what may be a pre-natal event in humans could well be equivalent to post-natal periods in the animal species commonly used in toxicology assays. More recently, the concept of the intrauterine environment influencing the onset of adult diseases such as coronary heart disease, hypertension and type-2-diabetes has come into prominence. A number of epidemiology studies have associated changes in birth weight (as a proxy for IUGR) with elevated risk levels of these diseases (Lau and Rodgers, 2005). The basis of the association is believed to be that alterations in nutrient availability (or other environmental stressors) lead to short term adaptive measures in the foetus (involving altered homeostatic set points) that later result in metabolic disorders when the stressor is relieved. The metabolic alterations, in turn, then gradually contribute to increased risk with aging. It is important to note that evidence of functional developmental toxicity may be evident only after long latency periods, and may be difficult to ascertain due to the functional reserve capacity of many organ systems.

4.3 Specific Organ Systems

4.3.1 Nervous System
The developing nervous system is more vulnerable to the disrupting effects of toxic chemicals than the adult brain. Levels of exposure that produce few, or no, obvious effects on the mature nervous system in adults may pose a serious risk to the developing nervous system (Faustman et al., 2000). The lengthy period of brain development and the extensive number of neural processes available for disruption during development contribute to the vulnerability of the developing nervous system to toxicants (Rodier, 1994). The process by which normal CNS development unfolds requires the precise orchestration of neuronal proliferation, migration, differentiation, synaptogenesis, gliogenesis, myelination and apoptosis (Rice and Barone, 2000). These developmental processes need to occur in specific brain regions at specific times. This complex array of processes can be disturbed by both genetic and environmental influences and lead to long-term losses in the structural and functional integrity of the nervous system (Barone et al., 2000, Rodier 1995). To understand the action of neurotoxicants on the developing child, it is important to explore both normal and abnormal pathways of brain development. Chapter 3, section 3.6.2 describes the normal development of the nervous system. This section provides a brief overview of how exposure to neurotoxicants at early life stages can alter the biological foundations of behavior.

4.3.1.1 Periods of vulnerability and consequences of exposure

To understand the consequences of chemical exposure on the CNS and ultimately, the implications of such exposures on childhood growth and development, it is important to consider the concept of critical or sensitive periods in development. Critical periods, in the context of neurotoxicology, are used to describe time points when the brain is highly susceptible to perturbation from exposure to chemicals. The complexity and vast number of processes that take place during CNS development provide multiple opportunities for differential effects of chemical exposures. When evaluating toxicological studies in animal models for their relevance to humans, it is also important to keep in mind differences in the timing of critical events in nervous system development between humans and common laboratory animal species. For example, in rodents considerable brain development occurs during the neonatal period, whereas most of this development occurs during the foetal period in humans.
Neurogenesis of different brain regions continues to occur throughout gestation and post-natally. The period of vulnerability to agents that affect proliferation and migration will thus vary depending on the brain region. For example, initial proliferation in the cerebellum occurs during the foetal period in humans and in rats. A second period of proliferation begins during the foetal period in humans and continues well into childhood, whereas in rats it occurs entirely post-natally (Rice and Barone 2000). Disorders in neuronal proliferation, migration, and maturation, owing to both genetic and environmental causes, can lead to both lethal and nonlethal congenital anomalies. Barone et al. (2000) and Rice and Barone (2000) have provided extensive reviews of early brain development in which the timing and sequence of processes such as proliferation, migration, synaptogenesis and apoptosis, as well as the critical roles of signaling and trophic molecules, are discussed. Perturbation of these processes in CNS development can result in malformations. For example, microcephaly (small brain and skull with mental retardation) is caused by faulty neuronal proliferation, and agenesis of the corpus callosum (usually associated with seizures and mental retardation) is caused by defects in neurulation and neuronal migration. The number of foetal brain malformations that has been clinically identified is too extensive to list in full. In the case of neuronal migration disorders alone (i.e. failure of neurons to move from their site of birth to where they will function in mature neural circuits), there are over 25 recognized clinical syndromes (http://www.ninds.nih.gov). Defects in genes that control neuronal migration are thought to play a leading role in the onset of these disorders but the mechanisms that control these genes are not yet delineated.

Neurobehavioral (functional) deficits: Low-level exposure to environmental chemicals such as methylmercury, lead, or pesticides can result in physical malformations but more commonly, can produce cellular or molecular changes that are expressed as neurobehavioral deficits (Adams et al., 2000) or as increased susceptibility to neurodegenerative diseases much later in life (Cory-Slechta et al., 2005). Scientists and health professionals must grapple with the fact that not all early CNS damage stemming from neurotoxic insult can be visualized with the eye or under the microscope. Functional loss, whether taking the form of mental retardation or subtle behavioral deficits, is a reflection of abnormal development and impaired CNS functioning. Moreover, neurotoxic insults during development, which result in no observable phenotype at birth or during childhood, could manifest later in life as earlier onset of neurodegenerative diseases like...
Parkinson’s disease. Only a small number of neurotoxins have been adequately studied to
tackle their specific neurobehavioral consequences after pre-natal or perinatal exposure. The
developmental consequences of some of these compounds will be briefly discussed below.

While behavior is frequently difficult to tie to specific brain regions, there are some important
generalities that can be gleaned from the fields of cognitive neuroscience and developmental
neurobiology. As summarized by Rice and Barone (2000), working memory and executive
functions are controlled by the prefrontal region, some aspects of learning and memory are
dependent on medial temporal lobe structures, and sleep/wake cycles, autonomic nervous system
functions and regulation of arousal are a function of the brainstem. Each of these neural areas
has a course of maturation that can be qualitatively (stages) and quantitatively (timing) distinct
from other structures within the brain. Behaviors that depend on different brain systems will
therefore be differentially affected by chemical exposure depending on when the exposure
occurred during development. The timing of the exposure is therefore critical in establishing
what type of functional loss is likely to occur. While early developing neural systems may be the
most vulnerable to chemical insult, recent evidence from pediatric functional MRI studies
suggests that the behavioral and physiological foundations of cognition continue to develop
during childhood and adolescence (Casey et al., 2005). Chemical exposures that occur late in
childhood or adolescence should not be dismissed as inconsequential.

The effects of pre-natal chemical exposure can be expressed across several domains of behavior
and can include adverse effects on intelligence/cognition, social behavior or temperament,
sensory development (vision, hearing) and physical growth (Vreugdenhil et al., 2004).
Behavioral changes can be difficult to detect and even more difficult to link to specific pre- or
perinatal risk factors such as exposure to a drug or chemical. Depending on the timing and
nature of the neurotoxicity, behavioral deviations in the developing child can range from mild
(e.g. learning disabilities) to severe (e.g. mental retardation). Common neurodevelopmental
disabilities such as autism, mental retardation, attention deficit hyperactivity disorder (ADHD)
and dyslexia affect approximately 3-8% of the babies born in the US each year (Weiss &
Landrigan, 2000). The basis of the neurological damage in most developmental disorders can
only be established in about 25% of affected children. Given our expanding knowledge of global chemical exposures, it remains plausible that there is a relation between early neurotoxicant exposure, subtle CNS damage and the rising number of children with major and minor neurodevelopmental disabilities.

Exposure to retinoic acid, methyl nitrosourea, and clomiphene during the early embryonic period, prior to the induction of the neural plate (before day 18 in the human), results in an increased incidence of neural tube defects and other malformations in experimental animal models (Bennett and Finnell, 1998). In addition, exposure of rodents to teratogens such as retinoic acid, arsenic and valproic acid during the period of neurulation result in neural tube defects such as spina bifida and encephaloceles (Adams and Lammer, 1993; Bennett and Finnell, 1998). Of these, therapeutic use of valproate acid has been associated with elevated rates of spinal bifida in humans. A number of structural defects of the brain, including exencephaly, result from the failure of the rostral neuropore to properly close during the fourth week of human embryonic development. Various teratogenic agents such as ionizing radiation (X ray) and hydroxyurea, both antimitotic agents capable of stopping cell division, can experimentally induce these conditions in animal models (Hicks, 1954; Rodier, 1986). As the rhombomeres form and the neural tube closes later during the embryonic period, valproic acid and thalidomide target the hindbrain, brainstem, and the cranial nerve nuclei (Rodier et al., 1996; Rodier, 2004). Indeed, it has been speculated that insult to the cranial nerve motor nuclei during rhombomere formation may play a role in the development of autism, a serious developmental disorder affecting thousands of children.

The majority of malformations of the spinal cord are the result of the failure of the caudal neuropore to properly close by the end of 4th week of development. The defective closure of the caudal neuropore results in serious neural tube malformations known generally as spina bifida. There are many types of spina bifida and the clinical presentation, including neurological deficits, can range from minor (e.g. spina bifida occulta) to severe (e.g. spina bifida with myeloschisis). Spina bifida cystica has been associated with large doses of retinoic acid (vitamin A) (Moore, 1988).
4.3.1.2. Specific Examples

Methylmercury. Catastrophic episodes of human methylmercury poisoning have occurred in both Japan and Iraq, providing much of the information that is known about high-dose methylmercury developmental exposure in human infants. At present, environmental methylmercury contamination is widespread, and low-level exposure to this toxicant occurs primarily through the consumption of contaminated fish. The foetus is particularly sensitive to methylmercury exposure and adverse neurobehavioral effects in infants have been associated with exposure levels that result in few, if any, signs of maternal clinical illness or toxicity. Early life stage exposure to this metal produces a broad spectrum of neurobehavioral effects that are clearly dose dependent (Davidson et al, 2004, NRC, 2000b). High-level developmental exposure to methylmercury can result in cerebral palsy, seizures, blindness, deafness and mental retardation.

After the work by Kjellstrom and colleagues on low-level methylmercury exposure and child development (Kjellstrom et al., 1986; Kjellstrom et al., 1989), two large-scale longitudinal studies were undertaken to investigate the consequences of in utero methylmercury exposure from a maternal diet high in fish. The Faroe Islands study in the Northern Atlantic Ocean is an epidemiological study that enrolled approximately 1,000 children at birth (Grandjean, 1992). In Faroese neonates, neurologic optimality scores taken at 2 weeks of post-natal age showed that increased cord-blood mercury concentrations were associated with decreased neurologic function and that this effect corresponded to a gestational age of about 3 weeks (Steuerwald et al., 2000). Analysis of behavioral data from children at 7 years of age revealed significant methylmercury-related impairments in language, attention and memory (Grandjean et al., 1997). Methylmercury effects on the latency of brain stem auditory evoked potentials were found at both 7 and 14 years, suggesting that some neurotoxic effects from intrauterine methylmercury exposure may be irreversible (Murata et al., 1999, Murata et al., 2004). Decreased heart rate variability, an indicator of autonomic nervous system function, was also observed at 7 and 14 years (Grandjean et al., 2004). Overall, results from the Faroe Islands suggest that, in this population, low-level
pre-natal exposure to methylmercury from maternal consumption of fish and pilot whale is an important neurologic risk factor for impaired behavioral development in infants and children. The second prospective study of in utero exposure to methylmercury was initiated in the Republic of Seychelles and enrolled about 800 mother-infant pairs (Myers et al., 1995). In contrast to the Faroe Islands study, the investigation in the Seychelles has not found evidence of methylmercury-related adverse effects on the neurobehavioral development of children through 9 years of age (Myers et al., 2003). In some instances, pre-natal mercury exposure was actually associated with precocious behavior and important developmental milestones were reached more quickly in the most highly exposed subjects. The differences in the outcomes of the studies in the Faroes and the Seychelles have been the subject of much deliberation (NTP, 1998; NAS, 2000). Both assessments concluded that the two studies were credible and provide valuable insight into the potential health effects of methylmercury. Differences in the study designs and in the characteristics of the study populations might explain the differences in findings between the Faroe and the Seychelles studies. Differences include the ways methylmercury exposure was measured (i.e., in umbilical-cord blood versus maternal hair), the types of neurological and psychological tests administered, the age of testing (7 years versus 5.5 years of age), and the patterns of methylmercury exposure. The NAS noted that the Faroe Islands population was also exposed to relatively high levels of polychlorinated biphenyls (PCBs). However, on the basis of an analysis of the data, the committee concluded that the adverse effects found in the Faroe Islands study, including those seen in the Boston Naming Test, were not attributable to PCB exposure and that PCB exposure did not invalidate the use of the Faroe Islands study as the basis of risk assessment for methylmercury.

Lead. Lead is perhaps the best-studied toxicant that has been clearly linked to CNS injury and adverse neurobehavioral outcomes in exposed children. It has long been recognized that high-level exposure to lead can result in encephalopathy, coma, and death (for review of lead poisoning, see Needleman, 2004). In the 1970s, Needleman and colleagues began to examine the neurobehavioral consequences of developmental exposure to lead in exposed children. This body of work established that chronic, low-dose exposure to lead is associated with a significant decrease in intelligence quotient (IQ) as measured by standardized psychometric instruments (Needleman et al., 1979). In a recent publication, the relation between environmental lead...
exposure and intellectual deficits in children was confirmed in an international pooled analysis (Lanphear et al, 2005). Children exposed pre-natally to lead also display impaired performance on specific cognitive tests such as reaction time and vigilance (Rice 1996). These experimental findings suggest that in utero exposure to lead results in slower basic information processing and deficits in attention. These functional losses may play a large role in the decreased global IQ scores observed in lead exposed children.

Research has also demonstrated that as early as age two, lead exposed children exhibit more problem behaviors than their unexposed peers (Sciarrillo et al., 1992, Wasserman et al., 1998). In a prospective study, the behavior of lead-exposed children at eight years of age was measured by teacher ratings in the classroom and total problem behavior scores were significantly related to tooth dentine lead levels (Bellinger et al., 1994). These data suggest that social and emotional difficulties may be correlates of early lead exposure. One of the most significant relationships to emerge from the lead literature is the relation between antisocial behavior and developmental lead exposure. In a longitudinal study of boys, a modest relationship was found between bone lead levels and teacher ratings of behavior such as aggression and delinquency at 7 years of age (Needleman et al., 1996). At 11 years of age, parental reports suggested an increase in antisocial behavior and health complaints in children with higher lead levels. Teacher ratings corroborated the parental reports in that children with higher bone lead levels were found to have more health concerns, depression/anxiety and behavioral problems in the classroom. In a prospective, longitudinal study conducted by Dietrich and colleagues, both pre-natal and post-natal lead exposure was related to antisocial and delinquent acts in adolescents (Dietrich et al, 2001). It is generally accepted in the scientific and medical communities that the adverse neurobehavioral consequences associated with developmental lead exposure are not reversible and remain in place across the human lifespan (Bellinger, 2004).

Polychlorinated biphenyls (PCBs) are prevalent environmental pollutants that pose potential health risks to both humans and wildlife. In episodes of human PCB poisoning in Japan (Yusho) and Taiwan (Yucheng), people became ill after ingesting rice oil that was highly contaminated with PCBs and polychlorinated dibenzofurans (Kuratsune et al., 1971; Hsu et al., 1985). Infants born to mothers who consumed PCB contaminated rice oil during pregnancy were at increased
risk for low-birth-weight, abnormal brown pigmentation of the skin and clinical abnormalities of
the gingiva, skin, nails, teeth and lungs (Yamashita and Hayashi, 1985; Rogan et al., 1988).
Neurobehavioral deficits such as delayed attainment of developmental milestones, lower scores
on intelligence tests, and higher activity levels were also reported in both cohorts of children
(Guo et al., 2004). In most contemporary exposure scenarios, human infants are exposed to low-
level complex PCB mixtures through the placenta during pre-natal development and via breast
milk during post-natal development. Although the effects of these compounds on the CNS are
not well described, there is mounting evidence of developmental neurotoxicity from studies in
Taiwan, US, Holland, Germany and the Faroe Islands (Schantz et al., 2003).

Studies of newborns suggest lower levels of PCB exposure can affect a number of newborn
behaviors. Exposed infants are more likely to exhibit signs that are consistent with immaturity of
the CNS (e.g., increased startle response, abnormal reflexes) (Rogan et al., 1986; Huisman et al.,
1995). In a longitudinal investigation in the US of infants born to mothers who consumed fish
contaminated with low level PCBs, investigators found early recognition memory deficits in
exposed infants, poorer scores on a preschool IQ test and reduced verbal IQ and reading
comprehension at 11 years of age (Jacobson and Jacobson, 2002). The authors also found that
adverse effects of developmental PCB exposure were observed less frequently in breast fed
infants, suggesting a protective influence of breast-feeding on the behavioral development of
exposed infants. Further studies of this cohort at 11 years of age have found evidence of
increased impulsivity as well as deficits in concentration and working memory in exposed
children (Jacobson and Jacobson, 2003). Again, adverse effects were primarily seen in subjects
who had not been breast fed. A study from Holland found that pre-natal PCB exposure was
related to longer and more variable reaction times in childhood, suggesting persistent deficits in
basic cognitive processes (Vreugdenhil, 2004). Although these studies and others provide
significant evidence of a relation between low-level PCB exposure and intellectual impairment,
some studies have not observed such effects and PCB-related deficits on childhood cognition
remain controversial (Gladen and Rogan, 1991; Gray et al 2005).

Ethanol. Pre-natal exposure to ethanol is the leading preventable cause of mental retardation in
the US, if not the world. The consumption of beer, wine or spirits during pregnancy can have a
profound impact on childhood development (Burbacher and Grant, in press). The effects of
ethanol are dose-dependent and children born to to alcoholic or ethanol-abusing mothers are at
highest risk for poor developmental outcome (Stratton et al, 1996). The most serious clinical
outcome for infants who have been exposed in utero to alcohol is the development of foetal
alcohol syndrome (FAS) (Jones and Smith, 1973). Infants with this condition have a common
facial dysmorphology that includes shortened palpebral fissures (eyelid openings), smooth
philtrum (area between the nose and upper lip), thin upper lip, low nasal bridge, and minor ear
anomalies (Abel, 1984). It is now understood that some alcohol-exposed infants do not express
the facial features commonly associated with FAS but do exhibit significant neurobehavioral
delays. Children with this less severe constellation of behavioral effects are commonly referred
to as having foetal alcohol effects (FAE). It is now recognized that maternal intake of one drink
per day, on average, is sufficient to result in neurologic disturbances in exposed infants
(Streissguth, 1993). Pre-natal exposure to ethanol is clearly associated with CNS injury and a
broad spectrum of behaviors can be affected, including deficits in intellectual functioning
(particularly arithmetic), language, abstract problem solving, working memories, attention, and
executive functioning (e.g. planning, flexibility) (Streissguth et al., 1994, Mattson et al, 2001,
Jacobson and Jacobson, 2002). Attention appears particularly vulnerable to the effects of alcohol
exposure and a recent study found that standardized tests of attention and distractibility can
discriminate between exposed and control subjects with 92% accuracy (Lee et al., 2004). In
general, children with a history of intrauterine ethanol exposure tend to lack the ability to stay
focused and attentive over time and have difficulty analyzing problems and forming effective
response strategies. New evidence from South Africa indicates that gestational ethanol exposure
also results in the disruption of the infant visual system (Carter et al, 2005). As children born to
drinking mothers mature, deficits in social behavior become more pronounced and are often
expressed in the form of classroom aggression, impaired social judgment and
antisocial/delinquent behavior (Allebeck and Olson, 1998; Famy et al., 1998). Pre-natal alcohol
exposure is also a risk factor for the development of drinking problems in young adulthood,
underscoring the intergenerational nature of ethanol abuse during pregnancy and the lifelong
consequences of gestational exposure (Baer et al, 2003). In addition, adolescents and adults with
a history of pre-natal ethanol exposure are more likely to experience adverse life outcomes such
as dropping out of school, arrest or confinement in a jail or psychiatric setting, or the expression of repeated inappropriate sexual behaviors (Stressguth et al., 2004).

Pesticide Exposure and Neurodegenerative Diseases. It has been hypothesized that neurotoxic insults during development, which result in no observable phenotype at birth or during childhood, could manifest later in life as earlier onset of neurodegenerative diseases like Parkinson’s disease. Recent studies in mice provide support for this hypothesis (Cory-Slechta et al., 2005). Mice were exposed as juveniles only (post-natal days 5-19), as adults only, or during both stages to the pesticides Maneb and Paraquat. These agents both damage the dopaminergic pathways involved in Parkinson’s disease, but the two agents act via different modes of action. Mice exposed as juveniles and again as adults had dramatically greater declines in nigrostriatal dopaminergic neurons at 7 months of age than did mice exposed during either period alone (Cory-Slechta et al., 2005).

It is important to take into account that besides the above examples, the number of environmental chemicals that might affect the neurological development of children is increasing. Recently, cognitive effects have been shown for environmental tobacco smoke (Yolton et al., 2005), arsenic (Calderon et al., 2001 and Wasserman et al., 2004), manganese (Wasserman et al., 2006), and some mixtures of arsenic and manganese (Wright et al., 2006).

4.3.2 Reproductive system

4.3.2.1 Periods of vulnerability

Reproductive organs develop throughout gestation as demonstrated in Figure 4.1. Many gene activities, such as sry, sox9, and amh, are strictly time-dependent, and disruption of those activities can occur only in a narrow time-frame, whereas some other developmental phases take a long time (e.g., testicular descent). It starts with a transabdominal migration in mid-gestation and ends with a inguino-scrotal migration during the late gestation. This long developmental period may partly explain why testicular maldescent cryptorchidism is so common (2-9% of newborn boys; (Boisen et al., 2004)). Male-type development is hormonally regulated, whereas
female-type differentiation occurs in the absence of reproductive hormone action. Because the male phenotype is dependent on an induced, rather than default, pattern of gene expression, the male foetus tends to be vulnerable to hormonal perturbations that modulate function of the androgen signaling pathways. Likewise, genotypic females will be masculinized by exposure to sufficient amounts of androgens. These effects do not need to be all or none, as the phenotype could be anywhere in the continuum between male and female (IPCS, 2002). Due to accurate timing of gene activities, the same agent can cause very different effects at different times of
development; i.e. an antiandrogen exposure early in pregnancy would cause hypospadias,
whereas later in pregnancy it would only cause cryptorchidism.

Puberty is a period of interrelated neuroendocrine processes that culminate in a physiologically
mature reproductive system and, therefore, is another period of vulnerability to environmental
influences. The form of pubertal alteration (maturational delay or acceleration) is a function of
the nature of the insult (Colon et al., 2000; Dendlond and Schoeters, 2006). Effects on puberty
can be the result of earlier life stage exposure, or exposure concurrent with the maturational
process. Comprehensive reviews of normal puberty and perturbations by exogenous influences
in experimental animal models are available for the female (Goldman, et al., 2000) and male
(Stoker et al., 2000). Body composition changes across lifestages, and the endocrine control of
body composition by such factors as gonadal sex steroids and growth hormone (GH) and insulin-
like-growth factor 1 (IGF-1) also play important roles in puberty (Veldhuis et al., 2005).

4.3.2.2 Consequences of exposure to chemicals

Outcome after chemical exposure depends on the mechanism and type of action, the timing of
exposure, and the dose of the chemical. Adverse effects can be manifest at birth, like
hypospadias and cryptorchidism in humans, or they may appear in puberty as delay or precocity,
or in adulthood as infertility, alterations in accessory sex organs, disturbances in pregnancy
maintenance, endometriosis, or premature reproductive senescence (Buck et al., 2005; Pryor et
al., 2000). In the following we discuss some specific chemicals that have been reported to affect
one or several of reproductive outcomes. Where possible, the discussion will focus on effects
that are observed in humans. The list of chemicals that have been reported to have adverse
effects in reproductive organs is much longer than presented here and the selected examples
serve only to illustrate the types of reproductive toxicants and their different effects. The general
order in which they are presented is based on the lifestage in which exposure is associated with
the outcome, regardless of whether the outcome was observed concurrent with the exposure, or
in a subsequent lifestage.
Diethylstilbestrol (DES), and other estrogen agonists. In utero exposure of men to DES has been linked to increased incidence of meatal stenosis, epididymal cysts, testicular hypoplasia, cryptorchidism, microphallus and sperm abnormalities (Gill et al., 1977; Gill et al., 1979; Henderson et al., 1976; Stillman, 1982). In females, adenosis, clear cell adenocarcinoma, and structural defects of the cervix, vagina, uterus, and fallopian tubes have been linked to in utero exposure to DES (Stillman, 1982).

Phthalates. Foetal exposure of male rats to some phthalate esters (e.g., DEHP, DBP and BBP) results in many changes in the male reproductive tract, such as decreased anogenital distance, hypospadias, cryptorchidism, disturbed development of prostate, epididymis, vas deferens and seminal vesicles, retained nipples and decreased sperm production (Gray et al., 2000; Kavlock et al., 2002a,b,c,d,e,f; Koch et al., 2006; Lottrup et al., 2006; Skakkebaek et al., 2006; Mylchreest et al., 1998; 1999; 2000; 2002). The critical window for all of these effects is the latter half of gestation (days 12-21), which is the time during which male sexual differentiation occurs (Mylchreest et al., 1999). The critical window has been further refined for undescended testes and the reduction of anogenital distance in males to gestational days 15-17 (Ema et al., 2000 and Selevan et al., 2000).

In utero exposure of male rats to dibutyl phthalate (DBP) on gestational days 13-21 permanently alters testis and produces foci of testicular dysgenesis (immature seminiferous tubules with undifferentiated Sertoli cells, Sertoli cell-only tubules, Leydig cell hyperplasia, morphologically distorted tubules, and the presence of abnormal germ cells) which persist in the adult animal (Fisher et al., 2003). Subsequent research demonstrated a coordinated, dose-dependent reduction in expression of key genes and proteins involved in cholesterol transport and steroidogenesis and a corresponding reduction in testosterone in the foetal testes (Lehman et al., 2004). In humans similar dysgenetic changes in the histology of testis have been found in patients with testicular cancer, subfertility or cryptorchidism (Berthelsen & Skakkebaek 1983; Hoei-Hansen et al., 2003; Skakkebaek et al., 2003; Sohval, 1954, 1956). Furthermore, men with rare genetic abnormalities that cause testicular dysgenesis (e.g., 45X/46XY and androgen insensitivity) also have high risk of testicular cancer, often combined with cryptorchidism and hypospadias (Savage and Lowe 1990). It has been proposed that all these human disorders (testicular germ cell cancer,
cryptorchidism, hypospadias and low sperm counts) have common origins in foetal life and thus they all represent different symptoms of the same underlying entity called the testicular dysgenesis syndrome (TDS) (Aarskog, 1970; Scully, 1981; Asklund et al., 2004; Sharpe 2003; Skakkebaek et al., 2001; Sharpe and Skakkebaek, 1993; Skakkebaek et al., 2006). Since the testicular and other changes in DBP-exposed rats have also been reported in human TDS, it has been proposed that in utero exposure of the rat to dibutyl phthalate (DBP) is a possible model for studying human testicular dysgenesis syndrome (Fisher et al., 2003).

Perinatal exposure of rats to butyl benzyl phthalate (BBP) causes reduced anogenital distance, reduced testis weight, permanent nipples, hypospadias, cryptorchidism, and testicular malformations (Gray et al., 2000). Also reduced daily sperm production has been linked to gestational and lactational exposure to BBP in rats (Sharpe et al., 1995).

Polybrominated Biphenyls (PBBs). In humans, exposure to high levels of polybrominated biphenyls (PBBs) in utero and via breastfeeding has been linked to an earlier age at menarche. Perinatal exposure to PBB has been associated with earlier menarche (pubic hair stage) in breastfed girls (Blanck et al., 2000).

Anti-neoplastic Agents. Many anti-neoplastic agents are well-known to cause amenorrhea and premature ovarian failure in women and oligospermia or azoospermia in men (Howell and Shalet, 1998). The likelihood of ovarian failure after chemotherapy treatment increases with increasing age and is thought to be due to lower ovarian reserves in older women (Howell and Shalet, 1998). There are few data on the effects of in utero exposure to antineoplastic drugs on reproductive function in adulthood. However, evidence from animal studies suggests that the foetal ovary may be more sensitive to these agents than the prepubertal or adult ovary. Foetal exposure to busulfan causes reduced numbers of oogonia and primordial follicles in rats (Hirshfield, 1994; Merchant, 1975) and high dose exposure causes preterm ovarian exhaustion (Shirota et al., 2003). Also in humans, high dose busulfan regimenes cause ovarian failure in young women (Cicognani et al., 2003). Complete destruction by the alkylating agent cyclophosphamide of primordial and primary follicles is achieved at lower doses in prepubertal mice than in adult mice (Shiromizu et al., 1984; Plowchalk and Mattison, 1991). In young men,
the use of multiple chemotherapy regimens is associated with a risk of permanent sterility and
the cumulative dose of cyclophosphamide has been shown to be an important determinant of
recovery to normospermic levels after azoospermia (Meistrich et al., 1992).

Lead. Rats chronically exposed to lead (starting in utero) show delay in sexual maturity (Ronis
et al., 1998). In a study concerning U.S. girls, higher blood lead levels were associated with
delayed attainment of pubic hair and menarche (Wu et al., 2003). As compared with
concentrations of 1 microgram per deciliter, lead concentrations of 3 microgram per deciliter
were associated with decreased height (P<0.001), after adjustment for age, race, and other
factors, but not with body-mass index or weight. Blood lead concentrations of 3 microgram per
deciliter were associated with significant delays in breast and pubic-hair development in
African-American and Mexican-American girls. The delays were most marked among African-
American girls; in this group, the delays in reaching Tanner stages 2, 3, 4, and 5 associated with
a lead concentration of 3 microgram per deciliter as compared with 1 microgram per deciliter
were 3.8, 5.3, 5.8, and 2.1 months, respectively, for breast development and 4.0, 5.5, 6.0, and 2.2
months, respectively, for pubic-hair development; the associated delay in age at menarche was
3.6 months. In white girls, there were non-significant delays in all pubertal measures in
association with a lead concentration of 3 microgram per deciliter (Selevan et al., 2003).

Polycyclic Aromatic Hydrocarbons (PAHs) and smoking. Women who smoke have decreased
fecundity and earlier menopause than non-smokers (Jick & Porter 1977; Baird and Wilcox,
1985). Women whose mothers smoked while they were in utero also have reduced fecundity
compared to women whose mothers did not smoke (Weinberg et al., 1989). Treatment of mice
with PAHs, which are present in tobacco smoke, has long been known to cause dose-dependent
destruction of oocytes (Mattison and Thorgeirsson, 1979). More recently, it has been appreciated
that lower doses of PAHs, which cause limited oocyte depletion in adult mice, cause much
greater oocyte depletion in the offspring when given to pregnant mice (Matikainen et al., 2002).
Exposure to PAHs induces the expression of the gene Bax in oocytes, which is followed by
apoptosis. This results in fewer oocytes at birth and premature ovarian failure. The same cascade
can be induced also in human ovarian explants (Matikainen et al., 2001; Matikainen et al., 2002).
In men exposed to tobacco smoking during in utero development, reduced semen quality,
smaller testis size and reduced fecundability odds ratios have been observed (Jensen et al, 1998; Jensen et al, 2005).

Atrazine. Peripubertal exposure to atrazine causes delayed vaginal opening in rats (Ashby et al., 2002; Laws et al., 2000), indicating delayed puberty. Studies examining both the effect of prenatal and lactational exposure to atrazine on pubertal indicators in rats have shown that in utero exposure can cause delays in the development of the mammary gland, whereas delayed vaginal opening seems to be mediated via lactational exposure (Rayner et al., 2004). Atrazine has been shown to alter serum LH and prolactin levels in female rats by changing hypothalamic control of these hormones (Cooper et al., 2000).

Alcohol. Consumption of alcohol during early adolescence has been linked to delays in the onset of female puberty. The response appears to be related to alcohol’s effect on the function of insulin-like-growth factor 1 (IGF-1), which is synthesized in the liver and which is active in the brain to coordinate overall physical growth. Long term consumption of alcohol inhibits the production of IGF-1, and short term consumption of alcohol may alter IGF-1 function within the brain (Dees et al., 1998).

4.3.3 Endocrine and Metabolic Disorders

The diverse glands, hormones, and other chemical messengers that make up the endocrine system exert effects on virtually every organ system and cell within the body. Endocrine systems regulate metabolic, nutritional, reproductive, and behavioral processes, as well as growth, responses to stress, and the function of the digestive, cardiovascular, renal, and immune systems. Disruption of endocrine function can have severe health consequences in adults, and exposures that interfere with the development of the endocrine system during early life stages can have even more far-ranging consequences (Barr et al., 2000). Like the other systems discussed in this chapter, the development of the endocrine system involves intricately orchestrated processes of cell proliferation, migration, and death, which if disrupted can lead to permanent consequences. In addition, programming of endocrine set-points is a unique aspect of endocrine system development, which is also vulnerable to disruption. This section will discuss periods of
vulnerability and consequences of exposures for non-reproductive components of the endocrine
system. The endocrine regulation of reproduction and consequences of its disruption are
discussed in Chapter 3 and in Section 4.3.2, respectively.

4.3.3.1 Periods of vulnerability

The endocrine glands have early windows of vulnerability during the embryonic period, when
the glands first begin to develop. The later period of differentiation of the glands, which occurs
mostly during the foetal period, constitutes another set of vulnerable periods for the endocrine
system. While homeostasis via feedback loops is central to the functioning of all endocrine
systems, the set-points or narrow ranges within which the levels of hormones are regulated must
first be programmed. The term programming generally describes a process whereby a stimulus or
insult when applied at a critical or sensitive period of development results in a long term or
permanent effect on the structure or function of the organism. Importantly, it is during
foetal/neonatal development that programming of the endocrine systems occurs. Exposures to
toxicants during this critical period of programming can result in permanent abnormalities in
endocrine function.

Periods of vulnerability during pancreas development and consequences of disruption of normal
development. In human embryos, weeks 4 to 8 of gestation, when the pancreatic buds first
appear and then begin to proliferate, represent an early window of susceptibility for the pancreas
(Sadler, 2000). Weeks 10-14 during the foetal period, when differentiation is occurring and
alpha, beta, and delta cells of the endocrine pancreas appear, constitute another period of
vulnerability during the development of the pancreas (Sadler, 2000) (Table 4.2). The formation
of distinct islets of endocrine cells occurs during the later foetal period, but the islets continue to
grow and rearrange until about four years of age (Hellerstrom and Swenne, 1991). Similar
stages of pancreatic development occur in mice during equivalent developmental periods, and
much has been learned in recent years about the genes that control pancreatic development from
knockout mice that have disruption of these genes (see Figure 4.2).
Evidence from epidemiological studies demonstrates that exposures during early life stages can impact susceptibility to diabetes and obesity later in life (Lau and Rogers, 2005). In particular, numerous studies have linked poor maternal nutrition with later risk for these adverse health outcomes. The Dutch Hunger Winter was a short defined period of famine; therefore, it has been possible to assess both the role of early nutrition in future susceptibility to disease and to identify critical time windows (Ravelli et al., 1976; 1998). It was shown that poor maternal nutrition, especially during the last trimester of pregnancy, was associated with poor glucose tolerance and insulin resistance in the offspring. In terms of obesity, individuals who were exposed to the famine during the first half of pregnancy were more obese at age 19. In contrast, those who were exposed to the famine during the last trimester of pregnancy and in early post-natal life had reduced obesity (Ravelli et al., 1976). This suggests that the critical time windows for increased risk of obesity and type 2 diabetes differ.

Studies of twins have been used to address the importance of the intrauterine environment in determining future susceptibility to type 2 diabetes and insulin resistance. The advantage of carrying out studies in monozygotic twins is that they are genetically identical and are not
influenced by gestational age or sex. One such study was carried out on twin pairs in Denmark (Poulsen et al., 1997). Midwife recorded birth weights were traced for middle-aged twin pairs (identified from the Danish Twin Register) who were discordant for type 2 diabetes. Birth weights were significantly lower in both diabetic monozygotic and diabetic dizygotic twins who had diabetic mothers compared with their non-diabetic co-twins (Poulsen et al., 1997). It was therefore concluded that a non-genetic (environmental) intrauterine factor (such as intrauterine malnutrition) played an important role in the development of type 2 diabetes much later in life. A second study in Italian twins has reported similar findings. In this study both monozygotic and dizygotic twins with hyperinsulinaemia and/or hyperglycaemia during an oral glucose tolerance test were found to have a significantly lower birth weights than their co-twins with normal glucose tolerance and normo-insulinaemia. They also had higher levels of triglycerides, total cholesterol, insulin and C-peptide (Bo et al., 2000).

In an attempt to understand the molecular basis of the relationship between early growth restriction and development of subsequent disease, and to investigate the specific nutrients that may be involved, a number of animal models have been developed. Maternal protein restriction, maternal caloric restriction, maternal high fat feeding and maternal anemia have all been shown to result in features of the metabolic syndrome in the offspring (reviewed in Ozanne, 2001). The phenotypic outcomes of these different insults have been remarkably similar suggesting that these act through a common pathway. Elevation of glucocorticoid levels in the foetus has been suggested as a key element of this common pathway (Philips et al., 1998; Lau and Rogers 2005).

Periods of vulnerability in thyroid development and consequences of developmental hypothyroidism. In humans migration of endodermal cells from the pharynx to the site of the future thyroid during weeks 5-6 of gestation constitutes an early period of vulnerability to disruption by chemicals, and the subsequent period of thyroid differentiation during weeks 8-9 of gestation constitutes a second window of vulnerability (Sadler 2000). Foetal thyroid hormone synthesis begins by weeks 10-12 (Sadler, 2000) and is potentially susceptible to agents that affect thyroid hormone synthesis or metabolism.
Thyroid hormones are essential for normal CNS development. They play roles in neuronal and
glial proliferation and migration, in neuronal outgrowth and myelination, and in the development
of the dopaminergic and cholinergic neuronal systems. The developing CNS is sensitive to
disruption of thyroid homeostasis throughout the embryonic and foetal periods and continuing
through early post-natal life, as demonstrated by studies of infants whose mothers were
hypothyroid during pregnancy or who themselves have congenital hypothyroidism. Maternal
hypothyroidism, which causes inadequate levels of thyroid hormone to the embryo/foetus prior
to the onset of foetal thyroid hormone secretion, causes lowered intelligence quotient, poor word
discrimination, decreased reading comprehension, and learning deficits (Haddow et al., 1999).
Congenital hypothyroidism, if untreated, causes late disappearance of infantile reflexes, delayed
acquisition of acquired reflexes, speech and learning disorders, spasticity or hypotonia, and
tremors (Porterfield and Hendry, 1998). Even when treated from an early age, children born with
congenital hypothyroidism display increased learning and behavioral disorders, as well as
impaired memory, spatial perception, and fine motor coordination (Porterfield and Hendry,
1998). Even more severe effects are observed when both mother and foetus are hypothyroid,
once common in parts of the world that lacked adequate iodine in the diet, causing endemic
cretinism. These children have deaf-mutism, spasticity, gait disturbances, complete or partial
inability to stand, and profound mental retardation (Porterfield and Hendry, 1998).

Periods of vulnerability during pituitary gland development. An early period of vulnerability for
the pituitary gland occurs during weeks 4 to 9 of gestation in humans, when Rathke’s pouch
from the embryonic oral cavity grows up to form the anterior pituitary and the infundibulum
while a downgrowth from the floor of the diencephalon forms the posterior pituitary. Weeks 8 to
13 of gestation, when the differentiation of the five cell types that form the anterior pituitary
occurs, constitute another period of vulnerability. Corticotropes, which secrete ACTH, appear at
8 weeks; thyrotropes, which secrete thyroid stimulating hormone; somatotropes which secrete
growth hormone; and lactotropes which secrete prolactin all appear at 11 weeks of gestation.
Gonadotropes, which secrete luteinizing hormone and follicle stimulating hormone, appear at 12-
13 weeks of gestation (Sadler, 2000).
Periods of vulnerability during adrenal development. In humans, the adrenal cortex is sensitive to disruption by environmental chemicals during gestational weeks 5-8 when mesoderm from the urogenital ridge proliferates to form the embryonic adrenal cortex, which develops both a foetal zone that regresses during the neonatal period and a definitive zone (Sadler 2000; Hammer et al., 2005). A second period of vulnerability occurs during week 12 of gestation when the adrenal medulla begins to differentiate, a process that is completed 12 to 18 months after birth (Sadler 2000). The definitive zones of the adrenal cortex do not begin to differentiate until 7-8 months after birth in humans, constituting another critical window for the cortex; however, the adrenal cortex does not fully reach its adult form until puberty (Figure 4.3) (Sadler et al, 2000). While many of the same genes appear to regulate adrenal development in mice and other laboratory animal species as in humans, the pattern of adrenal development differs between mice and humans (as shown in Figure 4.3). Mice are born with less developed adrenal glands than humans. The mouse X-zone (analogous to the human foetal zone) appears 10 to 14 days after birth. In male mice, the X-zone degenerates at puberty, whereas in females it regresses during the first pregnancy (Hammer et al., 2005).

![Figure 4.3 Comparison Between Humans and Mice of Adrenal Gland Development](from Hammer et al., 2005)

Cortisol production by the foetal adrenal gland is critical for maturation of the lungs, glycogen production in the liver, and for synthesis of enzymes in the brain, pancreas, and gut (Sadler, 2000). Foetal stress has been associated with increased size of the foetal adrenal cortex (Barr et al., 2000) and with increased glucocorticoid hormone levels, leading to permanent
reprogramming of the hypothalamic-pituitary-adrenal axis (Lau and Rogers, 2005). Since the adrenal cortex continues to develop until puberty, one might expect that the vulnerability to exposures would extend into the neonatal and childhood periods. In the rat, maternal grooming behavior towards her pups during the neonatal period profoundly affects the pups’ ability to deal with stress later in life. Pups whose mothers groomed and licked them intensively as neonates retained methylation of a portion of the promoter region of the glucocorticoid receptor gene that binds the Egr-1 transcription factor (Gilbert, 2005). As adults these pups had more glucocorticoid receptors in the hippocampal region of the brain and were better able to respond to stress (Gilbert, 2005).

Consequences of alterations in growth hormone and glucocorticoid signaling. Congenital failure of growth hormone/somatotropin synthesis and secretion causes dwarfism, whereas over-secretion causes gigantism. Less severe perturbations of growth hormone and of the hypothalamic-pituitary-adrenal axis during development have been linked to the development of osteoporosis later in life. The long bones have their most rapid period of growth during the second trimester of pregnancy. The main adaptive response to a lack of nutrients and oxygen during this period of growth is to slow the rate of cell division. This results in permanent reduction in peak skeletal proportions attained later in life. Thus, several studies have shown that body weight in infancy is positively associated with adult bone mass (Cooper et al., 1997). Low rate of childhood growth has been associated with increased risk of hip fracture later in life (Cooper et al., 2002; Javaid and Cooper, 2002). Experiments in rats, mice, sheep, and pigs have also demonstrated that protein or calorie restriction of the mother during pregnancy and lactation is associated with smaller offspring that have lower bone mineral content and bone area in adulthood (Cooper et al., 2002; Javaid and Cooper, 2002). The effects of birthweight and weight in infancy on the pathogenesis of osteoporosis appear to be mediated by effects on growth hormone and cortisol in these individuals have lower basal levels of growth hormone and elevated cortisol, as well as increased rates of bone loss, in late adult life (Fall et al., 1998; Dennison et al., 1999). Offspring of female rats maintained on low protein diets have reduced bone marrow alkaline phosphatase activity and reduced responsiveness to growth hormone, IGF-1 and Vitamin D (Cooper et al., 2002).
Vitamin D deficiency during childhood leads to the disease rickets, a disorder of mineralization of the bone matrix that involves both the epiphysis (growth plate) and newly formed bone. Children with rickets commonly develop a bowed deformity of the legs, as well as deformities of the back, skull, and sternum. Evidence of a gene-environment interaction has been observed for the vitamin D receptor. The relationship between lumbar spine bone mineral density and vitamin D receptor genotype varies according to birth weight. Among individuals in the lowest third of birth weight distribution, spine bone mineral density is higher in individuals with vitamin D receptor genotype “BB”. In contrast, individuals with this genotype in the highest third of birth weight distribution have reduced spine bone mineral density (Keen et al., 1997).

Consequences of disruption of hypothalamic regulation of pituitary prolactin secretion:
Maternal prolactin via the milk is required for normal development of the tuberoinfundibular neuronal system of the hypothalamus during the neonatal period in rats (Shyr et al., 1986). Blocking the suckling induced rise in maternal prolactin levels causes abnormal tuberoinfundibular function with decreased inhibition by dopamine of prolactin secretion in the offspring later in life. The resulting chronically elevated prolactin levels cause prostatitis in the male offspring (Tangbanluekal and Robinette, 1993).

4.3.3.2 Consequences of exposures

The consequences of chemical disruption of the development of the endocrine system or of endocrine function during early life have only begun to be explored (Dietert et al., 2002). Importantly, exposures during gestation or childhood may not manifest immediately. In some cases the latency period between exposure and effect may be years. Some examples of environmental chemicals and other environmental factors that are known to affect the human endocrine, metabolic, and cardiovascular systems with developmental consequences are discussed in this section.

A number of environmental chemicals alter thyroid homeostasis. In animal models, many polyhalogenated aromatic hydrocarbons like dioxins and polychlorinated biphenyls (PCBs) suppress levels of thyroxine (T4) and triiodothyronine (T3) by upregulating metabolic enzymes,
leading to increased glucuronidation and excretion of these hormones (Brouwer et al., 1998). In rats, PCB exposure during gestation has been shown to increase enzymatic activity of type II T4’5-deiodinase in foetal brain (Brouwer, et al., 1998). This is likely a compensatory response to decreased circulating levels of T4, as this enzyme converts T4 to T3, the more active form. Hydroxylated metabolites of PCBs also inhibit binding of T4 to the serum binding protein transthyretin, resulting in increased availability of free T4 for metabolism (Brouwer et al., 1998; Cheek et al., 1999). Transthyretin binding of PCB metabolites has been shown in animal studies to result in increased transport of these compounds from the maternal to the foetal compartment and to the foetal brain (Brouwer et al., 1998). There is concern that alterations in thyroid hormone signaling by these compounds during foetal and neonatal life could disrupt CNS development. There have been several long-term epidemiological studies of the neurobehavioral effects of in utero exposure to PCBs. Some of these studies have found significant associations between PCB exposure and cognitive and behavioral deficits, while others have not (NRC, 1999; Jacobson and Jacobson, 2003; Winneke et al. 2002). Thus far, there have been few studies that have directly linked neurobehavioral effects of exposure to polyhalogenated hydrocarbons to disruption of thyroid hormone signaling. One example is a study which showed that low-frequency hearing loss caused by developmental exposure of rats to PCBs could be partially reversed by replacement of T4 (Goldey and Crofton, 1998).

Perchlorates, which inhibit iodine uptake by the thyroid gland, reducing T4 and T3 synthesis, constitute another class of environmental chemicals that affect thyroid function. In the past, high doses of these compounds were used to treat hyperthyroidism. In recent years, there has been concern that contamination of drinking water supplies with perchlorates from industrial sites, could suppress foetal thyroid hormone synthesis, disrupting CNS development. Several ecological studies have addressed this issue, with some finding increased rates of congenital hypothyroidism in communities with detectable perchlorate levels in the drinking water (Brechner et al., 2000), and others observing no such difference (Lamm and Doemland, 1999; Kelsh et al., 2003). Clarification of this issue awaits the results of additional studies.

Several environmental exposures have been associated with poor growth and increased risk of diabetes, osteoporosis, and hypertension later in life. Lead poisoning in children causes poor
growth and abnormal bone structure. The mechanism is thought to be interference by lead with the metabolism of 25-hydroxycholecalciferol to the active form of Vitamin D, 1,2,5-hydroxycholecalciferol (Osterloh, 1991). Maternal smoking and poor nutrition during pregnancy are associated with reduced neonatal bone mineral content, presumably via effects on foetal nutrient supply and subsequent bone accretion (Cooper et al., 2002). There is also direct evidence that poor maternal nutrition (Ravelli et al., 1998) and maternal smoking (Montgomery and Ekbom, 2002) cause both a reduction in birth weight and subsequent loss of glucose tolerance. Adult humans exposed to dioxins have increased risk of diabetes, hyperinsulinemia, and abnormal glucose tolerance (Henriksen et al., 1997; Michalek et al., 1999). In contrast, exposure to dioxins causes hypoinsulinemia and hypoglycemia in adult rats and rabbits (Gorski and Rozman 1987; Ebner et al., 1988). Unfortunately, the effects of exposure to dioxins during early life stages on glucose homeostasis and the risk of developing diabetes have not been studied in humans or animals. Low birth weight individuals also have increased risk of developing hypertension later in life. It has been suggested that reduced growth of the kidneys during gestation, leading to a decreased number of nephrons, may be a causal factor (Brenner et al. 1988). The reduction of the filtration area leads to systemic hypertension via sodium retention and subsequent increased extracellular fluid volume. These cause increased cardiac output, total peripheral resistance and so increased arterial blood pressure. This leads to increased glomerular capillary pressure and glomerular sclerosis exacerbating the reduced surface area. From this it can be seen that individuals who suffer poor conditions in utero could be more susceptible to hypertension as they already have a reduced number of nephrons.

The herbicide atrazine suppresses pituitary prolactin secretion in adult rats and suppresses suckling-induced increases in prolactin secretion in lactating rats by stimulating hypothalamic dopamine secretion (Stoker et al., 1999; Cooper et al., 2000). By altering prolactin secretion in the mother, atrazine exposure during lactation causes elevated prolactin levels in the male offspring at puberty (Stoker et al., 1999). Subsequently, these males develop persistent inflammatory changes in the lateral prostate (Stoker et al., 1999).

The formation of the heart is one of the earliest events in development, as it is essential for the delivery of oxygen and nutrients to the rapidly developing cells of the embryo. The molecular
decision to form cardiac cells is made at the time of gastrulation. The heart begins to beat at 3
weeks of embryonic age. Important elements of cardiac formation include formation of the heart
forming fields as cells migrate out of the primitive streak, the segregation of cell lineage
(myocardial and endocardial) within the fields; the elongation and segmentation of the tubular
heart, the internal differentiation/ septation of first the atria, and later the ventricle, and
development of the conducting system. The heart also descends as it is developing, starting
cephalic to the somites and winding up at the mid-thoracic level. All this development takes
place while the heart is performing a critical function to the rest of the developing embryo
(O’Rahilly and Muller, 1992; Markwald et al; 1997). Molecular control factors for cardiac
morphogenesis are being elucidated, including T-box transcription factors (Stennard and Harvey,
2005), homeobox transcription factors (Akawaza and Komoro, 2005); and growth factors and

Periods of Vulnerability: The most vulnerable period of prenatal development of the heart
occurs between weeks 2 and 8 in humans. Congenital cardiac anomalies occur in about 5:100-0
to 1:8000 live births, and one-third of these are severe. Two percent of the anomalies are
thought to be primarily environmental in origin, with the vast majority arising from multi-
factorial causes. The most frequent types of defects are intraventricular septal defects,
coarctation of the aorta, transposition of the great vessels and tetralogies. With the significant
changes in blood distribution at birth (closing of the foramen ovale and ductus arteriosus that
shunts blood to the lungs of the newborn), at time prenatally induced changes in cardiac
development do not manifest until the neonatal period. During this transition to neonatal life,
there is also a progression of functional changes that include decreasing pulmonary vascular
resistance, increasing pulmonary blood flow, increasing volume of the left atrium, and increasing
systemic vascular resistance (O’Rahilly and Muller, 1992).

Consequence of Early Exposure: In experimental animal models, developmental cardiac
anomalies have been associated with a broad spectrum of agents and altered metabolic
conditions. For example, cardiomegaly is induced by thyroid hormone, carbon monoxide or
monocrotaline; cardiac glycosides and catecholamines produce arrhythmogenic effects; and
agents that affect the plasma osmolality such as mirex, trypan blue and 2-methoxymethanol
induce characteristic changes in the foetal electrocardiogram (Lau and Kavlock, 1992). There is evidence from mechanistically oriented experimental studies that malformations and other effects in the foetus induced by antihypertensive, antiarrhythmic, and sympathomimetic drugs are secondary to hemodynamic alterations causing foetal hypoxia rather than direct effects on the heart (Danielson and Weber, 1997). However, as the chronotropic (rate) and inotropic (contractile force) responses of the heart are regulated by the autonomic nervous system, and adrenergic innervation in particular has been implicated in controlling growth and development of the cardiac muscle, direct or indirect changes in adrenergic tone during critical periods can produce long lasting effect in the cellular development of the myometrium. In experimental animal models, several factors that alter adrenergic tone have been known to influence the prenatal maturation of the heart, including hormones (thyroid and glucocorticoid); therapeutic drugs (opiates and antihypertensives) and environmental compounds (e.g., mercury). This period of vulnerability also extends into the neonatal period in experimental animals (Lua and Kavlock, 1994). For instance, Meyer et al., (2004) studied the immediate and long term effects of exposure of neonatal rats to the organophosphate chlorpyrifos on gestation days 9-12, 17-20 or postnatal days 1-4 or 11-14. Cardiac responses were measured by pharmacologic challenge of adenylyl cyclase activity with forskolin, isoproteronol, and glucagons. Effects were most prominent in adulthood, with exposure of the three earlier stages the most effective. Interestingly, the effects on cell signaling pathways did not appear too related to the inhibition of acetyl cholinesterase, the pesticidal mode of action of chlorpyrifos (Song et al., 1997).

There are suggestions that environmental contaminants can affect cardiac development in humans. The effects of ambient air pollution (carbon monoxide, nitrogen dioxide, ozone, and particulate matter less than 10 microns) on the risk of cardiac malformations were evaluated using the California Birth Defect Monitoring Program in Southern California between 1987 and 1993 (Ritz et al., 2002). The odds ratio for a ventricular septal defect increased with increasing concentration of carbon monoxide during the second month of pregnancy. For the fourth quartile of exposure, the odds ratio was 2.95 (95% CI 1.44-6.05). They also observed increased risks for aortic artery and valve defects during the second month of pregnancy.

4.3.4 Cardiovascular System
The cardiovascular system is, out of necessity, the first of the organ systems to develop. The heart itself forms from a primordium from mainly splanchnic mesoderm (Kaufman and Navaratnam, 1981) and begins as a tubular structure which becomes a four-chambered structure after looping and septation. The development of a primary vascular plexus begins by the differentiation of precursor cells into endothelial cells that, after proliferation, cluster and form long extending endothelial processes. This primitive plexus outgrows into a complex network by the process of angiogenesis (Carmeliet, 2000). These processes require a variety of growth factors, genes, and transcription factors including vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β), ephrins and integrins (Risau, 1998). However, these do not exclusively control the process, both haemodynamic and metabolic factors can also alter the development of the cardiovascular system. Hypoxia acts via the redistribution of cardiac output to cause “brain sparing” (Cohn et al., 1974). This results from an increase in peripheral sympathetic outflow as well as catecholamine release. The process of generating arteries is by no means completed by the formation of the endothelial tube. Cells from the mesenchyme differentiate into vascular smooth muscle cells and this process is controlled by a different set of growth factors (Hellstrom et al., 1999). The thickness of the vascular smooth muscle cells is linked with cardiovascular disease and may continue to change in early postnatal life.

4.3.5 Immune System

The development of the immune system as it relates to comparative developmental toxicology has been considered in several recent publications (Barnett et al., 1996; Dietert et al., 2000; Holladay and Smialowicz, 2000; Chapin, 2002; Holsapple, 2002; Holsapple et al., 2003; Luster et al., 2003; Holladay and Blaylock 2002 ; Luebke et al., 2004). Immune development is a dynamic process involving cellular proliferation, migration, recognition, selection, apoptosis, clonal expansion, dissemination to peripheral sites, and finally cell cooperation and function. Because many of the changes require exquisitely timed differentiation events occurring in more than one site, there is ample opportunity for environmental interventions that can alter, delay or abrogate specific elements of immune development. Environmentally induced events altering immune development can be directed at immune cells. However, immunotoxic changes can also
arise if the physiological micro-environment necessary to promote immune cell maturation is
modified through environmental exposure. An example of the latter would be perinatal changes
in immune function linked to the pituitary-thyroid-immune axis (Rooney et al., 2003).
Obviously, such indirect changes could include the endocrine, neurological, hepatic or lymphoid
organ supporting tissues. Operationally, one may need to consider only the relative
developmental stage of toxicant exposure and the potential for serious outcomes later in life. But
mechanistically, it is useful to understand the potential for both direct immune cell insult and
indirect developmental alterations in supportive non-immune tissues such as thymic stroma, bone
marrow, and reticuloendothelial cell components that contribute to immune cell development.
Ironically, potentially subtle or transitory changes in these non-lymphoid cells, if occurring at a
critical time of immune development, might produce severe and prolonged immunotoxicity.
From a risk assessment perspective, it is helpful if those windows of greatest immune system
vulnerability can be identified.

4.3.5.1 Periods of Vulnerability
If one examines immune development, there are specific functionally distinct windows during
which the immune system might be expected to have different vulnerabilities based on critical
biological events. Five such windows were identified as likely candidates for differential
immune sensitivity (Figure 4.4) (Dietert et al., 2000; Landreth, 2002). By defining functionally
distinct immune developmental windows, it is possible to make direct comparisons of
differential immunotoxic vulnerabilities using exposure assessment.
The immune “windows,” along with developmental windows for the respiratory system, were identified at an EPA/March of Dimes sponsored workshop (see Dietert et al., 2000; and Landreth, 2002). They include: 1) initiation of hematopoiesis, 2) migration and expansion of stem cell populations, 3) colonization events including bone marrow colonization, pre-T cell seeding to the thymus, T cell education, T-cell repertoire establishment then seeding of the periphery by mature T cells, 4) acquisition of immunocompetence, 5) and finally the capacity to develop immunological memory. While the exact placement of these five “immunological boxes” on a gestational/perinatal timeline would differ between rodents and humans, the sequence of immune developmental events is similar (Figure 4.4). This approach of carving up development into distinct segments, or boxes, for direct sensitivity testing would seem to offer advantages that are not restricted solely to the immune system. Recently, additional developmental immune windows have been described which emphasize heightened perinatal vulnerabilities (Dietert and Pipenbrink, 2005).

Exposure to a low-level of an immunotoxicant during these different immune developmental windows might be expected to produce different outcomes. This is based on such factors as the
specific target cells present at the time of exposure, the cellular interactions in progress during exposure, the dependency on cell proliferation or migration and the extent to which immunotoxic damage might be contained or possibly reversed after environmental insult. As will be discussed later, the limited data available to date suggest that the developmental timing of exposure is critical in determining the nature and extent of environmentally-induced immune alteration.

One of the tenets of early immune development that has emerged in recent years is the apparent differential timeline for the development of certain immune capacities. In particular, several investigators have proposed that T-helper-2 (Th2) driven capacity is the earliest to form during ontogeny while T-helper-1-dependent (Th1) functions emerge in later development (Peden, 2000; Bellanti et al., 2003). The implication of this differential timeline is that Th2-associated capacity is a likely default function with useful immune balance being achieved when Th1 capacity can develop fully. If environmental exposures delay, impair or reduce the efficiency of timely Th1 development, then the neonate and potential subsequent life stages might face a skewed immune capacity toward Th2 driven functions. This could result in an increased risk of allergy and atopy as well as certain types of autoimmunity at the expense of protective anti-viral and anti-tumor Th1 driven responses (Bellanti et al., 2003). Therefore, chemical exposures that interfere with optimum Th1 maturation could leave the individual at increased risk for diseases requiring effective Th1-driven immune function. Additionally, some allergic and autoimmune conditions exacerbated by over-zealous Th2 function could become prevalent in segments of the population experiencing chemically-induced Th1 depression. Neonatal production capacity for interleukin 12 may be a factor in juvenile Th1/Th2 balance among responses (Prescott et al., 2003). The status of the maternal system appears to be an important risk factor for allergic disease at least within some subpopulations. Maternal stress has been identified as a contributing factor to altered T cell differentiation and potential risk of postnatal allergic disease (Von Hertzen, 2002). This suggests that foetal and perinatal environmental exposures may not need to target the immune system directly to disrupt subsequent T-associated immune capabilities. Several studies on endocrine disrupting gestational exposures support this likelihood (Ahmed et al., 1999; Karpuzoglu-Sahin et al., 2001; Dietert et al., 2003). Furthermore, some early environmental exposures during specific windows of gestation appear to result in unexpected
physiological responses when the juvenile or adult offspring encounters post-natal stessors (Karpuzoglu-Sahin et al., 2001; Lee et al., 2002).

An additional component of the T helper balance issue is a postnatal concern known as the ‘hygiene hypothesis.” This states, in its purest form, that exposure of the neonatal immune system to certain infectious agents and/or their immunostimulatory components (such as lipopolysaccharide of gram-negative bacteria) is an important component of immune maturation and may be necessary for appropriate Th1 development (Qi et al., 2003; Cremonini and Gasbarrini, 2003). However, other researchers dispute the “hygiene hypothesis” in favor of a tenet that it is the development of robust anti-inflammatory responses early in life which helps protect against or minimize allergic disorders (Yazdanbakhsh et al., 2002).

4.3.5.2. Consequences of Early Exposure

Numerous toxicants have been reported to alter the immune response capabilities and health outcomes following early exposure. The majority of these appear to alter thymus-associated T cell development and/or T cell-dependent functions. However, changes are not restricted to T cell function and some, like the pesticide, chlordane, target other immune cell lineages (Theus et al., 1992; Blyler et al., 1994). Even in the case of the heavy metal, lead, most notably known as a T cell toxin, early foetal exposure seems directed more against macrophages than T cells (Lee et al., 2001; Bunn et al., 2001a). Therefore, for some chemicals, it may be useful to specify the primary immune target of toxic exposure in the context of developmental life stages.

As might be expected, the outcomes of early-life-stage induced immunotoxicity take several forms. T-dependent functions are frequently impaired, and these alterations may be more persistent than with similar adult toxicant exposure (Dietert et al., 2003; Luebke et al., 2004). The heightened sensitivity of T dependent function to early life immunotoxicant exposure may be linked to the dramatic reversal of Th balance and changes in dendritic cell maturation that occur before and after birth (reviewed in Dietert and Piepenbrink, 2005). This can lead to increased susceptibility to both infectious diseases and cancer. Additionally, asthma, atopy and some forms of autoimmunity may be at an increased risk based on specific cell-mediated
immune changes. Gender differences have been seen following certain exposures (Blyler et al., 1994; Bunn et al., 2000; 2001b,c; Chapin et al., 1997). Little is known presently about the impact of early toxicant exposure on the onset and rate of immune senescence.

While the database for developmental immunotoxicants is relatively modest, several chemicals have received considerable research attention. For example, Luebke et al. (2004) in a report to the EPA, reviewed the comparative age related sensitivities of the human and rodent immune systems for four developmental immunotoxicants: diethylstilbestrol (DES), lead, diazepam, and tributyltin. These authors concluded that DES, a strong estrogenic compound, produced similar immune alterations at similar doses following exposure of adult rodent and embryos or neonates. However, the immune changes persisted following early exposure, while adults appear to be able to recover post exposure.

With the heavy metal, lead, the evidence suggests that rodent foetuses are sensitive to lower doses of lead than are required to produce adult immune alterations. Immunotoxicity is persistent following early exposure and, depending upon the timing (the critical window) of exposure, different combinations of juvenile and adult immune changes will result. Immune sensitivities appear to be comparable to those reported for the neurological system (Canfield et al, 2003; Dietert et al., 2003). Rodent data suggest that early exposures to lead (producing <8 ug/dL blood lead levels at or near birth) are associated with subsequent immunotoxicity (Snyder et al., 2000; Bunn et al., 2001b; Dietert et al., 2003; Luebke et al., 2004). Furthermore, in rats, lead exposure from mid-gestation appears to reduce Th1 capabilities skewing immune responses toward Th2 (Heo et al., 1997; Miller et al; 1998; Bunn et al., 2001c; Dietert and Lee 2005; Dietert et al., 2004). This would be expected to increase the risk of allergy and asthma while decreasing certain anti-viral and anti tumor responses. Additionally, changes in autoimmune responses have been reported (Bunn et al., 2000). There is an immune developmental period in which the capacity of low level lead exposure to modulate T cell response in the offspring appears to be the greatest. This period of increased sensitivity (compared against adult-induced immunotoxicity) corresponds to the developmental period during which stem cells migrate, progenitor cell populations expand and the bone marrow and thymus are colonized (Dietert et al., 2000; Landreth, 2002).
In a study on diazepam, a drug in the benzodiazepine family (Luebke et al., 2004) concluded that late gestational and early neonatal exposure of rodents produced severe immunosuppression at lower doses than were required for similar effects in adults. Furthermore, the cellular and humoral immunosuppression following foetal and neonatal exposures was persistent, while the adult-induced immunosuppression appeared to be short-lived. With the organotin wood preservative compounds found in some paints (tributyltin oxide and tributyltin chloride) the results appeared to be similar (Luebke et al., 2004). The tributyltin compounds appear to target the thymus, producing thymic atrophy and widespread immunosuppression in rodents with exposure at sufficient doses. However, the doses required for adult-induced suppression appear to be significantly greater than those found to produce immunosuppression after perinatal exposure (Smialowicz et al., 1989).

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD dioxin) is another important developmental immunotoxicant that has been extensively examined in animal models (Vos and Moore, 1974; Faith and Moore, 1977). TCDD can target very early precursor T-cells in the bone marrow (Fine et al., 1989), cause profound atrophy of the thymus (Gehrs and Smialowicz, 1999; Gehrs et al., 1997), inhibit thymocyte maturation (when given during gestation) (Holladay et al., 1991; Blaylock et al., 1992), persistently depress T–dependent immune responses including delayed type hypersensitivity (DTH) (Gehrs and Smialowicz, 1999) and increase susceptibility to infectious diseases and tumor cells (Holladay and Smialowicz, 2000). It appears that early life stages are not only more sensitive to lower doses than are adults (at least in rodents), but that early exposure seems to produce more persistent immunosuppression (Holladay et al., 1991; Gehrs and Smialowicz, 1999; Holladay and Smialowicz, 2000). Smialowicz (2002) discusses the capacity of TCDD to suppress the DTH response following perinatal (Gehrs and Smialowicz, 1997; 1999; Gehrs et al., 1997) vs. adult (Fan et al., 1996) exposure. He concludes that placental plus lactation exposure were the most sensitive exposure windows for DTH depression in the rat, and the differential age-related sensitivity for TCDD exposure is greater than two orders of magnitude for this DTH suppression.
Two organochlorine pesticides have also been evaluated in animal models using early life stage exposures. Methoxychlor (Chapin et al., 1997) and heptachlor (Smialowicz et al., 2001) were evaluated for immunotoxicity after perinatal plus juvenile exposure of rats. In the case of methoxychlor, T-dependent antibody responses were depressed persistently in males but not females (Chapin et al., 1997). For heptachlor, early exposure of Sprague-Dawley rats, using doses relevant to human exposure, produced persistent impairment of antibody responses in males but not females. No adult-exposure immunotoxicity was observed at the doses examined, suggesting there is an increased susceptibility of the pre-natal and/or early post-natal life stages to this pesticide (Smialowicz et al., 2001; Smialowicz, 2002).

Two recent studies have reported on immune effects of human exposure to PCBs and DDE (a metabolite of the organochlorine insecticide DDT) in humans (Dewailly et al, 2000; Dallaire et al, 2004). In both of these studies, Inuit infants were recruited and followed during the first year of life for the occurrence of infections of various kinds. This population has high exposure to PCBs and other organochlorines because of their high consumption of carnivorous fish and marine mammals. Various PCBs and DDE were measured in cord blood and/or maternal plasma at delivery (Dewailly et al, 2000; Dallaire et al, 2004) and in the infants’ blood at follow-up (Dallaire et al, 2004). In both studies, the risk of otitis media during the first year of life increased with increasing pre-natal exposure to PCBs and DDE (Dewailly et al, 2000; Dallaire et al, 2004), although most of the associations did not reach statistical significance. In the latter study, the risk of all infections combined was significantly increased with increasing pre-natal PCB level (Dallaire et al, 2004).

In summary, early life stage exposure to environmental hazards can produce significant and persistent immunotoxicity. For some chemicals, adult-induced immunotoxicity has not been observed or the effect is transitory. Consequences following early exposure can include increased susceptibility to infectious disease and cancer, increased risk for asthma and atopy and an increase in some forms of autoimmune disease. For some chemicals, gender differences have been noted and the developing immune system may have greater than an order of magnitude difference in dose sensitivity compared against adults. Additionally, evidence suggests that the expected outcome of exposure can differ depending upon the window of immune development.
when exposure occurs. Hence, the developmental status of the immune system during environmental insult is a key factor in determining the likely health risk.

4.3.6 Normal Development Respiratory System

Development of the human lung begins in the embryo and continues until the age of 18-20 years. Cellular differentiation and formation of the primary lung structures occur in stages during foetal development, but the majority of growth and maturation of the lung occurs postnatally through the processes of branching morphogenesis and alveolarisation. The major antenatal and postnatal developmental milestones are summarized in Chapter 3, Table 3.2.

Many of the studies on the effects of chemical exposures on the growth and development of the lungs have been performed in experimental animals; but patterns of lung development differ between animals and humans. Because of these differences, extreme care must be taken when extrapolating the results from animal studies to human situations.

4.3.6.1 Periods of Vulnerability

The respiratory system has a number of critical windows of exposure and periods of vulnerability (Pinkerton et al., 2000). Lung development occurs through 6 stages: embryonic, pseudo-granular, canalicular, saccular, alveolar, and vascular maturation. The first four of these stages are complete during foetal development, and about 85% of alveoli are present in the human at birth as shown in Figure 4.5 (Zoetis and Hurtt, 2003a). As lung development is a continuous process from embryo to adolescence it is logical to surmise that children may be more vulnerable to the effects of respiratory toxicants than adults whose lung growth is complete. Alveoli number and lung surface area begins to level off between 2 and 4 years of age, while lung expansion continues to up to 8 years of age. Immature (neonatal) differentiating cells of the respiratory tract are more sensitive to injury following exposure to respiratory toxicants than mature cells, and at dose levels that cause no effects in adult cells (Plopper et al. 1994). Lung injury in the early post-natal period impairs cellular differentiating capacity and proliferation, producing abnormal post-natal lung growth and development in rabbits (Smiley-Jewell et al., 1998).
Exposure of neonatal primates to oxidative insult (via ozone exposure) has been shown to impair the development of pulmonary gas exchange units and bronchioli (Tyler et al., 1991). Experimental studies in rats clearly defined the critical window of exposure to environmental tobacco smoke as both the pre- and post-natal periods (Joad et al. 1995; Joad et al. 1999). Clinical parallels are seen in studies of human infants born to smoking mothers, demonstrating reduced lung function in those exposed to ETS in utero and post-natally (Hanrahan et al., 1992; Tager et al., 1983).

Figure 4.5 Parallel Time Lines for Development of the Lung in Mouse/Rat and Human (from Dietert et al., 2000)

Many studies try to distinguish between environmental exposures that induce disease in previously normal hosts or trigger exacerbations of pre-existing disease. From the above discussion, we contend that this is an artificial distinction and is more likely to result from the developmental phase of the host when the exposure took place rather than an intrinsic property of the exposure agent. More research has concentrated on relating environmental exposures to triggering exacerbations of respiratory disease, as this is technically easier. Studies designed to understand the role of environmental exposures on the induction of disease are more difficult and
longitudinal assessment of exposures and disease outcomes are required. Birth cohort studies are particularly powerful in this respect but are expensive and require a long-term commitment.

4.3.6.2 Consequences of exposures

Air pollution, both outdoor and indoor, has been identified as a potential risk factor for both the initiation/induction and the exacerbation of respiratory diseases, especially asthma. Pollutants or irritants that may influence immune system development and the induction or exacerbation of respiratory diseases include:

- Combustion related products formed by the burning of organic fuels, including nitrogen dioxide, particulate matter and diesel exhaust particulates;
- Bio-aerosols including molds, allergens and bacterial products (e.g. lipopolysaccharide - LPS);
- Air toxics including formaldehyde and other volatile organic compounds;
- Pesticides, PCBs and heavy metals.

Indoor Air. Indoor air is potentially the most important source of pollution affecting child respiratory health given that children spend up to 90% of their time indoors and the large range of pulmonary irritants found in the home (Woodcock and Custovic, 1998). Infants and young children in particular have little control over their exposure to the home environment and are vulnerable to the impact of lifestyle activities (e.g., tobacco smoking) of the adult occupant(s). The potential for exposure to pollutants in the home environment has increased with improved insulation and reduced ventilation, and the use of chemical detergents and building or furnishing constituents that contain noxious pulmonary irritants. Common indoor air pollutants include NO$_2$, formaldehyde, volatile organic compounds (VOCs) and environmental tobacco smoke (ETS).

Studies examining the effect of indoor NO$_2$ on the respiratory health of children, though inconsistent, identify NO$_2$ as a potential hazard. The principal source of NO$_2$ in westernized homes is gas cooking and heating appliances, though combustion of unprocessed fuels such as wood and coal, and tobacco smoke contributes to NO$_2$ exposure globally. The extensive use of
gas appliances in homes suggests large populations of children are at risk of exposure (IEH, 1995). NO₂ levels have been associated with increased risk of cough and wheezy bronchitis in children (Dodge, 1982; Pershagen, 1995), and rises in ambient NO₂ levels of 30 micrograms/m³ are reported to increase the risk of respiratory illness in children by 20% (Hasselblad et al., 1992). Asthmatic children may be particularly vulnerable to the effect of NO₂, experiencing airflow limitation and increased use of inhalant therapy (Jarvis et al., 1998; Ng et al., 2001). Studies have linked indoor NO₂ levels to increased asthma risk and increased bronchial hyper-responsiveness in preschool children (Salome et al., 1996; Volkmer, 1995).

VOCs are components of household detergents, adhesives and furnishings. Ambient levels of VOCs, including formaldehyde (HCHO) have been linked to reported asthma in children (Ware et al., 1993; Krzyzanowski et al., 1990; Garrett et al., 1999; Rumchev et al., 2000; Rumchev et al. 2002). The weight of evidence currently available linking VOCs to ill health in adults suggests that ambient levels found in homes are unlikely to cause adverse effects, but research focusing on the impact of VOCs on the respiratory health of children is sparse. HCHO has been associated with increased prevalence of atopy (Garrett et al., 1999) and higher levels of specific IgE (Wantke et al., 1996) in children. HCHO, at levels typically encountered in homes, is associated with airway inflammation in both healthy children (Franklin et al., 2000) and adults (Wieslander et al., 1997). Exposing animals to HCHO (250 ppb) enhances sensitization to inhaled allergens (Riedel et al., 1996) an observation that supports the role of HCHO as a co-factor for post-natal Th2 boosting. A study by Lehmann et al. (2002) found that maternal exposure to VOCs was associated with increased Th2 cytokines (IL-4) and decreased Th1 cytokine (IFN-γ) in the cord blood of neonate.

One of the most important combustion pollutants in Western environments, particularly for indoor air, is environmental tobacco smoke (ETS), which contains not only combustion related pollutants but also a large number of air toxics and carcinogens, including particulate matter, NOx, aldehydes and oxygen free radicals that act as pulmonary irritants or cilio-toxins. The effects of passive smoking begin in utero where constituents of tobacco smoke, such as PAHs, nicotine and carbon monoxide, cross the placenta and are concentrated in the foetal circulation (Perera et al., 1999). Foetal enzymatic pathways are immature and do not effectively detoxify
and clear tobacco smoke leading to an accumulation of toxic metabolites at a period of intense cellular differentiation and growth (Ruhle et al., 1995). The potentially mutagenic effects of tobacco smoke may impair normal cellular division and differentiation in the respiratory tree leading to reduced lung function and increased bronchial hyper-responsiveness (Collins et al., 1985; Young et al., 1991; Cook et al., 1998). Animal studies demonstrate histological changes including hyperplasia of bronchial muscles and prematurity of lung tissues in the foetal lung secondary to the effects of in utero exposure to tobacco smoke (Neslon et al., 1999). The decreased lung function and increased bronchial hyper-responsiveness observed in infants born to mothers who smoked in pregnancy may predispose infants to wheezing and lower respiratory illnesses (Hanrahan et al., 1992; Tager et al., 1983; Tager et al., 1995).

Prenatal and postnatal exposures to ETS are independently related to an increased risk of incident asthma (Infante-Rivard et al., 1999; Strachan and Cook, 1998a; Cook and Strachan, 1999). Parental smoking greater than 10 cigarettes per day increases the risk of asthma amongst children 2.5 times (Martinez et al., 1992), and may increase their risk of atopic sensitization in a dose-response pattern (Braback et al., 1995; Ronchetti et al., 1990). Major meta-analyses show that lower respiratory tract illness is up to 60% higher amongst children exposed to ETS during the first 18 months of life (NHMRC, 1997), and risks of chronic and recurrent otitis media are significantly increased (Strachan and Cook, 1998b).

Bio-aerosols that have also been implicated in the development of asthma and other allergies include inhaled allergens (house dust mite and other insects, moulds, pets, pollens) and bacterial (LPS) and fungal (glucans) products. Sensitization to one or more common inhalant allergens is consistently associated with childhood asthma, especially in western countries (Sporik and Platts-Mills, 2001). However, the relationship between exposure to inhaled allergens in early life and the development of asthma or wheeze in childhood is controversial (Sporik et al., 1995; Lau et al., 2000; Burr et al., 1993). Indeed it seems that early exposure to some allergens may be protective of later development of asthma (Hesselmar et al., 1999) (see also discussion of the “hygiene hypothesis” in Section 4.3.4). For example, some studies have found that exposure to LPS in early life protects against the development of allergy in children who have regular contact with farm animals compared to those without contact (von Mutius, 2002). Children raised in
farming environments with livestock are exposed to very high levels of LPS (Gerda et al.,
2000a). The mechanism for the protective effect of exposure to farm animals is thought to be via
induction of Th1 immune responses (Gerda et al., 2000b). Interestingly, increased exposure to
LPS in non-farming domestic environments has been associated with an increased risk of
recurrent wheeze during the first year of life in children with a family history of allergy (Park et
al., 2001); however, the levels of exposure are substantially less than those experienced by
farmers’ children. A recent study of rural Iowa children seems to contradict the “hygiene
hypothesis” that early life exposure to farm animals is protective against allergic diseases.
Merchant and coworkers (2005) found that exposure to farm animals early in life was not
protective against asthma. Those children living on farms that raised swine actually had
significantly higher rates of diagnosed asthma and/or asthma symptoms compared to those not
living on farms or to those living on farms that did not raise swine (Merchant et al, 2005). These
authors suggested that previous studies which considered only doctor-diagnosed asthma may
have underestimated the true prevalence of asthma among farm children (Merchant et al, 2005).

Another source of exposure to pollutants in the indoor environment is via the combustion of
biomass (such as dung, charcoal, wood or crop residues) or coal. The WHO estimates that
worldwide approximately 50% of all households and 90% of rural households use such fuels for
cooking or heating. Biomass fuel emissions include respirable particulates, carbon monoxide,
nitrogen oxides, benzene, formaldehyde, 1,3-butadiene and PAH compounds such as
benzo(a)pyrene. Women and young children are most heavily exposed to indoor air pollution
from biomass combustion. Children under five years of age have been estimated to have a
strong elevated risk of acute lower respiratory tract infections, particularly in developing
countries. One example of such a study in India provided an elevated risk of 2.3 fold (WHO,
2004b).

Ambient Air. There is a consistent body of evidence that exposure to ambient air pollution is
associated with increased respiratory symptoms of cough, bronchitis, respiratory infection and
upper respiratory symptoms in children (Raizenne et al., 1996; Dockery et al., 1989). Although
the effects of pollutants appear small, they occur at levels within the national ambient air quality
standards (NAAQS) of most countries, and have the potential to affect large populations of
children. Most studies attribute respiratory symptoms to particulate matter (PM), though the
close correlation of PM levels with NO2 and SO2 levels makes the contribution of individual
pollutants difficult to determine. In Switzerland, moderate levels (below NAAQS) of PM10, SO2
and NO2 were associated with increased reporting of chronic cough and bronchitis (Braun-
Fahrlander et al., 1997). This relationship was stronger amongst those children with a family
history of asthma. Estimated relative risk for reported cough of 1.16 for every 20 microgram/m3
rise in PM10 was reported in Swiss preschool children (Braun-Fahrlander et al., 1992). Similar
results were seen in a review of 3 cross-sectional surveys of children in East Germany between
1990 and 1998 with odds ratios for cough and bronchitis of 1.20 per 10 mg/m3 increase in PM10
(Heinrich, 2003). The decline in pollution levels over this period was associated with an age-
adjusted decrease in respiratory symptoms of bronchitis by 16%, cough by 1.2%, otitis media by
4% and upper respiratory tract illness by 8%. This suggests the respiratory symptoms caused by
air pollution are potentially reversible (Heinrich, 2003). Pollutant exposure has also been linked
to increased reporting of upper respiratory tract infection (Heinrich, 2003; Jaakkola et al., 1991).
Ozone may cause a small increase in reported respiratory symptoms of cough and bronchitis at
peak levels greater than 80 ppb (Braun-Fahrlander et al., 1997). Diesel exhaust emissions have
been associated with increased reporting of cough and non-specific respiratory symptoms in
children (Hirsch et al., 1999; Wjst et al., 1993; Oosterlee et al., 1996; Nitta et al., 1993;
Nakatsuka et al., 1991). Children living in close proximity to major freeways with high truck
density experience increased cough, wheeze and rhinitis (Van Vliet et al., 1997), and children
less than 2 years of age may be most susceptible (Gehring et al., 2002).

Most of the literature to date on air pollution and asthma has concentrated on acute exacerbations
resulting from environmental exposures. The role of environmental irritants in the induction of
asthma is much less well understood. Various air pollutants can enhance allergic sensitization in
animal models (Gilmour, 1995) and are thought to play an important adjuvant role in the
development of asthma. Several studies have demonstrated that pre-natal exposure to air
pollutants have an affect on cytokine profiles in cord blood (Perera et al., 2003; Lehmann, 2002),
and on the inflammatory effects in human airways (Parnia et al., 2002). Further research is
required to elucidate the mechanisms by which environmental irritants modulate the
development of asthma and atopy.
Oxidant gases such as NO₂ and ozone have also been studied with regards to their role in the development of asthma. Both of these gases have been associated with asthma exacerbations (Parnia et al., 2002), increased airway responsiveness to inhaled allergens in asthmatic subjects (Jenkins et al., 1999), and can produce inflammatory changes in the airways (Davies and Devalia, 1993; Devalia et al., 1999). Asthmatic children exposed to exhaust emission experience more symptoms and reduction in peak flow measurements, and increased hospital admissions for asthma exacerbations (Wyler et al., 2000).

Acute exposure to criteria pollutants is known to cause transient reversible decreases in lung function (Vedal et al., 1998; Pekkanen et al., 1999). It is plausible that cumulative exposure to pollution throughout childhood could adversely affect airway maturation and therefore lung function. Reduced lung function in children and adolescents associated with chronic exposure to ozone (Kunzli et al., 1997) and annual mean particulate matter levels (Raizenne et al., 1996) has been shown, though studies are not consistent (Roemer et al., 1999; Dockery et al., 1989) and are limited by assumptions that measured ambient levels reflect personal exposure. Particulate matter, NO₂, SO₂ and ozone (individually and synergistically) have been implicated in reductions in lung function growth in children and adolescents. Expiratory flows, calculated from maximal forced expiratory maneuvers (proxy measurements of large and small airway growth) appear primarily affected. Decrement in average annual growth rates of forced expiratory flow between 25% and 75% of exhaled vital capacity (FEF₂₅₋₇₅) and the forced expiratory volume in one second (FEV₁) at a magnitude of 11% and 5% per year respectively were found in pre-adolescent children exposed to high levels of acid vapor and PM₂.₅ in California (Gauderman et al., 2002). Studies in Austria found a 2% reduction in FVC and FEV₁ for every 10 ppb rise in ozone in 7 year olds exposed to relatively low levels of ambient ozone (Horak et al., 2002) and large decrements in FEV₁ (-84mls/year) and FEF₂₅₋₇₅ (-329mls/yr) for every 10 microgram/m³ increase in PM₁₀ in children exposed to particulate levels below NAAQS. Increments in FEF growth rates in children have been observed upon relocation to residences with lower ambient pollution levels (Avol et al., 2001) again suggesting the detrimental effect of pollution is potentially reversible. Reduced lung function amongst children living close to freeways has been described
(Brunekreef et al., 1997). Personal exposure studies are required to confirm these findings and their clinical implications to determine whether lung function deficits persist into adulthood.

4.3.7 Kidney

Chapter 3, Section 3.6.8 describes the normal development of the kidney.

4.3.7.1. Periods of vulnerability

Development of the metanephric kidney begins with an outgrowth of the ureteric bud from the distal region of the mesonephric (Wolffian) duct during the embryonic period. The ureteric bud must grow into the mesenchyme of the nephrogenic cord. Upon contact the mesenchyme epithelializes to form a nephron, and this process is repeated over and over as the ureteric bud branches. Ultimately, the branching of the ureteric bud results in the formation of the major and minor calyces (the large ducts that empty into the renal pelvis) and the system of collecting tubules. The two major calyces form from the first branching of the ureteric bud, around the end of the sixth week in humans. Secondary branches arise from these, which in turn give rise to tertiary branches. About twelve generations of branching occur by the end of the fifth month, which essentially completes the formation of the collecting duct system.

Although the general pattern of nephron formation is similar across mammals, there are marked species differences in the timing of development. The onset of nephron development starts at approximately the same stage of embryogenesis in all species that have been evaluated, but because of differences in the length of the embryonic period, the days of gestation differs. In humans, metanephric kidney developments starts around gestation day 35 while in the rat it starts on gestation day 12, and in the mouse, day 11. Induction and differentiation of nephrons occur continuously through the 38th week of gestation in humans and for 10-12 days post-natally in rats and mice. There are approximately 1.5 million nephrons per kidney in humans, and 1000-2000 in mice. The end of anatomical development of the kidney is marked by the completion of nephrogenesis, and as noted, is complete before birth in humans, but not in rodents. Functional development including glomerular filtration, concentrating ability, acid-base balance and urine
volume control generally do not mature until after birth in most species. Zoetis and Hurtt (2003b) have published a review on comparative aspects of kidney development.

4.3.7.2 Consequences of Exposure

As evident from the developmental sequence of ureteric bud outgrowth, induction, proliferation and sculpting, the critical period for vulnerability of the developing kidney to toxicants is prolonged. Lau and Kavlock (1994) provide a representative list of renal teratogens and related critical periods for renal teratogenesis. In general, exposures during the time of early kidney development tend to cause the most severe structural and functional alterations, perhaps because they alter the earliest interactions and therefore have the most profound consequences on kidney development. Direct acting cytotoxicants (e.g., chlorambucil) are very effective at disturbing development during periods of rapid cell proliferation that occurs during induction of the anlagen. Alteration of differentiation of the ureteric epithelium or inhibition of breakdown of the ureteric membrane (e.g., by dioxin) can result in hydronephrosis. In the foetus, excessive disturbance of normal physiology due to pharmacologic agents working on immature feedback can alter amniotic fluid production with secondary consequences to the lungs. Guignard and Gouyon (1988) reviewed effects of a variety of vasoactive or diuretic drugs, ACE inhibitors, adrenergic agents, and noted that several drugs used to treat sick, and often premature infants, also posed a risk of compromising renal function due to poorly developed feedback loops. Alteration in trophic signals that regulate cell proliferation and differentiation during the rodent post-natal period (e.g., by methylmercury) can result in altered organ growth. Finally, direct effect on cell proliferation during histogenesis (e.g., by gentamicin) can also alter renal development. Because nephrogenesis continues post-natally in the rat, toxic insult during the neonatal period may still produce permanent developmental effects. For example, treatment with DFMO (Gray and Kavlock, 1991) or gentamicin (Gilbert et al., 1987) reduces growth and differentiation of the kidney when given to newborn rats.

A species comparison of the effects of angiotensin converting enzyme inhibition has been published (Tabacova et al., 2003). It is noteworthy in understanding why the human foetus suffers adverse consequences from exposure whereas rodent foetuses do not. The greater
susceptibility of the human foetus to pharmacologic agents such as enalapril and other ACE inhibitors is a function of the relative maturity of the kidney and the renin-angiotensin system, which are the specific targets during intrauterine development. In humans, these systems begin developing at the end of the first trimester, with continuing vulnerability throughout the pregnancy. The timing is quite different in most animal species tested: these target systems develop close to birth and after the exposure period in standard developmental toxicity protocols has ended. At these later stages, these systems are relatively more mature and less vulnerable to the pharmacologic effects. For this reason, animal studies that follow standard protocols and evaluate developmental toxicity only for exposures during embryogenesis miss developmental effects arising secondary to disruption of target systems that develop after the period of major organogenesis. Thus, differences in the timing of development of the critical target organ systems, the renal system and renin-angiotensin system, explain the absence of definitive structural abnormalities in test animals.

It is important to note that there does not appear to be a good concordance between those agents that induce renal toxicity in the adult versus those that induce developmental renal toxicity. This is the result of the different cellular processes involved in organ development versus organ function. It is also interesting to note that the developing kidney is not always more sensitive to a toxicant than the adult kidney. Thus, while the proximal tubular toxicant gentamicin treatment has been used to study reduced glomerular function in the neonatal rat, the age class is remarkably resistant to other proximal tubular toxicants (mercuric chloride, sodium fluoride, and dichlorovinylcysteine). The former result is probably due to the immature status of the brush border in the neonatal rat, while the later is probably reflective of immaturity in biochemical differentiation, as the agent requires activation by β-lyase.

4.4 Cancer

Cancer is uncommon during the first two decades of life, but is nonetheless a substantial concern. In the USA cancer is diagnosed in approximately 12,400 children and adolescents annually, and is the most common cause of death from any kind of disease between 1 and 19 years of age. In the US and other western countries, lymphoid neoplasms (leukemia, lymphoma)
and cancers of the central nervous system are the most common pediatric malignancies. Other kinds of childhood tumors include embryonal tumors of the retina, sympathetic nervous system, kidney and liver; tumors of bone and soft connective tissues; and certain gonadal neoplasms. Different kinds of cancer (e.g., carcinomas of liver or thyroid) may predominate in children in parts of the world where specific environmental risk factors are more prevalent.

Exposures to cancer-causing agents preconceptionally, during intrauterine life, or in early childhood may develop cancer during later childhood or during subsequent adult life (Figure 4.6). Cancers that arise many years after carcinogenic exposures early in life may originate from different cell types and occur in different organs than the cancers that characteristically occur in infants and children.

![Figure 4.6 Impact of Timing of Developmental Exposure on the Type of Cancer Developed (from Anderson et al., 2000)](image)

There is direct evidence that children are more susceptible than adults to at least some kinds of carcinogens, including certain chemicals and various forms of radiation. Data from controlled experimental studies in animals also support the concept that susceptibility to some chemical carcinogens and to various forms of ionizing radiation is greatest during the early stages of life, both before and after birth (Tomatis and Mohr, 1973; Napalkov et al., 1989; Birnbaum and
There is also evidence of increased or even unique early-life susceptibility to cancers that result from infection with certain oncogenic viruses, including Epstein-Barr virus and hepatitis B virus. Anderson et al., (2000) have reviewed epidemiological and experimental animal studies that investigated the relative susceptibility to carcinogens at different life stages. Windows of enhanced susceptibility were observed for many agents and organ systems during the embryonic, foetal, and neonatal periods (Anderson et al., 2000).

4.4.1 Childhood cancers that may have environmental causes

Lymphoid tissues. Leukemia was the first cancer to be linked with exposure to radiation from the atomic bombings at Hiroshima and Nagasaki. Excess relative risk for leukemia was higher than for any other neoplasm in bomb survivors, and for people exposed as children. Radiation-related leukemia started to occur 2-3 years after the bombing, reached its peak within 6-8 years, and has declined steadily since then. For people exposed as adults, the excess risk was lower than that of people exposed as children but appears to have persisted throughout the follow-up period (IARC, 2000). Small increases in childhood leukemia may also have occurred in some populations that were exposed to radioactive fallout from nuclear weapons tests, but different studies of this possible association have not produced consistent findings (Chow et al., 1996).

Prenatal diagnostic x-irradiation has also been linked to increased risk of leukemia in offspring, as has therapeutic, high dose, ionizing radiation in childhood for other cancers and for various non-neoplastic conditions (Ron et al., 1988a; Chow et al., 1996).

Exposure to non-ionizing radiation in the form of extremely low frequency magnetic fields is statistically associated with a small increased risk of leukemia during childhood (2-fold or less), but no carcinogenic effect of such electromagnetic fields has been convincingly demonstrated in experimental animals and no biologically plausible mode of carcinogenic action for non-ionizing radiation has been identified (IARC, 2002a).
Chemical exposures that are related to childhood leukemia include high-dose therapies for cancer and other serious diseases. Cancer chemotherapeutic regimens that included topoisomerase II inhibitors have caused acute myeloid leukemia in children with acute lymphoblastic leukemia who were treated with these regimens (Pui et al., 1991). Cancer chemotherapeutic regimens that included alkylating agents for treatment of various childhood cancers increased the risk of developing leukemia as a second malignancy (Tucker et al., 1987a). Use of the antibiotic chloramphenicol has also been associated with subsequent development of acute leukemia in children (Shu et al., 1987; Chow et al., 1996).

Infection with Epstein-Barr virus (EBV) in early childhood is common worldwide, but in combination with malaria infection it causes a characteristic non-Hodgkin lymphoma in equatorial African children. African Burkitt lymphoma (small non-cleaved B-cell lymphoma, Burkitt type) accounts for 30-70 percent of childhood cancers in equatorial Africa, with about 5-10 cases per year per 100,000 children below the age of 16 years and a peak incidence between five and 10 years of age. The disease is an extranodal lymphoma, involving the kidneys, ovaries, adrenals and characteristically the jaws and the orbit. It is overwhelmingly associated with EBV infection and holoendemic malaria: the neoplasm occurs most frequently in a geographic belt across Africa that has high rainfall and abundant mosquitos. It is thought that malaria infection stimulates the B-cell system and facilitates the emergence and proliferation of EBV-infected and potentially neoplastic B-lymphocytes (IARC, 1997). African Burkitt lymphoma is an example of a locally common pediatric malignancy that is caused entirely by infectious processes, without a discernable etiologic contribution by chemical agents, ionizing radiation, or major predisposing genetic factors.

Liver. Hepatitis B virus (HBV) infection is spread by transfusion of blood from infected individuals and from mothers to children during the perinatal age period. HBV can cause a chronic active infection that leads to hepatocellular carcinoma (Blumberg 1997). Risk of hepatocellular carcinoma is greatly increased by the combination of HBV infection and dietary exposure to naturally occurring aflatoxins which can heavily contaminate certain staple food crops including maize and peanuts (Turner et al., 2002; IARC, 2002b). In regions of the world
where prevalence of chronic HBV infection is high and aflatoxin contamination of foodstuffs is also common, hepatocellular carcinoma often develops during childhood. Liver cancer is sufficiently common in children 6 to 14 years of age in Taiwan and that this age group has been used to evaluate the efficacy of hepatitis B vaccination for prevention of infection in offspring of infected mothers and to confirm that successful vaccination reduces the incidence of hepatocellular carcinoma (Chang et al., 2000). Aflatoxin has been detected in cord blood and breast milk in areas of Africa and Asia that have high rates of food contamination with this fungal toxin. In Thailand, 17 of 35 samples of cord sera contained aflatoxin at concentrations of 0.064 to 13.6 nmol/ml, whereas only 2 of 35 samples of maternal sera contained aflatoxin (Denning et al, 1990). Aflatoxin was detected in 37% of Sudanese, 28% of Kenyan, and 32% of Ghanaian breast milk samples (Maxwell et al., 1989). In Gambia, the amount of aflatoxin excreted in the breast milk is estimated to be 0.09% to 0.43% of the dietary intake (Zarba et al., 1992). Further, infants in Gambia receive not only in utero exposure but also continuing postnatal exposure to AF, as the AFB1 level in the sera of children does not differ markedly from that of adults (Wild, 1990). To what extent embryonic, foetal, and neonatal exposures compared to later exposures to aflatoxin contribute to the development of liver cancer in children is not known.

Thyroid. The Chernobyl nuclear reactor accident in April 1986 caused an epidemic of thyroid carcinomas in children. Vast quantities of radionuclides including $^{131}$I, other short-lived isotopes of iodine, and $^{137}$Cs were released, mainly during a period of 10 days following the accident, and contaminated large areas of the Ukraine, Belarus, and the Russian Federation. A significant increase in the incidence of thyroid cancer, generally of the papillary type, has occurred as a result in children in these three countries since 1990 (Table 4.1). Most of the tumors have been observed among individuals who were very young at the time of the accident (IARC, 2001). In Belarus, over half of the tumors occurred in people who were < 6 years old at the time of the accident. In a series of 472 children with thyroid cancer diagnosed up to 1995 in Belarus, only two percent had been conceived after the accident; nine percent had been exposed in utero, and 88 percent were under 15 years of age at the time of diagnosis (Pacini et al., 1997). What is unique about thyroid cancers resulting from the Chernobyl accident is the very early age at which the cancers have begun to be diagnosed. The unique vulnerability of the thyroid gland to...
external ionizing radiation during childhood is one of the most striking examples in human
experience of special sensitivity to a carcinogen other than an infectious agent occurring
exclusively during pre-adult life.

Brain and nervous system. Brain tumors have occurred in children who had received therapeutic
doses of ionizing radiation to the head, especially for the treatment of tinea capitis (Ron et al.,
1988b) or lymphoid neoplasms (Brustle et al., 1992). Iatrogenic tumors are however a very
small fraction of all pediatric brain tumors. Except for certain genetic factors, causes of the most
common brain tumors of childhood, including primitive neuroectodermal tumors and
astrocytomas, remain unknown (Rice, 2004).

Other organ sites. Survivors of the Hiroshima and Nagasaki atomic bombings who were
exposed to ionizing radiation in utero have experienced a significantly increased risk of solid
tumors of childhood. Individuals exposed after birth have no such increased risk. It has been
suggested that this difference may result from the tumor precursor cells being susceptible to
neoplastic transformation only during the pre-natal period (Wakeford and Little, 2003). Both
radiotherapy and chemotherapy for cancer are associated with increased risk of second cancers at
various sites in children. Increased risk of bone sarcoma has been documented in children with
various initial tumors who were treated with radiation or with cancer chemotherapeutic regimens
containing alkylating agents (Tucker et al., 1987).

4.4.2 Adult cancers related to childhood exposures

Exposures to carcinogens during childhood have caused tumors that appear chiefly in adulthood.
Examples include tumors of the brain, cranial nerves and meninges after therapeutic irradiation
of the head; thyroid carcinoma after therapeutic and environmental exposures to ionizing
radiation; leukemia and solid tumors in adult survivors of the Hiroshima and Nagasaki atomic
bombs who were exposed in childhood; and skin cancer after intense childhood exposures to
solar radiation. In addition, treatment of pregnant women with synthetic non-steroid estrogens
(e.g., DES) has caused tumors of the female reproductive tract in the adolescent and young adult
offspring of these pregnancies.
Brain and nervous system. Therapeutic ionizing radiation to the head during childhood has been shown to cause tumors of the meninges and of the brain and cranial nerves in later life. For meningiomas a clear dose-effect relationship has been recognized, with higher radiation dose leading to increased risk. Meningiomas typically occur in middle-aged and elderly individuals, but also occur in children and in the very old. Meningiomas that result from radiation exposures during childhood can arise in late adult life, after latencies of 30 years or more, and can have extraordinarily long latencies; a case with a 63-year latency period has been reported (Kleinschmidt and Lillehei, 1995). As with chemical carcinogenesis in experimental animals, tumor latency is inversely proportional to intensity of exposure: average latencies of 35, 26, and 19-24 years of age have been reported for meningiomas induced by low-, moderate-, and high-dose radiation, respectively (Harrison et al., 1991; Kleinschmidt and Lillehei, 1995). Irradiation to treat tinea capitis at a retrospectively estimated mean X-ray dose per patient of 1.5 Gy was associated with a significantly increased relative risk of 8.4 for neurogenic tumors of the head and neck, including meningiomas (Ron et al., 1988a). This is a relatively low therapeutic dose, compared to what is used for anti-tumor therapy.

Thyroid. Between 1920 and 1960 radiotherapy was widely used to treat a variety of non-neoplastic conditions, including tinea capitis, tonsillar hypertrophy, and “thymic enlargement” which was not then recognized as the normal state of the thymus in childhood. Significantly increased risks of thyroid carcinomas later in life have resulted from these relatively low-dose therapeutic radiation exposures in childhood. Excess relative risks per Gy averaged 32 for thyroid carcinomas following X-ray treatments for tinea capitis. Individuals exposed as very young children, less than 5 years old, had an excess relative risk more than twice that of children exposed between 5 and 15 years of age. In contrast, external exposures to ionizing radiation during adult life have not been linked convincingly to thyroid cancer (Ron, 1996). High-dose radiation exposures of pediatric cancer patients have also resulted in thyroid carcinomas as second cancers, with a positive linear dose-response above doses as low as 0.1 Gy.

Thyroid carcinomas are also increased in survivors of the atomic bombs at Hiroshima and Nagasaki who were children at the time of the bombings in 1945, and in inhabitants of the
Marshall Islands who were accidentally exposed as children to fallout from one of the above-ground US nuclear weapons tests in 1954 (Ron, 1996). These thyroid cancers, like the thyroid cancers in individuals exposed to radiotherapy as children, have occurred mostly in adults. Among survivors of the atomic bombings, the most pronounced risk for thyroid cancer occurred in individuals with an external radiation dose to the thyroid greater than 1 Sv before the age of 10 years, and the highest risk was seen 15-29 years after exposure (IARC, 2001). Radiation from the Chernobyl accident and from atomic bomb detonations differs fundamentally from what is used in therapeutic radiology, in that the exposures from Chernobyl and from atomic detonations included both external gamma radiation and internal irradiation from radionuclides of iodine that were selectively deposited in the thyroid.

Female breast. Ionizing radiation is a known environmental cause of female breast cancer. Breast cancer risk is significantly elevated in female survivors of the atomic bombings at Hiroshima and Nagasaki, but varies significantly depending on age at the time of radiation exposure. Relative risk at estimated exposure levels of 1 Sv was approximately 3-4 for women exposed before 10 years of age or between 10 to 20 years of age, but decreased to approximately 2 in women irradiated between 20 and 40 years of age and decreased even further in women exposed after 40 years of age (Boice et al., 1996).

Female reproductive tract. Diethylstilbestrol (DES), a synthetic non-steroid estrogen, was extensively prescribed in Western countries from the late 1940s through the 1970s to women with high-risk pregnancies to prevent miscarriage and other complications of pregnancy. In 1971, Herbst et al. reported that pre-natal DES exposure was associated with a rare form of female reproductive tract cancer, clear cell adenocarcinoma of the vagina, in daughters of women who had taken the drug during pregnancy (Herbst et al., 1971). Vaginal clear cell adenocarcinoma occurs in only 0.1 percent of women who were exposed to DES in utero, but this represents a 40-fold excess risk in comparison to the non-exposed general population. As of 1985, 519 cases of clear-cell carcinoma of the vagina and cervix had been recorded by the Registry for Research on Hormonal Transplacental Carcinogenesis at the University of Chicago (USA). Of these, 311 cases had a definite history of exposure to DES in utero, and 91 per cent of these 311 cases were diagnosed between 15 and 27 years of age (Melnick et al., 1987). Cases
were diagnosed up to 34 years of age (later extended to 48 years of age) at which time more than 700 cases had been recorded by the Registry (Herbst, 1999). In contrast, men who were exposed to DES in utero do not have a clearly increased risk of any cancer, although a statistically non-significant 3-fold increased risk of testicular cancer has been reported (Strohsnitter et al., 2001).

Integument. Solar radiation and sunburn in childhood are significant risk factors for malignant melanoma of the skin. Numerous studies have assessed the carcinogenic effect of sunburn at different ages and concluded that childhood exposures were the most significant. A study in the U.K. assessed sunburn at different ages and found that the strongest association between elevated cancer risk and sunburn occurred at 8-12 years of age (Elwood et al., 1990). Duration of residence in Australia and the associated exposure to intense solar radiation is strongly associated with risk of developing malignant melanoma; and numerous studies indicate that childhood is an especially vulnerable life stage (IARC, 1992). For example, the Western Australia Melanoma Study showed a strong inverse correlation of age on arrival in Australia as an immigrant with risk of developing melanoma, indicating that individuals arriving there as early as 10 years of age were less likely to develop this cancer than native-born Australians of European descent or immigrants arriving at ages younger than 10 years, and that childhood exposure to the intense solar radiation of Australia was therefore a primary contributor to risk of melanoma (Holman and Armstrong, 1984).

Other organ sites. The Radiation Effects Research Foundation’s Life Span Study of atomic bomb survivors has reported that for all solid tumors combined, there is clear evidence of a radiation dose response relationship. Both excess relative risk and excess absolute risk are larger for individuals exposed as children than for those exposed as adults, and solid tumor risk continues to increase in later years (Kodama et al., 2003). Survivors of the atomic bombs also have increased risk of all kinds of solid tumors, including those of adult life, although the degree of susceptibility varies with age at the time of the bombings and is generally highest early in life.

4.4.3 Chemical exposures of special concern
Ionizing and solar radiation cause cancers in adults by damaging cellular DNA, and clearly have also caused cancers in children. Exposures to chemicals or chemical mixtures which are known to be carcinogenic to adults, especially DNA-reactive drugs, have also caused cancers in children. This occurs principally under the intense and prolonged exposure conditions that are associated with antitumor chemotherapy and certain other medical treatments (Chow et al., 1996).

Chemicals with DNA-damaging modes of action are likely to cause cancers in children if the children themselves, or their mothers during pregnancy, suffer sufficiently intense or prolonged exposures. It is prudent to regard any exposure to such substances as potentially carcinogenic. Whether children are likely to be more or less susceptible than adults to such chemicals is likely to depend on how a given chemical is absorbed, distributed in the body, and metabolized in younger versus older individuals, at the exposure levels encountered by children and pregnant women (Neri et al., 2005).

Tobacco smoke contains many DNA-damaging chemicals, including PAHs and 4-aminobiphenyl, and is categorized as a Class I carcinogen by the International Agency for Research on Cancer (IARC). These compounds are transplacentally transferred to the foetus. The genotoxicity of tobacco smoke to the foetal liver has been tested in an animal study. Sister chromatid exchange (SCE) in the liver cells of foetal mice was analyzed at the 16th day of gestation after short-term exposure (twice, on the 15th and 16th days of gestation), long-term exposure (starting 4 weeks before mating and stopping on the 16th day of gestation), and pre-pregnancy exposure (4 weeks before mating). The number of SCEs was significantly increased in all exposure groups, and long-term exposure caused a significantly higher increase than did short-term exposure (Karube et al., 1989)

PAHs are metabolically activated to diol epoxides by phase I drug metabolizing enzymes (e.g, cytochrome P450 and monooxygenase) and form DNA adducts by binding to genomic DNA. The formation of DNA adducts is an initiation step in mutagenesis and carcinogenesis as well as a useful marker of exposure to mutagens. The metabolism of carcinogens by foetal tissues and their extracts has been extensively studied in experimental animals (Anderson et al., 1989), and
10 of 28 human placentas examined had DNA adducts containing diol epoxide metabolites of the
PAH benzo[a]pyrene (Manchester et al., 1988). Enzyme-linked immunosorbancy assays
identified PAH–DNA adducts (0.63 to 2.51/10⁷ nucleotides) in the livers of 4 of 15
spontaneously aborted human foetuses (gestational age, 17 to 23 weeks) in the US (Hatch et al.,
1990). Administration of the PAH 3-methylcholanthrene to pregnant mice causes hepatic tumors
in the offspring (Anderson et al., 1985).

Alkylating agents such as nitroso compounds are potent mutagens that bind to a nucleotide on
genomic DNA without metabolic activation by monooxygenase. Some of these compounds are
transplacentally transferred and cause mutagenesis and carcinogenesis. Administration of N-
nitrosodimethylamine to pregnant mice induces DNA fragmentation in the livers of foetuses
(Bolognesi et al., 1988). In addition, the treatment of pregnant rats with a single dose of ethyl
methanesulfonate, ethyl-N-nitrosourea, N-nitrosodiethylamine, or methyl-N-nitrosourea causes
alkylation and fragmentation of DNA (Robbiano et al., 1989). N-nitroso compounds have been
shown to exert transplacental carcinogenic effects (Anderson et al., 1989). For example, pregnant
C3H mice were injected with N-nitrosodimethylamine (NDMA; 7.4 mg/kg bw), N-
nitrosodiethylamine (NDEA; 51 mg/kg bw), or N-nitrosoethylurea (NEU; 41 mg/kg bw) on day
16 or 19 of gestation. Administration of NDMA on either gestational day 16 or 19 significantly
increased the number of female offspring with hepatocellular carcinoma, and the incidence of
hepatocellular carcinoma in male progeny was significantly elevated by administration of
NDMA on gestational day 19. In addition, exposure to NEU on day 19 of gestation significantly
increased the incidence of hepatocellular carcinoma among female offspring. These results
indicate that sensitivity to N-nitroso compounds is dependent on gestational age.

Inorganic arsenic was long considered to be carcinogenic to adult humans but not to rodents, but
has recently been shown to be a multitissue transplacental carcinogen in mice. Sodium arsenite
(As III) given ad libitum in drinking water to pregnant mice caused a significant dose-dependent
increased incidence and multiplicity of hepatocellular adenomas and carcinomas and increased
incidence of adrenal cortical adenomas in male offspring, and dose-dependent increases in
ovarian and lung tumors in females (Table 4.1) (Waalkes et al., 2003). Inorganic arsenic in
various forms (including arsenic trioxide, As₂O₃ [As III]) has been previously tested extensively
for carcinogenicity in adult rodents, with negative or equivocal results. At least in mice, inorganic arsenic is a much more potent carcinogen to the foetus than to adults.

Inorganic arsenic (arsenate plus arsenite) in drinking water has recently been evaluated by the WHO as carcinogenic to humans, increasing risk of tumors of urinary bladder, lung, kidney and skin (IARC, 2004). Inorganic arsenic is an important human carcinogen in parts of the world where very high levels of arsenic contaminated drinking water occurs. The mode(s) of carcinogenic action of inorganic arsenic in rodents and in humans are not yet fully understood, but the possibility exists that inorganic arsenic in drinking water poses a special concern for pregnant women and their unborn infants. The toxic effects of arsenic in pregnant women and in young children may possibly include increased risk of carcinogenesis.

Vinyl chloride, a known occupational carcinogen, has been studied at different life stages in animals. Rats treated with vinyl chloride beginning during the neonatal period for one year had much higher rates of liver hemangiosarcomas than did rats treated for the same duration of time starting at age 11 weeks (Maltoni et al., 1981). Males treated from the neonatal period had a 16.7 fold higher incidence of the tumors than did males treated as adults. Females treated from the neonatal period had an even higher increased incidence; however, the ratio was not calculable because no cancers occurred in the females treated as adults only (Maltoni et al., 1981).

4.5 Conclusions

In this chapter we have attempted to highlight the importance of the timing of exposure to chemicals or other insults in determining the consequences to children’s health. As illustrated in Table 4.1, the special vulnerabilities of children relative to adults is clearly manifest looking across the various life stages, organ systems, and exposure scenarios. The fact that these vulnerabilities exist, combined with the fact that the impacts are felt throughout the affected individuals’ lifetime, raises the importance of understanding the contributions of environmental chemicals so that preventative measures can be taken to relieve the burden of disease. Several key points emanate from the analysis of the current state of the science:
• The windows of vulnerability of children are broad, and extend from the pre-conception period through to the end of the adolescent period.

• Given the special nature of development, it is sometimes difficult to make predictions about which exposures might pose greatest risks to children. However, those agents that influence key signal transduction pathways, modify cell proliferation or cell differentiation rates, activate apoptotic pathways, or react with DNA seem to present the most concern.

• There are both qualitative and quantitative aspects to the potential heightened sensitivity of children. Thus, the target organ effects observed following exposure during early life stages are not necessarily the same as those seen following exposure during adult life. Whether or not there are similar target organs, exposures that are relatively without effect in adults can prove damaging during early development.

• For the early post-fertilization life stages, the true extent of susceptibility is difficult to gauge due to methodological difficulties in diagnosing early pregnancies, but several studies have shown this to be a period of high pregnancy loss, and the role of environmental factors cannot be ruled out.

• The manifestations of the effects are often delayed in appearance from the life stage where the critical exposure occurred, thus making determination of cause and effect relationships problematic. The greatest challenge is to detect effects related to functional alterations of organ systems. This is due to a combination of lack of easily applicable methods and to the reserve capacity of organ systems that can modulate deficits in function. As epidemiological studies that have tracked children longitudinally are rather sparse, there is a tendency to rely on animal models to understand the potential extent of the problem,

• The comparative rate of development has to be understood when examining the effects of exposures observed in laboratory animal models with human development. Major
differences can occur in the interspecies extrapolation of both toxicokinetic and
toxicodynamic information depending on the comparative rates of organ maturation.
This must be done on a organ system by organ system basis, as the relative rates of
development vary among organ systems and across species.

• Unlike the general situation in adult, transient changes in physiology or endocrinology at
critical periods of development can result in permanent changes in organ function.

• Even for adverse outcomes which have been clearly linked to exposures during specific
life-stages, there is a very limited understanding of the mechanisms by which the
exposures cause the outcomes. For example, additional studies are needed on the
mechanisms by which intrauterine growth restriction causes subsequent diabetes,
cardiovascular disease, obesity, and hypertension, and to understand the role of
alterations in foetal thyroid function on subsequent neurobehavioral outcomes.

• There is clearly the potential for gene - environment- chemical interactions that can either
ameliorate or enhance the risk of an adverse health outcome depending on the nature of
the influences and the involved life stage. Non-chemical environmental factors, such as
socioeconomic status, also are capable of interacting with these chemical exposures.

• There is a pressing need to develop and validate sensitive, specific, and cost effective
biomarkers of exposure, susceptibility, and effect that can be applied to human studies so
that the gaps in our understanding of the role of environmental stressors on children’s
health can be closed.
CHAPTER 5 - EXPOSURE ASSESSMENT OF CHILDREN

5.1 Introduction

Children’s environmental health risks result from exposure before conception, during the prenatal period, and through childhood and adolescence. In this context, a child can be defined by a series of life stages from conception through adolescence where each life stage has distinct anatomical, physiological, behavioral, and/or functional characteristics that contribute to potential differences in vulnerability or exposures.

Children, like adults, may be exposed to chemicals through the air they breathe, the water they drink, the foods they eat, and the surfaces and materials they contact. Children also have unique routes of exposure including transplacental exposures for the developing fetus, and ingestion of breast milk for infants. Because of their unique physiology and behavior, children’s exposures may be higher than adults and as a result children may have greater health risks than adults in the same environments. In this chapter, we will review general principles of exposure assessments with a discussion of methods for conducting exposure assessments, including biomonitoring of internal dose and other biomarkers. The unique characteristics of children that would result in differential exposures for children at different ages or life stages are then discussed. Finally, we will describe those situations that are likely to result in high exposures to children throughout the world because of their location, culture, socioeconomic status, or unique activities.

5.2 General Principles of Exposure Assessments

Exposure is defined as the contact (at visible external boundaries) of an individual with a pollutant for specific durations of time. For exposure to occur, an individual must be present and must come in contact with a contaminated medium. Exposure results in absorbed dose when chemicals enter the body. Exposure is described in terms of the intensity, frequency, and duration of contact (USEPA, 1992a). The intensity of contact is typically expressed in terms of the concentration of contaminant per unit mass or volume in the medium to which humans are
Exposed. Exposure is expressed as mass per unit time. Absorbed dose is expressed as mass per unit volume (e.g., body weight) or surface area.

Exposure is one element in the environmental health framework depicted in Figure 5.1 that links contaminant sources to health effects. Exposure assessment evaluates the processes for identifying potentially exposed populations; identifying potential pathways of exposure; and quantifying the magnitude, frequency, duration, and time-pattern of contact with a contaminant.

The processes that are important for exposure assessment are shown in the boxes on the left-hand side of Figure 5.1. Starting in the upper left-hand corner, chemical or biological contaminants are released from a source. Ambient sources are those that release contaminants into the outdoor environment and include automobiles, power plants, manufacturing facilities, waste sites, and agricultural spraying. Other sources release contaminants directly into indoor environments where people live, work, and play. These include consumer products, building materials, pesticide products, combustion sources for heating and cooking, and environmental tobacco smoke (ETS).

Many contaminants can be transformed in the environment through a number of processes including chemical reactions and biological degradation. Contaminants from ambient sources or their transformation products are transported through the environment to locations where people spend time and will be found in environmental media at these locations including air, water, food, dust, and soil. Contaminants from indoor sources also distribute themselves among these media in the location where they are used. Exposure occurs when an individual actually comes in contact with the contaminated environmental media and the contaminant is transferred to the individual. The amount of exposure depends upon the concentration of contaminants in the media as well as the activity pattern of the individual that define the frequency, duration, and intensity of the contact. Exposure becomes dose when the contaminant moves across the body barrier; it becomes target tissue dose when the contaminant or metabolite interacts at the target site that ultimately leads to an adverse health outcome.
The text under each pink box in Figure 5.1 shows the information that is needed to characterize the process represented in the box. This information is usually developed through laboratory and field measurement studies. The orange boxes show the types of models that are used to link the processes and to estimate exposure, absorbed dose, or target tissue dose.

Many chemicals such as lead, dioxins, PCBs, and organochlorine pesticides are persistent in the environment. Thus, exposures will continue to occur over time as long as there is contact with the contaminated medium. Longitudinal exposure can also occur for non-persistent chemicals since sources for most chemicals provide reoccurring releases into the environment. Understanding both the frequency and the timing of exposures is very important for the developing fetus and young child. For many contaminants, there are discreet developmental windows during which exposure may lead to adverse health outcomes. For other pollutants, exposure accumulated over time is the important exposure metric.
Children’s exposure to environmental contaminants is a complex process that can occur as a result of release of pollutants from many sources that can reach the child through a number of different routes and pathways (Hubal et al., 2000). Aggregate exposure assessments evaluate exposure from all sources, routes, and pathways for a single contaminant. For example, a pesticide exposure may result from an agricultural application to foods as well as applications in homes, schools, daycare centers, and recreational areas to control pests. For children of agricultural workers, exposure can occur if the child stays with the parent in the field or if the parent transports pesticides into the home via their skin or clothing. For older children, direct occupational exposure to pesticides is an additional route.

Cumulative risk assessments evaluate the health risk for aggregate exposures accumulated over time and for multiple contaminants or stressors. Cumulative risk is best applied to populations to understand the impact of all stressors on that population or individuals in the population. Figure 5.2 illustrates the concept of cumulative exposure showing both exposure and dose to multiple contaminants as well as considering susceptibility factors and other stressors. There is currently no internationally accepted framework for assessing such scenarios. Populations may be defined by their location relative to sources, their activities and customs, and their vulnerability to exposures. In this context, populations can include different ethnic groups, different communities, or different age groups. Cumulative risk is a very important concept in understanding environmental health risks to children in different settings, particularly in underdeveloped countries where children may be facing multiple stressors.

Route of exposure is defined as the portal of entry to the body. Pathway is defined as the course that the contaminant takes from its source to the exposure medium, and then to the portal of entry. For a given source, exposure media and exposure routes can define the pathways. Depending upon the life stage of the child, exposure media can include amniotic fluid, breast milk, air, water, soil/dust/sediments, food, and objects/surfaces. Exposure routes include transplacental transfer, inhalation, ingestion, dermal absorption, and indirect (non-dietary) ingestion.
Exposure media will also change with life stage. For example, the fetus will be exposed via amniotic fluid, the infant to breast milk, the teething child to many objects (both intended and unintended) for mouthing, the school age child to contaminants in the classroom, and the adolescent to vocational or recreational hazards.

Exposure factors are those factors related to human behavior and characteristics that determine an individual’s exposure to a contaminant. In a simple case, a child’s exposure to ozone by the inhalation route is determined by factors that include the duration of time spent in different indoor and outdoor locations during the day and the child’s breathing rates during the period of exposure. Differences in exposure factors, including location, activity, and behavior are primarily due to differences in age, gender, culture, geographical location, or socioeconomic status. Relative to body weight, children eat more food, drink more water, and breathe more air than adults. They also eat different foods. An extreme example is the almost total reliance of infants on breast milk for nutrition. Children are often in different environments than adults; but even when in the same environment they interact differently to give more direct contact. A more complete discussion of the characteristics of children that affect exposure is given in Section 5.4.
5.3 Methods for Conducting Exposure Assessments

5.3.1 Direct Methods

Direct assessments measure the contact of the person with the chemical concentration in the exposure media over an identifiable period of time. Direct assessments are made through field monitoring studies of children in their everyday environments. In such studies, data are collected on pollutant concentrations in a variety of exposure media (i.e., air, drinking water, food, house dust, surface residues), activities, and exposure factors so that exposure can be measured or estimated for each child in the study. When measurements are made on multiple people, the interpersonal variability in exposures can be evaluated. When the group of individuals for measurement is selected using probability-sampling methods, then exposure distributions for the population can be estimated. Information on the highest exposures and the factors associated with these exposures is important for understanding and mitigating risks. Intrapersonal variability can be estimated by conducting repeated measurements on the same person over time. This information can be used to estimate intermittent or chronic exposures for individuals.

For assessing exposure, it is important to collect all of the data on exposure media concentrations, activities, and exposure factors that are required to quantify exposure (Cohen Hubal et al., 2000a, USEPA 2001). As an example, Table 5.1 shows the data requirement for estimating exposure by several routes. For inhalation and dietary exposure, personal samples can be used to estimate exposure. For inhalation exposure, the concentration of contaminant in personal air samples is combined with breathing rate. For dietary ingestion, the concentration of contaminant in duplicate diet samples is combined with the amount of food eaten. Methods for indirect ingestion as a result of mouthing or eating soil or dust are less straightforward. Here information on contaminant concentration in the environmental media (surface residue, dust, soil) is combined with factors that estimate the contact rate and the transfer rate. For dermal exposure, information on surface residues is combined with factors that estimate contact rate and transfer rates to the skin.
Table 5.1. Summary of the Algorithms and Data Collection Requirements by Exposure Route (USEPA 2001b)

<table>
<thead>
<tr>
<th>Parameter - Measurement</th>
<th>How Collected</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhalation Exposure</strong> (E&lt;sub&gt;ime/ma&lt;/sub&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;ame&lt;/sub&gt; - Air Concentration in microenvironment (&lt;em&gt;ame&lt;/em&gt;)</td>
<td>Measured with active sorbent collection</td>
<td>µg/m&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;me/ma&lt;/sub&gt; - Time spent in each microenvironment/ macroactivity (&lt;em&gt;me/ma&lt;/em&gt;)</td>
<td>Time-activity diary, questionnaire</td>
<td>h/d</td>
</tr>
<tr>
<td>IR&lt;sub&gt;ma&lt;/sub&gt; - Inhalation Rate (&lt;em&gt;ma&lt;/em&gt;)</td>
<td>Estimated from size, age, and activity data collected with diaries and questionnaires using reference values</td>
<td>m&lt;sup&gt;3&lt;/sup&gt;/h</td>
</tr>
</tbody>
</table>

**Dietary Ingestion Exposure** (E<sub>f</sub>)

\[ E_f = C_f W_f \]

C<sub>f</sub> - Concentration of pesticide in the food(<em>f</em>) item | Measurement in individual food items or composite duplicate diet samples | µg/kg |

W<sub>f</sub> - Weight of food(<em>f</em>) item consumed | Measured in duplicate diet sample | kg/d |

**Indirect Ingestion (Dietary and Non-Dietary) Exposure** (E<sub>ingmi</sub>)

\[ E_{ingmi} = C_{x} T_{Ex} S_{Ax} E_{F} \]

C<sub>x</sub> - Concentration surface(surf) loading (total or transferable) on object <em>x</em> | Measure by a wipe or press method | µg/cm<sup>2</sup> |

T<sub>Ex</sub> - Transfer Efficiency<sup>a</sup> (<em>x</em>) | Empirically determined from laboratory experiments | unitless |

S<sub>Ax</sub> - Surface Area contacted(<em>x</em>) | Visual observation or videotape | cm<sup>2</sup>/event |

E<sub>F</sub> - Frequency of mouthing Events | Visual observation or videotape | events/d |

**Dermal Exposure - Macroactivity Approach** (E<sub>dme/ma</sub>)

\[ E_{dme/ma} = C_{surf} T_{Cme/ma} A_{Dme/ma} \]

C<sub>surf</sub> - Concentration surface(surf) loading (total or transferable) in each microenvironment | Measured by wipe, press, or roller methods | µg/cm<sup>2</sup> |

T<sub>Cme/ma</sub> - Transfer Coefficient<sup>a</sup> (<em>microenvironment/macroactivity</em>) | Empirically determined for each me/ma from laboratory experiments or field studies | cm<sup>3</sup>/h |

A<sub>Dme/ma</sub> - Activity Duration for in a specific microenvironment/macroactivity (<em>me/ma</em>) | Time-activity diary, questionnaire | h/d |

---

<sup>a</sup> This parameter must be calculated using the same surface loading measurement method as used to measure C<sub>surf</sub>, factors that estimate the contact rate and the transfer rate. For dermal exposure, information on surface residues is combined with factors that estimate contact rate and transfer rates to the skin.
5.3.2 Biomarkers of Exposure

Biomarkers do not measure exposure directly, but are an indicator of absorbed dose. A biomarker of exposure is defined as a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism and can be related to exposure. Urine, blood, nail, saliva, hair, and feces are common media collected for biomarker measurements. Maternal biomarkers of exposure can also be measured in amniotic fluid and breast milk. These matrices can also provide a measure of exposure for children, both prenatally and postnatally. Biomarkers in first teeth have also been used to assess early childhood exposure while biomarkers in meconium and cord blood have been used to assess in utero exposures. Biomarkers of genetic damage (e.g., DNA adducts) have been extensively used to assess exposure to genotoxic agents (Neri et al., 2006).

When appropriately validated and understood, biomarkers present unique advantages as tools for exposure assessment (Gundert-Remy et al., 2003). Biomarkers provide indices of absorbed dose that account for all routes and integrate over a variety of sources of exposure (IPCS, 1993; IPCS, 2001). Certain biomarkers can be used to represent past exposure (e.g., lead in bone), recent exposure (e.g., arsenic in urine) and even future target tissue doses (e.g., pesticides in adipose tissue). Once absorbed dose is determined using biomarkers, the line has been crossed between external exposure and the dose metrics that reflect the pharmacokinetics and toxicokinetics of an agent (see section 5.3.3).

Currently, there are only a few cases where biomarkers can be used for quantitative exposure assessment. Biomarkers can be used to indicate that a person has been exposed and that the chemical has been absorbed into the body. They can often be used to rank exposure among individuals. Biomarkers alone cannot provide information on the source, route, and duration of exposure. Only in certain cases can biomarkers be used to give information on the frequency, duration, and intensity of exposure. Even with these limitations, biomarkers when appropriately validated can effectively be used to evaluate trends in these exposures (CDC, 2005), and determine the effect of exposure mitigation strategies as well as predict target tissue dose.
5.3.3 Modeling

Models use mathematical expressions to quantify the processes leading to exposure and dose. Models that predict dispersion, fate, transport, and transfer of chemicals are based on physical and chemical principles. Models that describe activities of individuals as they interact with the environment are based on statistical data from observational measurement studies. In Figure 5.1, the processes that must be accounted for from source to dose are described; the text above the boxes shows the types of models that can be used to quantify these processes. These models can be applied to predict exposure and dose for an individual; however, they are most effectively applied at the population level.

Deterministic models use a single value for input variables and provide a point estimate of exposure or dose. Probabilistic models take into account the fact that most input variables will have a distribution of values. These models use probability distributions to develop a range of plausible exposures for the population of concern. Understanding exposure distributions will allow understanding of the range of exposure as well as predicting risk for the entire population. It will also allow prediction of risk for the most highly exposed individuals. Sophisticated models can be used to develop distributions for different pathways and populations. They can also be used to develop information on inter-individual variability and uncertainty in the estimated distributions and to predict the variables that are most important for both exposure and dose.

Exposure models use available information on concentrations of chemicals in exposure media along with information about when, where, and how individuals might contact the exposure media to estimate exposure. For population assessments, distributional data on exposure factors and environmental concentrations are used to estimate exposure distributions for a population. Examples of various exposure models are summarized in Table 5.2.
Table 5.2 Examples of Models for Estimating Exposure

<table>
<thead>
<tr>
<th>Model</th>
<th>Type</th>
<th>Application</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrated Exposure Uptake Biokinetic (IEUBK) model</td>
<td>Aggregate; stochastic with probabilistic output; sensitivity analysis.</td>
<td>Estimates long-term lead exposure for children (6mos. To 7 years old) across multiple pathways, routes and environmental media, estimates lead blood concentrations</td>
<td>USEPA, 2004a</td>
</tr>
<tr>
<td>Stochastic Human Exposure and Dose Simulation (SHEDS) model</td>
<td>Aggregate; mechanistic modeling framework with statistical components and probabilistic capabilities. Sensitivity analyses, uncertainty estimations and inferences of source or pathway contributions.</td>
<td>Estimates exposure and absorbed dose of several pollutants types across multiple pathways and routes of exposure and various environmental media. Daily through annual absorbed dose is simulated for any aged individual considering time-series of exposure (up to 1 minute resolution).</td>
<td>Burke et al., 2001; Graham et al., 2005; Zartarian et al., 2000</td>
</tr>
<tr>
<td>LifeLine</td>
<td>Aggregate and cumulative; stochastic</td>
<td>Simulates daily pesticide exposures for periods from birth up to 85 years to pesticide applicators, residents of homes where pesticides are used and the general population (dietary and tapwater consumption)</td>
<td>LifeLine, 2005</td>
</tr>
<tr>
<td>RISK and Indoor Air Quality and Inhalation Exposure (IAQX)</td>
<td>Indoor air mass balance with multiple sources and sinks</td>
<td>Risk from inhalation exposure of volatile organic compounds; solvents; airborne particulate matter from indoor and outdoor sources.</td>
<td>Guo, 2002</td>
</tr>
<tr>
<td>Dietary Exposure Potential Model (DEPM)</td>
<td>Model and database system for deterministic dietary exposure.</td>
<td>Exposure from pesticides in diet; combines food consumption and residue data</td>
<td>USEPA, 2003a</td>
</tr>
<tr>
<td>Complex Exposure Tool (ComET)</td>
<td>Deterministic;</td>
<td>Risk screening model; estimates high-end exposure to chemicals from both consumer products and environmental background (multiple media).</td>
<td>LifeLine, 2004</td>
</tr>
<tr>
<td>Hazardous Air Pollutants Model (HAPEM)</td>
<td>Semi-stochastic, sequential simulation I producing aggregate exposure distributions</td>
<td>Used by USEPA to evaluate national air toxic exposures to hazardous air pollutants (part of Trim.Expo model)</td>
<td>Palma et al., 1996</td>
</tr>
<tr>
<td>Air Pollutants Exposure (APEX) model</td>
<td>Stochastic time-series simulation model producing probabilistic exposure distributions</td>
<td>Used by USEPA to evaluate National ambient air quality standards. Also part of Trim.Expo.</td>
<td>USEPA, 2005b</td>
</tr>
</tbody>
</table>

Physiologically-based pharmacokinetic models (PBPK models) are used to estimate the dose of toxic metabolites reaching target tissues. Model outputs provide accurate internal dose estimates for specific life stages and differences between genders, species, dose routes, and exposure patterns. These models provide a tool for understanding the physiological and biochemical characteristics of children that influence metabolism and disposition of chemicals at different stages of development. PBPK models can aid in evaluating the impact of parameters such as
tissue growth rates and biochemical parameters such as enzyme induction on dose and health outcome. PBPK models can also be used as a tool for estimating exposure and dose for the developing embryo, fetus, or newborn (Corley et al., 2003).

PBPK models can also be used for toxicokinetic assessments. Such assessments provide more precise estimates of internal dose which, in turn, can be used to replace interspecies defaults for more precise risk assessments. Probabilistic assessments account for the range of interindividual variability and can be used to estimate the central tendency and upper bound of internal dose. Toxicokinetic assessments can also provide an understanding of the mechanism of toxicity by providing various estimates of internal dose that can be correlated with health effects (Ginsberg et al., 2004).

5.4 Unique Characteristics of Children that Affect Exposure

Children's exposure and dose to environmental contaminants are expected to be different and, in many cases, much higher than adults. Both physiological and behavioral characteristics influence children’s exposure to environmental contaminants.

Physiological characteristics influence exposure by affecting a child’s rate of contact with exposure media or by altering the exposure-uptake relationship (as described in Chapter 3). The developing fetus is the most unique case and is primarily exposed to chemicals through cord blood and amniotic fluid. For infants and young children, the primary source of food is often breast milk. Children have a much larger surface area relative to body weight than do adults. In addition to providing more area for dermal absorption, the larger relative surface area of children means that body heat loss will be more rapid, requiring a higher rate of metabolism. Children also need extra metabolic energy to fuel growth and development. The higher basal metabolic rate and energy requirements in children means that both oxygen and food requirements are greater per kilogram of body weight. The higher breathing rate and food consumption rate required to meet these physiological needs can result in higher exposures to environmental contaminants in air and food relative to adults.
The distinct life stages for children, the major routes of exposure, as well as, maternal exposures all affect total exposure levels as a child ages. During the prenatal stage, exposures to chemical and biological contaminants occur through the placenta and the mother is the primary pathway for exposure. For the developing fetus, all maternal exposures are important, including occupational exposures, drug and alcohol consumption, and smoking. During infancy and young childhood, children may be exposed to chemicals in breast milk as well as through direct contact with all environmental media. Indirect ingestion may occur when children handle and eat foods that have come in contact with the floor or other contaminated surfaces. Young children's mouthing activities (hand-to-mouth and object-to-mouth) will result in indirect ingestion if the hands or objects are contaminated. For older children and adolescents, the mother is no longer a pathway for the child’s exposure. In many countries, older children may enter the work force and occupational exposures may become important.

Children’s behavior and the way that they interact with their environment may have a profound effect on the magnitude of exposures to contaminants and differences in exposure at different age. In other words, a child’s exposure is greatly affected by where the child is, what the child is doing, and what the child ingests. Children crawl, roll, and climb over contaminated surfaces resulting in higher dermal contact than would be experienced by adults in the same environment. They eat different foods that may result in higher dietary ingestion (Cohen Hubal et al., 2000b).

The U.S. EPA’s Risk Assessment Forum has proposed “Guidance on Selecting the Appropriate Age Groups for Assessing Childhood Exposures to Environmental Contaminants” (USEPA, 2003b). The development of these groupings takes into account behavior as a function of developmental age and the impact it will have on exposure. Figure 5.3 gives the age groups in the document and illustrates how selected behaviors change within these age groups.

Not only are children’s activities different than adults but children (especially young children) can demonstrate very wide ranges in those activities that may affect exposure. Thus, children’s exposures will be higher and more variable with very high exposure at the upper tail of the distribution, as demonstrated by children’s pica behaviours. It is important to understand that stages of biological development and susceptibility do not necessarily parallel the developmental stages that are important for exposure. Table 5.3 attempts to show the overlap between these
Figure 5.3 Children’s Activities that Impact Exposure as a Function of Development Age

stages. Information in the table extends the concept of exposures important for children to
preconception (parental exposures) and embryo/fetal development. Methods that can be used for
exposure assessment for different stages are also given.

Although developmental age is a critical element in determining children’s activity patterns,
other factors such as country, culture, gender, season of the year, and areas (i.e., urban vs. rural)
can also have an important impact on activities and exposure. Thus, there is a need for caution
when data about activity patterns generated in one region are used to estimate exposures in
another region.
Table 5.3 Considerations for Exposure Assessment at Different Developmental Stages

<table>
<thead>
<tr>
<th>Developmental Stages</th>
<th>Exposure Stages (US EPA, 2003b)</th>
<th>Exposure Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-conception</td>
<td>Reproductive age adult</td>
<td>Maternal or paternal exposure measured or modeled using adult methods (WHO, 2000a)</td>
</tr>
<tr>
<td>Pre-implantation Embryo</td>
<td>Conception to birth</td>
<td>Maternal is the primary exposure route, - maternal exposure measured or modeled using adult methods; - fetal exposure modeled from maternal exposure or biomarker measurements (cord blood, amniotic fluid, meconium)</td>
</tr>
<tr>
<td>Post-implantation Embryo–Implantation to 8 week of pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus – 8 week of pregnancy to birth</td>
<td>Birth to 3 months</td>
<td>Child’s exposure measured or modeled, Biomarker measurements may be effected by differential uptake and metabolism depending upon age and birth condition</td>
</tr>
<tr>
<td>Perinatal Stage - 29 week of pregnancy to 7 days after birth</td>
<td>3 to 6 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 to 12 months</td>
<td></td>
</tr>
<tr>
<td>Neonate – birth to 28 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant – 28 days to 1 year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young Child – 1 to 4 years</td>
<td>12 to 18 months</td>
<td>Exposure measured or modeled, differential exposure due to differing behaviors and contact with different environmental media.</td>
</tr>
<tr>
<td></td>
<td>18 to 24 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 to 30 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 to 36 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 to 5 years</td>
<td></td>
</tr>
<tr>
<td>Toddler – 2 to 3 years</td>
<td>24 to 30 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 to 36 months</td>
<td></td>
</tr>
<tr>
<td>Older child – 5 to 12 years</td>
<td>5 to 10 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 to 15 years</td>
<td></td>
</tr>
<tr>
<td>Adolescent - usually 12 to 18 years</td>
<td>15 to 17 years</td>
<td></td>
</tr>
</tbody>
</table>

As an example, a comparison of activities, lifestyles, habitats, and diets in tropical areas and regions in the Arctic illustrates the differences that might exist in geographical regions. Likewise, different cultures have different diets and lifestyles that would be expected to impact exposures. Some folk medicines that contain chemical contaminants are commonly used. A good example is the use of azarcon (a lead-based product) for the treatment of gastrointestinal symptoms in Mexico (Yáñez et al., 1994). Traditional cosmetics used in some cultures can be sources of metals like lead (al-Hazzaa and Krahm, 1995) or mercury (Sin and Tsang, 2003). Other exposures to harmful chemicals can occur through several sources, including the use of lindane-based shampoos that are used for the treatment of head-lice and scabies (ATSDR, 1999a) and contact with plastic toys and pacifiers that contain phthalates (Shea, 2003).
Gender has been identified as a factor influencing activity level and type of activity. Even for young children (ages 3 to 5 years), gender differences are observed in the types of games played, the frequency of play, and the activity level, with boys engaging in more active play. These patterns have also been observed for the activities of older children (Cohen Hubal et al., 2000b). In many cultures there are important gender differences in activity patterns. Boys are more likely to attend school while girls are more likely to work (Marcoux, 1994; UNICEF, 2005b). In other regions, it has been observed that females, especially young women, spend more time in contact with water. Thus, women would be at higher risk from exposure to contaminants via water.

5.5 Exposure as It Relates to Children around the World

5.5.1 Sources/Geographical Location

Proximity to sources, either natural or anthropogenic, is an important determinant for exposure to environmental contaminants. When considering ambient sources, contaminant concentrations in air, water, soil, and biota are highest in areas that are closest to sources. Children who live in the most contaminated areas throughout the world will have high exposures and health risks simply because they live in these areas. For example, in mining areas, children can be exposed to metals as a result of contact with contaminated air, soil, and dust. Similarly, in agricultural areas, children could have high exposures to the pesticides that are applied to crops in the area. In the Antarctic area, children in countries such as Chile and New Zealand are at a higher risk of exposure to ultraviolet radiation due to a thinning ozone layer (McGee et al., 2002). High levels of arsenic or fluoride in drinking water would be expected in areas receiving water from polluted aquifers. Finally, exposure to radon can occur in areas with a high natural concentration of radium in the soil (Vaupotic, 2002).

Figures 5.4 to 5.7 show areas of the world where pollutant levels are high and could impact exposure and health of adults and children in the area.
5.5.2 Pathways of Exposure

An exposure pathway is the course that a contaminant takes from its source to the individual. When contaminants are released from a source into the environment, they move through multiple environmental media to humans by many pathways. Air, water, soil, house dust, and food are important environmental media for human exposure. For children, several other media such as breast milk, amniotic fluid, and cord blood are also important. The remainder of this section discusses important pathways for children’s exposure.

5.5.2.1 Ambient Air Exposure Pathway

Contaminants in ambient air result in inhalation exposure either when the child is outdoors and breaths contaminated air or when contaminants in the air are transported indoors where the child spends time. Adverse health effects (acute and chronic) associated with inhalation of air contaminants are a common concern for people living in polluted cities, near hazardous waste sites, or close to point sources like smelters (Figure 5.4). Air emissions from past or current production processes, as well as volatilization of organic compounds, airborne particulates, and acid gases, may expose residents to contaminants at levels of health concern (ATSDR, 1994). In urban areas, mobile sources contribute substantially to organic, inorganic, and particulate air pollution. Fires, open burning, and wind-blown dust can also be major sources of ambient air pollution.

A causal relationship between ambient air pollution and daily mortality and morbidity rates has been reported for many cities throughout the world (Schwela, 2000; Stieb et al., 2002). The relevant contaminants include sulfur dioxide, suspended particulate matter, nitrogen dioxide, carbon monoxide, ozone, and lead (Schwela 2000). Ambient air pollution has been declared an important health problem, for developing countries. A considerable burden of disease has been reported for cities like New Delhi, India (Pande et al., 2002); Santiago, Chile (Ostro et al., 1999); and Mexico City, Mexico (Borja-Aburto et al., 1998). In 22 developing countries, the World Resources Institute (WRI) found 180 cities whose air quality did not reach the WHO guidelines (WRI, 2002).
5.5.2.2 Indoor Exposure Pathways

Indoor pathways are very important for exposure for two reasons. First, in many regions of the world, children spend more than 90% of their time indoors. Second, the indoor concentrations of many contaminants are much higher than those found outdoors. It is estimated that 70 percent of the poorest people in developing countries live in rural areas (World Bank, 2002). The indoor exposure pathway is especially important for these individuals (Figure 5.5). Approximately 50% of the world’s population and up to 90% of rural households in developing countries still rely on coal or unprocessed biomass material in the form of wood, dung, and crop residues for fuel (Bruce et al., 2000). High levels of indoor air pollutants result from the use of either open fires or poorly functioning stoves to burn biomass or coal (Ezzati et al., 2001). Women, especially those responsible for cooking, are the ones most heavily exposed. Young children who spend their time close to their mothers also have high exposures (Bruce et al., 2000). Many of the substances in smoke from either biomass or coal burning can be hazardous to humans. The most important
are suspended particulate matter, carbon monoxide, nitrous oxide, sulphur oxides (coal),
formaldehyde, and polycyclic aromatic hydrocarbons (Bruce et al., 2000; Smith et al., 2000).

Environmental tobacco smoke (ETS) is an indoor air pollutant that is a major concern. WHO estimates that there are around 1.1 billion smokers in the world, or about one-third of the global population who are 15 years of age or older. Of these, 800 million are in developing countries (WHO, 2005b). Especially in developing countries, people smoke indoors where they live and work, thus resulting in a high percentage of homes with ETS pollution and high inhalation exposures for children.

Several studies have identified house dust as an important route of exposure for many chemical contaminants (Butte and Heinzow, 2002, USEPA, 2004b). House dust is a sink for semivolatile
organic compounds and particle-bound matter (Butte and Heinzow, 2002). House dust and
compounds adsorbed to it may enter the body by inhalation of suspended and resuspended
particles, through nondietary ingestion of dust, through ingestion of particles adhering to food,
surfaces in the homes, and on the skin as well as by absorption through the skin (Butte and
Heinzow, 2002). High levels of pesticides, PCBs, PAHs, plasticizers (phthalates, phenols), flame
retardants, other organic xenobiotics, and inorganic constituents have been reported in house dust
(Butte and Heinzow, 2002; USEPA, 2004b). High lead levels in house dust have been found in
houses with deteriorating lead-based paint (USEPA, 2004c). High lead levels in house dust have
also been found in mining areas (Yáñez et al., 2003) and in the vicinity of smelters (Díaz-Barriga
et al., 1993). Ingestion of house dust though hand-to-mouth activities are the most likely pathway
of lead exposure.

Indoor concentration of pesticides in proximity to pesticide-treated farmland represents a risk for
children (Fenske et al., 2002). For example, it has been shown that homes in close proximity
(200 ft/60 m) to pesticide-treated farmland had higher dust concentrations of chlorpyrifos and
parathion than did homes farther away (Fenske et al., 2002). Clothing and cars of farm workers
can also be a source of pesticide exposures for children. Usually, applications of insecticides and
herbicides in and around the home are a more likely source for children’s exposures.

The indoor environment can serve as an important pathway for exposures to fungal molds as
well as chemical contaminants. Molds can be found almost anywhere; they can grow on virtually
any organic substance, as long as moisture and oxygen are present. There are molds that can
grow on wood, paper, carpet, foods, and insulation. It is impossible to eliminate all molds and
mold spores in the indoor environment. Controlling moisture indoors is the most effective
mitigation strategy. Moisture problems in portable classrooms and other temporary structures
have frequently been associated with mold problems. (USEPA, 2005c). Fungal toxins (e.g.,
aflatoxin) contaminate food and are a particular problem in African and Southeast Asian
countries (Ehal et al., 2005).
5.5.2.3 Water Exposure Pathway

Ingestion of contaminants is the primary exposure pathway for drinking water. Dermal absorption and inhalation of contaminants during bathing is another common pathway. When contaminated surface waters serve as recreational areas for children, accidental ingestion (water or sediment) and dermal contact become additional pathways for exposure. Finally, aquatic organisms can bioaccumulate contaminants in surface waters that can lead to dietary exposure through the food chain.

Industrial effluent, agricultural run-off (pesticides), and oil and mining wastes are important sources for surface water contamination. Hazardous waste sites are a recognized source of groundwater contamination. In many countries, the natural pollution of aquifers with metals such as fluoride and arsenic is an important source of contamination. High levels of fluoride in water sources have been identified in at least 25 countries, 23 of which are undeveloped nations like Mexico, Argentina, China, India, Pakistan, Bangladesh, Uganda, Kenya, and Tanzania (Ando et al., 1998; Grijalva-Hero et al. 2001; UNICEF, 2001b). In Mexico, approximately five million people live in areas where the fluoride levels in drinking water are higher than the national guideline of 1.5 mg/L (Díaz-Barriga et al., 1997a). In India, 62 million people, including 6 million children, are exposed to elevated levels of fluoride in the drinking water (Susheela, 1998).

High natural water levels of arsenic (Figure 5.6) have been found in undeveloped countries, including China, India, Bangladesh, Cambodia, Thailand, Vietnam, Mexico, Argentina, Chile and Romania (Smedley and Kinniburgh, 2002; UN, 2001b). In these countries, around 45 million individuals are exposed to arsenic through drinking water (Smedley and Kinniburgh, 2002; UN, 2001b). In Bangladesh, 80% of the population is estimated to be at risk of arsenic related diseases. Arsenic contaminated water poses a risk for millions of people globally.
Accessibility to drinking water and management of wastewater is an important health issue in developing countries. It is estimated that 2.4 billion people, including the poorest in the world, lack access to basic sanitation and 1.1 billion people lack access to even improved water sources. In the less developed countries, only 54% of the population in rural areas is using improved drinking water sources (World Bank, 2001).

5.5.2.4 Soil Exposure Pathway

Contaminated soils may expose children to multiple contaminants at levels of health concern (ATSDR, 1994). Ingestion is a primary exposure route of contaminated surface soil. Inhalation of contaminated dusts and direct dermal contact with contaminated soils can also lead to elevated exposure.
Worldwide, the most important sources of metals in soil are mine tailings, smelter wastes and atmospheric fallout (Nriagu and Pacyna, 1988). Poor waste management practices in the mining industry have important implications for exposures around the world. Less than 1% of mined ore produces metals; the remainder is waste (UNEP, 2000). The amount of waste produced is enormous given that world production of metals in 1999 was around one billion tons (UNEP, 2000). The United Nations Development Program (UNDP) estimates that there are 6 million artisanal miners worldwide with a further 30 million or more people dependent on these miners for their living (UNDP, 1999). If we assume that around 40 million individuals are working in the mining industry, then millions of children (including the children of the miners) may be directly exposed to environmental contaminants produced during mining. Most studies on children living in mining or smelter sites are limited to exposure assessments (Díaz-Barriga et al., 1997b; Hwang et al., 1997; Murgueytio et al., 1998). Few of them have described biological effects in the exposed children (Calderón et al., 2001; Counter et al., 1997). Further studies that evaluate exposures and link them with health effects are needed.

Organochlorine pesticides and other persistent organic pollutants (POPs) once applied will remain in the soil, thus providing the potential for children’s exposure through the soil pathway. In 1955, the World Health Organization started a global malaria control program using organochlorine pesticides. By 1958, 75 countries had joined and, at the peak of the campaign, 69,500 tons of pesticides, mainly DDT, were applied to 100 million dwellings each year (Wijeyaratne, 1993). For the control of malaria, houses were sprayed twice a year with DDT wettable powder to kill mosquitoes. Today, DDT has been substituted in many countries with agents such as pyrethroids, but is still used in special instances for vector control in a small number of countries. Due to past or present household spraying, levels higher than background concentration have been detected, both in outdoor and indoor soils throughout the world (Yáñez et al., 2002a; Díaz-Barriga et al., 2003). DDT is one of the 12 POPs being phased out under the Stockholm convention.

5.5.2.5 Food-Chain Exposure Pathway

Many contaminants in the environment are concentrated up the food-chain and result in dietary exposure. Both on- and off-site hunting, fishing, foraging, and farming activities may bring
people into contact with those contaminants. Some substances, particularly fat-soluble substances and heavy metals, may reach concentrations in animal tissues that are thousands of times higher than those found in water, soil, and sediment (Damstra et al., 2002). This pathway includes the exposure to chemicals by means of drinking animal milk or eating animal milk-based products.

Mercury is a good example of a metal for which the food-chain exposure pathway is important. Although much of the mercury in the environment is in the less toxic inorganic form, some microorganisms (plant and animals) can convert this to methyl mercury, which accumulates up the food chain (UNEP, 2002). People are then exposed for example, by eating contaminated fish. Environmental mercury contamination from mining practices that rely on mercury amalgamation for gold extraction is widespread (Eisler, 2004). Contamination is very high in the immediate vicinity of active gold extraction and refining operations. Very high environmental concentrations of mercury from this source have been measured in Canada, the U.S., Africa, China, the Philippines, Siberia, and South America (Eisler, 2004). In parts of Brazil, for example, mercury concentrations, in abiotic materials, plants, and animals, collected near ongoing mercury amalgamation gold mining sites, were far in excess of allowable mercury levels promulgated by regulatory agencies for the protection of human health and natural resources (Eisler, 2004). In industrial countries, sources of mercury contamination include: smelting processes, coal-fired power plants, incinerators and chlorine production (USEPA, 2004d; UNEP, 2002). Mercury, especially in the form of water-soluble methylmercury, may be transported to pristine areas by rainwater, water currents, deforestation, volatilization, and other vectors (Eisler, 2004).

POPs do not degrade in the environment, can be transported globally, and accumulate up the food chain. Ingestion of POPs through the food chain pathway is very important for a number of Native populations. The First Nations' people in the Arctic have markedly high levels of POPs in their diet (Health Canada, 2003). Studies in Canada have shown that average intakes are generally higher in the Baffin Inuit population as a result of a diet involving large amounts of marine mammals and fish, while the Sahtu Dene/Metis peoples who consume mainly caribou and fish have lower intakes (Health Canada, 2003).
Children can be exposed to biological as well as chemical contaminants through the food chain. For example, approximately 1.5 billion episodes of diarrhea occur globally each year, resulting in the deaths of 3 million children under the five years of age (mainly in developing countries) (WHO, 2005c). It is estimated that 70% of these annual cases of diarrhea worldwide have been caused by biologically contaminated food (WHO, 2005c). Foodborne parasitic diseases also present a major public health problem. For example, foodborne trematodes affect 40 million people, with more than 10% of the world's population at risk of infection (WHO, 2005c).

5.5.2.6 Human Exposure Pathways

Maternal Exposure Pathway. Most chemicals are able to cross the placental barrier and others are excreted through breast milk. Therefore, during the fetal or neonatal stages, exposed mothers become a major source of exposure to their offspring. There are multiple settings in which the potential of contaminated breast milk is relevant. These include: mining areas (metals), agriculture fields (pesticides), industrial areas (metals and organic compounds), rural areas (indoor pollution, for example, polycyclic aromatic hydrocarbons), urban areas (chemical mixtures, for example, gasoline), the arctic (POPs), and endemic malaria areas (DDT, pyrethroids).

As an example of this pathway, Figure 5.7 shows the worldwide importance of human milk as a source of DDT. Although ingestion of breast milk can be a pathway for exposure to chemicals, it is essential to state that overall, breastfeeding provides substantial benefits to both children and mothers. It significantly improves child survival by protecting against diarrhoeal diseases, pneumonia and other potentially fatal infections, while it enhances quality of life through its nutritional and psychosocial benefits. Breastfeeding also contributes to maternal health in various ways, including prolonging the interval between births and helping to protect against ovarian and breast cancers. Therefore, the benefits of breastfeeding clearly outweigh the risks of exposure to chemicals through this pathway (Pronczuk et al., 2004).
Since many women in both developed and developing countries work outside the home, maternal occupational exposures that may result in ingestion, inhalation, or dermal absorption can be an important pathway for many chemicals. In some occupations (i.e., agriculture, mining, and manufacturing), both mothers and fathers can transport chemical contaminants into the home environment on their skin and clothing. The child can then become exposed through contact with the parent or through contact with surfaces that the parent has contaminated.

5.5.3 Settings/Microenvironments

Most children spend their time in a few specific microenvironments including the home, school, and recreational areas (playgrounds). A study of these microenvironments or settings is critical to an understanding of exposure patterns in children. For children’s exposure there are factors specific to each microenvironment. However, these settings are also modified by external factors.
like those related to geographical areas, or to environmental equity factors. Finally, some
children are exposed to chemicals in special settings such as in child labor situations, refugee
camps, or on the street. These settings will be discussed further in the following sections.

5.5.3.1 Residential

Home is the most important setting for infants (children aged 1 month – 1 year) and young
children (aged 1-4 years). They often eat, play, and sleep in the same area. Examples of sources
of exposure to pollutants include building materials (e.g., wood treated with arsenic-based
pesticides); lead-based paints; insecticides that are sprayed indoors; fuel (e.g., coal and wood) for
indoor cooking; disposal practices for domestic waste (e.g., incineration); household chemicals
(e.g., solvents); and small-scale enterprises at the family residence (e.g., brick producers who
operate low-technology combustion kilns and makers of pottery using lead-based paints.

5.5.3.2 School

School is an important setting for many children and adolescents. Many of the residential factors
described above can apply to the school setting. However, there may be additional sources of
chemicals that are associated with laboratories, activity rooms, or school equipment. For
example, exposure to volatile compounds has been reported in art buildings (Ryan et al., 2002);
polybrominated diphenyl ethers (PBDEs), used in flame retardants, were detected in teaching
halls containing 20 computers (Sjodin et al., 2001); mercury intoxication resulting from use of
school barometers has been reported in a number of countries (Koyun et al., 2004); and in
Mexico, lead levels were higher in children who habitually bite colored pencils (López-Carrillo
et al., 1996).

5.5.3.3 Child Care Centers

In the US, The First National Environmental Health Survey of Child Care Centers was
conducted in licensed child care centers that serve children under the age of six (DHUD, 2003).
An estimated 14,200 or 14% of licensed child care centers have significant lead-based paint
hazards. Centers in older buildings are more likely to have significant lead and asbestos hazards
than those in newer buildings. In the US, daycare centers where the majority of children are
African American are likely to have significantly higher lead exposures and exposure to allergens than those where a majority of the children are Caucasian. Less than 22% of day care centers had detectable levels of any of the allergens measured. Data on child care center exposures from most countries is generally unavailable, and due to cultural factors, exposures may vary considerably.

5.5.3.4 Recreational

Playground environments provide opportunities for children’s exposure to pollutants. Like any other setting, children will be exposed to contaminants that are present at the site. Thus, playgrounds built near hazardous waste sites, mining waste sites, or agricultural fields may be contaminated and, hence, provide a pathway for exposure. In addition, children will have more direct contact with the contaminated environment through their play activities making exposures in recreational areas greater. In addition to exposures to contaminants present in the natural environment, there are two other risks associated with playgrounds: playground hazards, and materials used in playground equipment or in playground cover. A strong association between childhood injuries and the use of inappropriate surface materials under and around playground equipment has been described (Mowat et al., 1998). Significantly more hazards per play area were identified in playgrounds near low-income areas as compared with high-income areas.

Several materials commonly used on playgrounds may provide a health risk. Chromated copper arsenate (CCA) is a wood preservative that has been registered to protect wood from dry rot, fungi, molds, termites, and other pests (USEPA, 2003c). CCA-treated wood is most commonly used in outdoor settings for decks, walkways, fences, gazebos, boat docks, and playground equipment (USEPA, 2003c). New regulations have been put in place that will reduce the potential exposure risk to arsenic. Other materials that are used in playgrounds need further assessment. For example, 'Kieselrot' (red slag), a byproduct of copper production, is contaminated with leachable residues of PCDDs/PCDFs. This material has been used as surface cover for more than 1,000 sports fields, playgrounds and pavements in Germany and neighboring countries (Wittsiepe et al., 2001). Children can ingest this material directly by hand-to-mouth activities or soil-pica behavior. Transfer of leachable residues to the skin could result in dermal absorption of the residue on the skin or indirect ingestion through hand-to-mouth activities. The
bioaccessibility of PCDDs/PCDFs in this material was estimated in an “in vitro” assay to be more than 60% when using a model with higher bile content and in the presence of whole milk powder (Wittsiepe et al., 2001).

Exposures to chemical and biological organisms through ingestion, inhalation, and dermal absorption can occur as a result of swimming in contaminated water. Swimming pools typically use chlorine for disinfection resulting in high concentrations of chloroform and other disinfection byproducts. Fecal contamination and contamination with microorganisms may be a problem for beaches and other natural swimming areas. Industrial discharges, mine tailings, and untreated wastes all provide opportunities for contaminating recreational waters which can then lead to children’s exposures to these contaminants.

5.5.3.5 Special Settings

Some children in various parts of the world are exposed to toxics or to hazardous environments in unique circumstances. Globally, millions of children are exposed as a result of special socioeconomic and cultural settings. Examples include:

Child Labor. An estimated 246 million children between 5 and 14 years of age are engaged in child labor worldwide (UNICEF, 2004). Of those, 171 million work in hazardous situations or conditions, such as in mines or agriculture. In addition, many children do not have access to education, are not provided adequate health care or nutrition, are abducted, abused, and/or beaten, and are essentially reduced to slave labor. Information is limited, but includes the following:

- Over 19% of children (127.3 million) in the Asian and Pacific regions are engaged in child labor.
- Sub-Saharan Africa has an estimated 48 million child workers. Almost one child in three (29%) below the age of 15 works.
- Latin America and the Caribbean have approximately 17.4 million child workers.
- Fifteen per cent of young children in the Middle East and North Africa are engaged in child labor.
Street Children. A street child is any girl or boy who has not reached adulthood, for whom the street (in the broadest sense of the word, including unoccupied dwellings, wasteland, etc.) has become his or her habitual abode and/or source of livelihood, and who is inadequately protected, supervised or directed by responsible adults. Pollution, poverty, violence, discrimination, inadequate family support, and disease threatens the life, growth, and development of children living in the streets (UNICEF, 2001c). Their settings are unsafe environments with limitations on quality basic health services, clean water, and sanitation. In the end, the opportunities for recreation, learning, social interaction, psychosocial development and cultural expression are minimal. Furthermore, drug use may be high among this group. The well-being of street children is an important public health concern and the magnitude of the problem is only expected to increase. Cities are expanding at a rapid pace and the developing world is becoming increasingly urban. From 2000 to 2025, the number of people living in urban areas in the developing world will double from 2 billion to 4 billion (UNICEF, 2001c). Currently, one third of urban dwellers in the developing world lives in sub-standard housing or is homeless and this is not expected to improve. It is estimated that by 2025, six out of every ten children will live in urban areas (UNICEF, 2001c). Based on these statistics, it is anticipated that there may be a large increase in the number of street or homeless children over the next 20 years.

Refugee Children. Ample evidence exists to demonstrate that large-scale dislocation of people (characteristic of many recent refugee crises) creates adverse environmental impacts (UNHCR, 2001). The scale and suddenness of refugee flows can rapidly change a situation of relative abundance of natural resources to one of acute scarcity. Where the hosting environment is already under stress, as it is for instance in many arid regions of Africa and Asia, an influx of refugees can seriously threaten the integrity of local ecosystems, the economic activities dependent on them, and the welfare of local communities. Although deforestation tends to be the most apparent negative environmental feature of refugee situations, other visible impacts may include soil erosion, loss of wildlife and non-timber products, and loss of biodiversity (UNHCR, 2001). Indoor and outdoor air pollution caused by concentrated biomass burning, depletion or contamination of aquifers, and an altered pattern of transmission of certain diseases can be a serious threat to refugee health (UNHCR, 2001). Children, including adolescents under the age of 18, make up 45% of refugee populations world-wide (UNHCR, 2002). The estimated number
of refugees in 2003 was 20 million (UNHCR, 2003). In order to assess the environmental
exposure of these children in an integral way, we have to take into account the six most salient
and sometimes inter-related concerns facing refugee children today: separation of families;
sexual exploitation, abuse and violence; military recruitment; education; detention; and
registration (UNHCR, 2003).

5.5.4 Environmental Equity Factors (Vulnerable Communities)

Today, most poor people live in rural areas and have risks associated with agriculture and other
aspects of rural life. However, a rapid transition is occurring: many of the poor are moving to
large urban areas and entering the informal sector of the economy which may be accompanied by
new kinds of toxic hazards. Poverty is often accompanied by high rates of morbidity and
mortality of most diseases. However, poor people in developing nations are also more likely to
be exposed to toxic chemicals (Yáñez et al., 2002b). It has been estimated that 5 million to 6
million people die each year in developing countries due to water-borne microbiological diseases
and air pollution (World Bank, 2002), whereas, those who are more poorly nourished and who
have concurrent disease are more susceptible to toxic chemicals. For example, lead is known to
be more toxic to children whose diets are deficient in calories, iron, and calcium (Mahaffey,
1995). Environmental pollution can also contribute to poverty by making resources
unproductive. In Eastern Europe, for example, short-term decisions to allow high levels of toxic
pollution in a developing economy led to devastating economic impacts within just a few
decades.

With regard to environmental justice, it has been shown that there are clear differences among
racial groups in terms of disease and death rates; furthermore, racial minority and low-income
populations experience higher than average exposures to selected air pollutants, hazardous waste
facilities, contaminated fish and agricultural pesticides in the workplace (USEPA, 1992b).
5.6 Special Considerations for Children’s Exposure: Case Studies

5.6.1 Influence of Activities

Arsenic: In the communities surrounding the Rocky Mountain Arsenal (RMA), a Superfund site in Colorado, USA, pathways for exposure to arsenic were evaluated through analysis of residence history, occupation, hobbies, dietary habits, water supply, housing and activity patterns (Reif et al., 1993). Children of Hispanic origin or non-Caucasian children who drank less than three glasses of water daily, and children who spent more time outdoors had an increased risk of having \( \geq 10 \) ppb of arsenic in their urine (Reif et al., 1993).

Insecticides: In a study done in an endemic malaria area in Oaxaca, Mexico, it was found that children were exposed to deltamethrin (an insecticide used in the control program for malaria) (Yáñez, 2002b). Moreover, a negative correlation between urinary 3-PBA (biomarker for deltamethrin) and age was found in these children (Yáñez, 2002b). The results can be explained considering that deltamethrin was sprayed on the ceilings and walls, both indoors and outdoors, contaminating household dust and external surface soil. In tropical areas, these sites are important recreational zones for infants, since they have a lower temperature, levels of deltamethrin in surface soil and household dust in Oaxaca were higher than background levels (Yáñez, 2002b).

Environmental Tobacco Smoke (ETS): A Canadian study examined activity patterns and exposure to ETS in non-smoking respondents relative to age, sex, socioeconomic status and prevalence of asthma (Leech et al., 1999). Children experienced the most exposure at home, primarily between 4 p.m. and midnight. For children, the living room (22%) and the bedroom (13%) were the most common locations (Leech et al., 1999). Determining characteristic time and location patterns for ETS exposure is critical for developing educational strategies to help non-smokers avoid ETS exposure (Leech et al., 1999).

Lead: A study of pre-school children in New Jersey, USA, examined seasonal changes in residential dust lead content and its relationship to blood lead (Yiin et al., 2000). Blood and dust samples (floors, windowsills, and carpets) were collected to assess lead exposure. The geometric mean blood lead concentrations were 10.77 and 7.66 µg/dL for the defined hot and cold periods,
respectively (Yiin et al., 2000). The regression analysis, including the three representative dust variables in the equations to predict blood lead concentration, suggests that the seasonality of blood lead levels in children was related to the seasonal distributions of dust lead in the home (Yiin et al., 2000). In addition, the outdoor activity patterns indicate that children are likely to contact high leaded street dust or soil during longer outdoor play periods in summer (Yiin et al., 2000). Therefore, at least some of the seasonal variation in blood lead levels in children was probably due to increased exposure to lead in dust and soil.

5.6.2 Environmental Equity

Hazardous Waste Sites: The Agency for Toxic Substances and Disease Registry (ATSDR) has confirmed from more than 10 years of public health assessments, toxicological investigations, epidemiologic studies, and reviews by expert workgroups that children have unique characteristics that often place them at greater risk of adverse health effects (e.g., neurodevelopment disorders and respiratory disorders) when exposed to toxic substances emitted from hazardous waste sites or chemical releases (ATSDR, 1997). Children who live near hazardous waste sites may have greater exposures, greater potential for health problems, and less ability to avoid hazards than do adults (ATSDR, 1997).

In the US, an estimated 3 to 4 million children live within one mile of at least one hazardous waste site (HWS) (ATSDR, 2003). Furthermore, on the basis of data from 1,255 hazardous waste sites, there were 1,127,563 children under 6 years of age living within one mile of the borders of the sites, or about 11% of the potentially affected population. Women of child-bearing age account for about 24% of the population near waste sites (ATSDR, 2003). Some HWS are located in highly populated, largely minority or low-income areas; which most of the HWS are either in unpopulated areas with fewer minority or low-income people than the national average (Atlas, 2001). According to other publications, HWS are more likely to be found in tracts with Hispanic groups, primarily in regions with the greatest percentage of Hispanics (Anderton et al., 1994). A multivariate analysis of HWS distribution and a hazard regression analysis of the site prioritization process suggest that communities with a higher percentage of black residents are less likely to receive National Priorities List designation, delaying potential remediation (Anderton et al., 1997).
5.6.3 Aggregate Exposure

Many chemicals, both natural and man-made, are released into the environment and through dispersion and transport processes, may find their way into food, water, indoor and outdoor air, soil, and other environmental media. It has become increasingly clear in recent years, that for some chemicals, significant exposures may occur by more than one route (ingestion, inhalation, dermal absorption) and from more than one pathway. Thus, the concept of aggregate exposure refers to the total exposure of humans to a single chemical or to a mixture of chemicals through all relevant pathways and routes. Three examples that show the importance of assessing aggregate exposure in children in order to design risk reduction programs are described below.

**Chlorpyrifos:** A US study examined the aggregate exposures of preschool children to chlorpyrifos and its degradation product 3,5,6-trichloro-2-pyridinol (TCP) (Morgan et al., 2005). Samples that were collected included duplicate diet, indoor and outdoor air, urine, solid and liquid food, indoor floor dust, play area soil, transferable residues, and surface wipes (hand, food preparation, and hard floor). Generally, levels of chlorpyrifos were higher than TCP in all media, except for solid food samples. For these samples, the median TCP concentrations were 12 and 29 times higher than the chlorpyrifos concentrations at homes and day care centers, respectively. The median urinary TCP concentration for the preschool children was 5.3 ng/mL and the maximum value was 104 ng/mL. The median potential aggregate absorbed dose (ng/kg/day) of chlorpyrifos for these preschool children was estimated to be 3.0 ng/kg/day. The primary route of exposure to chlorpyrifos was through the dietary route, followed by the inhalation route.

**Smelter Areas:** Children living near lead smelter areas in Torreon, Mexico were exposed to lead by inhalation (lead in air particles), ingestion of soil, and ingestion of food cooked in lead glazed ceramics (García-Vargas et al., 2001; Albalak et al., 2003; Pineda-Zavaleta et al., 2004). Furthermore, in the smelter area, lead levels in household dust were higher than levels in control areas, thus, inhalation or ingestion of dust particles were additional pathways of exposure. Similar results were found for arsenic, in children living in the vicinity of a copper smelter in San Luis Potosi, Mexico (Diaz-Barriga et al., 1993; Carrizales et al., 2006).
Malarious Areas: In order to control malaria, DDT was used in Mexico until the year 2000. As a result, DDT contamination was widespread and shown that children can be exposed to this insecticide by soil ingestion, household dust ingestion/inhalation, fish consumption and human milk (Díaz-Barriga et al., 2003; Herrera et al., 2005). In these areas DDT levels in blood in children are higher to those in adults (Díaz-Barriga et al., 2003).

5.6.4 Cumulative Exposure

Cumulative risk is the combined risk resulting from exposures that accumulate over time, pathways, sources, or routes for a number of agents or stressors. This concept of cumulative risk addresses the fact that individuals are not usually exposed to a single environmental contaminant by means of a single exposure pathway. Multiple contaminants are released from sources as chemical mixtures. Environmental fate and transformation processes affect the nature, pathways, and extent of human exposure. Exposures by different pathways may result in differential absorption, metabolism, and toxic response even for the same chemical. Cumulative risks are difficult to assess and methods are still under development (USEPA 2003d). Most assessments for cumulative risk start with the receptor population and determine which chemicals, stressors, or other risk factors are affecting them. This is a particularly important and relevant concept when considering children’s exposures and risks especially in developing countries. Many of the examples presented here consider specific groups of children who may be exposed to high pollutant concentrations, poor nutrition, and poor sanitation at the same time and thus they may have a differential risk compared to populations that are influenced by only a single stressor.

A much narrower concept of cumulative risk considers the risk from exposures to pesticides (e.g., organophosphate pesticides: OPs) that have a common mechanism of toxicity (USEPA 1996a). OPs are powerful inhibitors of carboxylic hydrolases, including acetylcholinesterase, and several studies in different countries have shown higher exposure to OPs in children than adults (Wessels et al., 2003). Children of pesticide applicators, younger children within the 0-6 year age range, children living closer to pesticide-treated orchards, children living in urban areas, and those living where pesticides are used inside or outside the home have all been shown to have higher levels of a urinary biomarker for OPs (Wessels et al., 2003).
5.7 Conclusions

Because of differences in physiology and behaviors, exposures among children are expected to be different and often higher than exposures among adults. Furthermore, in terms of risk, children may also be more vulnerable to environmental pollutants because of differences in absorption, excretion, and metabolism. Given these important vulnerabilities, increased research in the area of children’s environmental health is needed, especially in developing countries.

More information is needed about children’s exposures and the factors that will bring them into contact with chemicals in the environment. Future studies must include in their analysis consideration of factors like occupation, smoking, cultural behaviors, nutritional conditions, etc. These factors, many of them present in non-developed nations, may modify the exposure to and response to modify toxicity or exposure to the contaminants.

Some children are exposed to toxics or to hazardous environments in unique circumstances. Thus, there is also a need for international assistance in order to study special settings in the world (child labor, street child, refugees). Globally, millions of children live under these conditions.

With the recognition of the special vulnerability of children, it is better to prevent than to treat environmental diseases in children. In developing countries the most important issue may be prioritizing which exposure reductions will have the greatest overall impact with the limited resources that are available. It is important to identify the exposures that pose the greatest health risk as well as the sources and pathways for these exposures. This information can then be used to make choices that lead to the health benefits for children around the world.
CHAPTER 6. METHODOLOGIES TO ASSESS HEALTH OUTCOMES IN CHILDREN

6.1. Introduction

6.1.1 Methodologic Approaches for Children’s Health

Children’s health status is an important population marker of environmental threats to human health. Children often are a sensitive subgroup of the population (Brent et al., 2004) necessitating the need for monitoring sentinel health endpoints followed by purposeful research for areas of concern. Only recently have investigators focused on methodologies designed specifically to address the unique characteristics of children and the need, more generally, to consider exposures in the context of life stages. Essentially, the same methodologies used for assessing adult health status in relation to environmental factors can be used for children, but they must be adapted to reflect the rapid rate of growth and development characteristic of infants and children. Added attention must be given to other unique aspects of children as discussed in previous chapters. Measurements of exposure and outcomes may need to be more frequent than in adults and timed to reflect the key stages of human growth and development, i.e., embryonic, foetal, neonatal, infant, childhood, adolescence, and adulthood (Table 2.1). Ideally, prospective follow-up of human conceptuses through adulthood (18-21 years of age) would permit a complete capture of health endpoints and exposures that may vary during the follow up interval. Data collection may need to rely on proxy reporting if children are unable to provide information in a valid and reliable manner. For example, an investigator interested in assessing dietary phytoestrogens may need to interview parents as a proxy for children. While proxy parental reporting may be prone to error, systematic differences in reporting by parents with regard to a child’s exposure or health status is unlikely. Investigators should attempt to devise methodologies that permit empirical evaluation of bias and to consider novel methodologies for correcting measurement bias (Sturmer et al., 2002; Thurigen et al., 2000). Appropriate study design benefits from the involvement of a multidisciplinary team of experts (e.g., epidemiologists and biostatisticians) to capture methodologic nuances unique to pediatric study populations. When characterizing exposures amongst children, investigators may need to rely on
proxy reports from parents, caregivers or teachers, especially for younger children who may be unable to accurately recall and report exposures such as diet or play.

**Epidemiologic methods.** Use of epidemiologic methods can ensure the validity and reliability of study results. Briefly, this includes formulation of a research question or testable hypothesis, selection of an appropriate study design with respect to the research question and type of study covariates, selection of an appropriate sample (determining whether a representative or population based sample is needed), standardized data collection instruments, and development of an analytic plan appropriate for the design and level of measurement of study covariates.

While several study designs are available for assessing environmental factors and child health, each has its own strengths and limitations that need to be weighed in making a final decision about research methodology. Study designs can be interchanged across health outcomes depending upon the research aims, characteristics of the exposure, incidence (new cases) or prevalence (new and existing cases) of the study outcome, fiscal considerations, and logistical issues as described below. Study design can tremendously impact the weight of evidence for a particular exposure and outcome (NRC 2001), with analytic studies adequately designed and statistically powered to test hypotheses weighing more heavily than descriptive studies (e.g., cross-sectional or linkage studies). Study findings need to be evaluated within an established paradigm for assessing causality. With regard to infants and children, biologic plausibility underlying the timing and dose of exposure at critical developmental windows needs careful attention in both designing the study and in the interpretation of the results (IPCS, 2002).

**Comparison of study designs.** Table 6.1 presents a comparison of basic epidemiologic study designs for assessing child health outcomes by their inherent methodologic strengths and limitations. While experimental study designs are included for completeness, none are appropriate for the evaluation of environmental influences on children’s health when the exposure(s) cannot be randomized within acceptable research practices.
<table>
<thead>
<tr>
<th>Study Design</th>
<th>Description</th>
<th>Strengths</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Descriptive*</td>
<td>Assessment of group exposures and health outcomes, thereby, gauging impact of a particular exposure in a defined population.</td>
<td>Hypothesis generating.</td>
<td>Cannot link exposure to diseases or study outcomes among individuals. Residual confounding may threaten validity.</td>
</tr>
<tr>
<td>Ecologic</td>
<td>Design that simultaneously ascertains exposures and outcomes. Appropriate for exploring research questions as a first attempt or to generate hypotheses for analytic study designs.</td>
<td>Can be completed in a relatively short period of time often at reduced cost. Useable initial approach for evaluating human health risks.</td>
<td>Disease prevalence only. Cannot assess causality or the temporal relation between exposure and health outcome. Residual confounding may threaten internal validity.</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>Physical merging of health and exposure datasets to explore research questions and conduct ecological analyses.</td>
<td>Can be completed in a relatively short period of time often at reduced research cost.</td>
<td>Completeness of case ascertainment varies by type (active vs. passive) of surveillance. Residual confounding may threaten internal validity.</td>
</tr>
<tr>
<td>Linkage</td>
<td></td>
<td></td>
<td>Residual confounding may threaten internal validity.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Useable as initial approaches for evaluating human health risks for a particular exposure.</td>
<td>Not applicable for many health outcomes without available registry data.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Useful for population based monitoring such as the detection of spatial or temporal trends &amp; patterns.</td>
<td></td>
</tr>
<tr>
<td>Analytic</td>
<td>Design that begins with disease status; cases refer to individuals, families or households with a particular health outcome while controls are similar to cases in every way save for the study health outcome. Potential confounders, if known, can be addressed by restricting study subjects in the design phase or through multivariate modelling techniques in the analytic phase. For disease outcome, cases need to comprise incident (newly diagnosed) cases and controls need to be selected from population at risk of developing the disease of interest.</td>
<td>Well suited for: 1) rare outcomes (&lt;5% prevalence in population) such as birth defects, neuro-developmental impairments or childhood cancer or 2) outcomes arising following a long latency. If conducted properly, summary statistics approximate those from cohort studies.</td>
<td>Typically, only one endpoint can be studied (though multiple exposures can be addressed). Response rates may vary by disease status with cases often more likely to participate than controls, thereby, introducing potential bias. Recall bias may stem from systematic differences in</td>
</tr>
<tr>
<td>Study Type</td>
<td>Design Description</td>
<td>Strengths</td>
<td>Weaknesses</td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Case Only</td>
<td>Design modified from the traditional case-control study that is intended for the investigation of gene-environment interactions. No external controls are used.</td>
<td>Relatively simple and efficient approach for estimating gene-environment interactions. Estimates multiplicative (not independent) effects of environmental factors and genes.</td>
<td>Requires population-based selection of cases. Stringent assumption that environmental factor and genes are independent. If violated, can produce biased estimates.</td>
</tr>
<tr>
<td>Cohort</td>
<td>Longitudinal design that begins with ascertainment of exposure(s) for cohort members with follow up for occurrence of health events. Such studies can be designed as historic (identification of a preexisting cohort whose exposure has been identified) or prospective (current exposure ascertainment) with prospective follow up for occurrence of health events. The cohort study offers two additional strategies for analysis – case-cohort and nested case-control study designs. The case-cohort comprises all individuals in the cohort with a particular disease and a random sample of unaffected individuals from the cohort. The nested case-control design includes cohort members with a particular disease (cases) and unaffected individuals (controls) matched on relevant study covariates.</td>
<td>Less subject to selection bias. Can establish a temporal order between exposure and outcome. Able to consider multiple exposures and outcomes within single design. Can incorporate time-varying covariates.</td>
<td>Loss to follow up may bias results. Depending upon length of follow up needed, can be costly.</td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Randomized Trial</td>
<td>Experimental trial in which the investigator randomly assigns study participants to receive or not a study treatment or intervention. Hence, every study participant has an equal chance of being assigned to the experimental treatment or intervention. Investigators can be blinded to randomization process dependent upon the characteristics of the study outcome.</td>
<td>Random assignment of study participants to groups, thereby, minimizing confounding by ensuring comparable groups. Design given most weight in establishing causality or treatment efficacy.</td>
<td>Not appropriate for study of most environmental agents. External validity or general findings may be limited to referent population.</td>
</tr>
</tbody>
</table>

*Excludes case reports and case series.
Descriptive and analytic study designs are observational in nature in that children cannot be randomly assigned to receive the environmental exposure (e.g., chemical). Analytic study designs are typically used when interested in the etiologic relation between an exposure(s) and health outcome. While experimental study designs remain the best approach for establishing causality or identifying effective disease prevention strategies, such designs are unethical for most environmentally oriented research. To this end, only observational designs are further discussed in this chapter.

Choice of study design is dependent upon a number of factors. The decision will lie primarily with the research question (for descriptive study designs) or hypothesis (for analytic study designs) to be assessed in the context of budget and other feasibility issues (e.g., the ability to ascertain data on relevant study factors regardless of whether the data already exists or needs to be collected). Unlike study design options for experimental animals, there are no universally recognized protocols (or set of guidelines) for assessing organ system specific toxicity; rather, an epidemiologic study design such as a case control or cohort study is tailored to the particular study exposure, age of the children, and health outcome to be considered. Such a design may be entirely devised for implementation in the field, within a clinical setting, or a combination of the two settings. Choice of study design should be sensitive to the voluminous body of literature supporting the clustering of human pregnancy outcomes (e.g., pregnancy loss, birth defects, preterm and low birth weight infants). This clustering or correlation needs to be addressed in designing the study and the accompanying analytic plan. Failure to appropriately address the underlying correlation may result in incorrect statistical conclusions and inferences (Buck et al., in press). Currently, modeling techniques are available that are responsive to this issue (e.g., generalize estimating equations, hierarchical models).

**Descriptive designs.** Descriptive studies are designed to generate hypotheses or assess research questions (not hypothesis testing, per se) for subsequent testing. Case reports and case series also may be categorized as descriptive studies; however, these approaches summarize data on an individual or a small group of individuals with a common set of symptoms or a health condition. Since neither of these approaches utilizes an appropriate comparison group for whom the investigator attempts to delineate a common exposure, no further discussion of these approaches
is offered. Key descriptive study design options include: 1) ecologic design; 2) cross-sectional design; and 3) linkage studies. These designs have been powerful in identifying patterns of disease occurrence and in identifying potentially at risk subgroups.

Ecologic studies examine factors in relation to health outcome at the population level given the absence of individual level data on these factors. While cautious interpretation of data is needed given the limited availability of information on potential confounders, important health concerns for children have been identified with such designs. For example, biomonitoring data for populations (e.g., ambient air monitoring) have been linked to live birth registries to assess the impact of air pollution on infant birth size (Wilhelm and Ritz 2003), gestation (Ritz et al., 2000) or birth defects (Ritz et al., 2002). Similarly, geographic proximity to hazardous waste sites has been associated with birth defects (Marshall et al., 1997) and the consumption of PCB contaminated fish with birth defects using a state birth defects registry (Mendola et al., 2005).

Cross-sectional designs collect information on study exposure and outcome at the same point in time so that a temporal ordering between the two cannot be established and often exposures at critical windows for various life stages cannot be captured. Cross-sectional studies typically employ survey research methodologies, especially if the study sample is to be population based or representative of the targeted study population. Various registries routinely collect information that can be analyzed to globally assess changes in the distribution or frequency of health endpoints such as infant birth weight and gestation, plurality of birth, secondary sex ratios, foetal deaths, stillbirths, birth defects, or childhood cancers. In some populations, hospital (in- and out-patient) visits and diagnoses can be used to monitor changes in the prevalence of diseases that may be affected by environmental influences (e.g., asthma). Cross-sectional surveys simultaneously collect information on exposure and health status and may reveal associations in need of formal hypothesis testing, such as those suggesting a relation between blood lead levels and pubertal delays in girls in the US (Selevan et al., 2003; Wu et al., 2003). Examples of other large cross-sectional studies in the US are the National Children’s Health and Nutrition Examination Survey (CDC 2003a), the National Survey of Family Growth (NCHS 1997), and the National Reports on Human Exposure to Environmental Chemicals (CDC 2003b).
Linkage studies can be performed by linking routinely collected registry information with existing health information to the extent such information exists in a formal registry or established data system. Such information can be analyzed to assess changes globally in the distribution or frequency of health endpoints such as infant birth weight and gestation, plurality of birth, secondary sex ratios, foetal deaths, stillbirths, birth defects, or childhood cancers. In some populations, hospital (in- and out-patient) visits and diagnoses can be used to monitor changes in the prevalence of diseases that may be affected by environmental influences (e.g., asthma). Often, one or more registries can be linked to assess childhood mortality and morbidity such as the use of linked birth and death certificate files or the linkage of birth registries to birth defects or cancer registries.

Analytic designs. Analytic studies are designed to test formal hypotheses requiring methodologies to establish a temporal ordering between the study exposure and health outcome. There are two major types of observational analytic designs: case controls and cohort studies. Hybrid designs also exist such as the case cohort design, which can be useful for analyzing rare failure events within a cohort study (Wacholder 1991; Barlow et al., 1999). The determining factor is whether the investigator will ascertain study participants on the basis of disease (case control design) or exposure (cohort). There are many practical considerations that impact the final choice of design such as prevalence of the exposure or health outcome in a population, presumed latency period between exposure and outcome, and the estimated benefit of a cohort design over a case control (e.g., ability to look at a spectrum of outcomes). Case control studies target incident (or newly diagnosed) cases of a particular disease in a specific population in time and individuals free of that disease will serve as controls. Selection of a control group is extremely difficult and a process that requires careful consideration with respect to what is known about the exposure. Controls come from the same target population as cases and are similar to them in every way except for the presence of disease. Investigators attempt to ensure the comparability of controls with regard to cases by matching them on potential confounders in the design phase (i.e., matched case control study) or in the analytic phase (i.e., multivariate modeling).
Prospective cohort studies are the design of choice when investigators can define a particular exposure or set of exposures in a population and follow individuals over time for disease incidence or newly occurring cases. Of particular note, the cohort study can be adapted to collect a multitude of data and biospecimens at critical windows or life stages along with the collection of other relevant study covariates (e.g., potential confounders). This aspect of exposure health outcome temporality is highly informative when assessing findings in relation to causality. A prospective cohort design can capture a spectrum of health endpoints and outcomes (e.g., onset and progression of puberty). Follow-up of study participants can be a challenge, especially for subgroups of the population that may be hard to follow (e.g., transient, young, mobile, or individuals with name changes). Name changes for children may present a particular challenge and investigators would do well to plan for such changes and to ascertain other information useful for tracking over long periods of time.

Retrospective cohort studies identify a historic exposure and focus on following up the study population to ascertain health status. An example of such a study is the identification of adults who were exposed to DES in utero to assess reproductive impairments or reproductive cancers. Prospective cohort studies ascertain exposure and follow the cohort for a defined time period, and present a unique option for assessing child health in that the latency period tends to be short (even shorter for intrauterine exposures and infant outcomes). For example, investigators interested in determining if EDCs adversely impact foetal growth could recruit couples at risk for pregnancy prior to conception, measure serum concentrations of EDCs at baseline and throughout pregnancy, measure foetal growth at relevant time periods, and obtain birth size measurements. Preconception recruitment of women and couples has been shown to be feasible (Buck et al., 2004) and the short latency period underscores the utility of cohort studies for answering related questions. Cohort studies can be designed to commence with birth with follow up of offspring until targeted developmental ages relevant for assessing growth and development (e.g., through 36 months), minor neurodevelopmental impairments (e.g., through 60 months) or onset and progression of puberty (e.g., through age 16 years). Cohort studies are not efficient when the disease of interest is rare, since a large cohort would be needed for ensuring a sufficient number of cases.
Given the increased recognition that diseases including adverse pregnancy outcomes may arise from the multiplicative effects of environmental factors and genes, variations of the traditional case control study have been developed. The implications of gene-environment interactions for human reproduction and development have been reviewed (Cummings and Kavlock, 2004). Polymorphisms of genes have been associated with differential susceptibility to environmental exposures underscoring the need for studies specifically designed to estimate gene-environment interactions and child health (Cummings and Kavlock 2004). Case only designs are responsive to this avenue of research and overcome methodologic limitations associated with an inability to recruit population based controls from the target population. Increasingly, it is difficult to find suitable controls or to enroll them in sufficient numbers in research necessitating the need to develop designs not dependent upon external comparison groups (Piegorsch et al., 1994; Khoury and Flanders 1996). A case only design is one such approach that uses cases of a particular disease (e.g., birth defect) to assess the association between an environmental factor and genes. Underlying this design is a strong assumption that the environmental factor and genes are independent.

Unique methodologic considerations. Regardless of the epidemiologic design selected, key issues facing analytic studies include errors associated in the measurement of exposure(s), completeness of ascertaining health outcomes, capture of relevant covariates, and the validity of the assumptions underlying choice of statistical model (i.e., role of exposure, intermediate variables and effect modifiers). Interpretation of study results with respect to potential biases or other sources or error is needed to rule out alternative explanations for the observed findings. For example, the validity of case control studies is threatened by the potential for selection and recall bias. Systematic attrition also threatens the validity of cohort studies such as in studies focusing on asthma in children utilizing hospital treatment data, which have been reported to overestimate morbidity and utilization of health services.

6.1.2. Methodologic Approaches for Animal Studies

Many different experimental methods exist for investigating toxic effects of chemicals on reproduction and development. Many of these methodologies incorporate standardized procedures
for which guidelines have been issued by governmental agencies and international organizations such as USEPA, USFDA, ICH, EU and OECD, while others are still undergoing scientific evaluation. In the following sections, methods internationally acceptable to regulatory authorities (i.e., the OECD test guideline methods) are discussed with emphasis on effects induced during the prenatal and postnatal period. Table 6.2 summarizes these methodologies, i.e., the Prenatal Development Toxicity Study (OECD 414), One- and Two-Generation Studies (OECD TG 415 and 416), Reproductive/Developmental Toxicity Screening Tests (OECD TG 421 and 422), and the Developmental Neurotoxicity Study (OECD TG 426).

Other tests can reveal effects indicative of potential reproductive toxicity. Examples include the dominant lethal test, fertility assessment by continuous breeding, repeated dose toxicity testing, and cancer studies where the gonads are subjected to pathological examination. These tests, however, provide information only on effects after dosing adult animals and are therefore not addressed below.

Insert Table 6.2 here.

Table 6.2. Overview of *in vivo* OECD guideline tests for reproductive toxicity testing

<table>
<thead>
<tr>
<th>Test</th>
<th>Design</th>
<th>Endpoints</th>
<th>Advantages/limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>OECD TG 414 Prenatal</td>
<td>At least from implantation to one or two days before</td>
<td>Implantation, resorptions, Foetal growth, Morphological variations and</td>
<td>+ malformations are assessed in all foetuses</td>
</tr>
<tr>
<td>Developmental Toxicity</td>
<td>expected birth 3 dose levels plus control N = 20</td>
<td>malformations</td>
<td>- the dosing period includes only the prenatal period</td>
</tr>
<tr>
<td>Study (OECD 2001a)</td>
<td>pregnant females</td>
<td></td>
<td>- the effects assessment includes only effects in foetuses</td>
</tr>
<tr>
<td>OECD TG 415 One-Generation</td>
<td>Exposure before mating for at least one spermatogenic</td>
<td>Fertility, Growth, development and viability, Histopathology and weight of</td>
<td>+ exposure covers most of the sensitive periods</td>
</tr>
<tr>
<td>Study (OECD 1983)</td>
<td>cycle until weaning of 1st generation 3 dose levels</td>
<td>reproductive organs, brain and target organs</td>
<td>- no exposure from weaning to sexual maturation</td>
</tr>
<tr>
<td></td>
<td>plus control N = 20 pregnant females</td>
<td></td>
<td>- not updated to include similar endpoints as the two-generation study</td>
</tr>
<tr>
<td>OECD TG 416 Two-Generation</td>
<td>Exposure before mating for at least one spermatogenic</td>
<td>Fertility, Oestrous cyclicity and sperm quality, Growth, development and</td>
<td>+ exposure covers all sensitive periods</td>
</tr>
<tr>
<td>study (OECD 2001b)</td>
<td>cycle until weaning of 2nd generation 3 dose levels</td>
<td>viability, Anogenital distance if triggered, Sexual maturation, Histopathology and weight of</td>
<td>+ effect assessment in F1 and F2</td>
</tr>
<tr>
<td></td>
<td>plus control N = 20 pregnant females</td>
<td></td>
<td>+ includes assessment of semen quality and oestrous cyclicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- anogenital distance only assessed in F2 if triggered</td>
</tr>
</tbody>
</table>
| OECD TG 421 and 422 Reproduction/Developmental toxicity screening test (OECD 1995 & 1996) | From 2 weeks prior to mating until at least day 4 postnatally 3 dose levels plus control N = 8-10 pregnant females | Reproductive organs, brain and target organs | - areola/nipple retention is not assessed  
- malformations of reproductive organs only investigated in 1 per sex per litter  
Fertility  
Pregnancy length and birth  
Foetal and pup growth and survival until day 3  
+ short-term test  
- limited exposure period  
- limited number of endpoints  
- limited sensitivity due to number of animals |
|---|---|---|---|
| OECD TG 426 Developmental Neurotoxicity Study (OECD 2003a) | At least from implantation throughout lactation (PND 20) 3 dose levels plus control N = 20 recommended, less than 16 not appropriate | Birth and pregnancy length  
Growth, development and viability  
Physical and functional maturation  
Behavioural changes due to CNS and PNS effects  
Brain weights and neuropathology | + exposure covers most of the sensitive periods  
- no exposure before mating and from weaning to sexual maturation  
- mating and nursing behaviour is not assessed |

A number of assays such as the Uterotrophic assay, the Hershberger assay and male and female pubertal assays and others are currently being developed for detecting potential endocrine disrupting activity and effects of chemical substances in juvenile or young animals. The Uterotrophic and Hershberger assays seem reliable for identifying chemicals with (anti)oestrogenic and (anti)androgenic activity, respectively. The pubertal assays provide knowledge on effects during the juvenile phase and some of the endpoints included are similar to those in the two-generation study. Generally, clear effects observed in these assays indicate a potential for developmental toxicity in the two-generation study (Hass et al 2004). The methodologies are summarized in Table 6.3.

During recent years, many in vitro test systems have been proposed as alternatives to animal testing for developmental toxicity. These tests may be useful for screening of closely related chemicals and for pinpointing mechanisms underlying developmental effects; however, they cannot replace animal testing, because of factors such as the limitations regarding metabolic activation of chemicals, developmental stage-specific vulnerabilities and the multiple number of mechanisms leading to developmental toxicity. Consequently, they are not considered in the following sections.
Developmental stage vulnerability, dosing periods and assessment of effects. The vulnerable windows for developmental toxicity effects start prior to conception, during prenatal development and during postnatal development to the time of sexual maturation (see Table 2.1). Developmental toxicity effects may become manifest at any time point in the life span of the organism. Figure 6.1 illustrates the vulnerable periods and the exposure periods covered in the OECD Guidelines, while Figure 6.2 illustrates the timing of assessment of effects. Table 6.4 gives an overview of the outcomes assessed in offspring in OECD test guideline studies.

Table 6.3. Assays for detecting endocrine disrupting activity in juvenile or young animals (examples)

<table>
<thead>
<tr>
<th>Test</th>
<th>Design</th>
<th>Endpoints</th>
<th>Advantages/Limitations</th>
<th>Guideline(s)</th>
</tr>
</thead>
</table>
| Uterotrophic Assay, detection of (anti)oestrogens | 3 day s.c. or p.o. to intact immature or adult ovariectomized female rats N=6 | Mandatory endpoints: Body weight and weight of oestrogen responsive tissue, i.e. uterus wet weight and blotted weight | +Used since 1935  
+Simple, robust and reproducible  
-Detects ER ligands only  
The first and second phase of the validation of the Uterotrophic assay within OECD is completed (OECD 2003b)  
The guideline preparation is in progress within OECD |                                                                                           |
| Hershberger Assay, detection of (anti)androgens | 10 day p.o. to immature castrated male rats N=6                          | Mandatory endpoints: Body weight and weight of ventral prostate, seminal vesicles plus coagulating glands, levator ani/bulbocavernosus muscle, Cowper’s glands, glans penis | +Used since 1940  
+Simple, robust and reproducible.  
+More sensitive than the pubertal assay to AR ligands.  
-Detects AR ligands only.  
-Inhibition of steroidogenesis not detected.  
-Surgical castration  
The first and second phase of the validation of the Hershberger assay is completed (OECD 2003c)  
The third phase is in progress within OECD |                                                                                           |
<table>
<thead>
<tr>
<th>Pubertal female assay</th>
<th>Daily dosing by oral gavage on postnatal days 22-42 At least 2 dose levels plus control N=15</th>
<th>Growth, serum T4 and TSH, age at vaginal opening, vaginal cytology, ovarian and uterus weight and histology, weight of liver, kidney, pituitary, adrenals. +Sensitive to modulators of the HPG-axis and the thyroid +Endpoints the same as in the generation studies - Does not detect 5α-reductase and some aromatase inhibitors - Growth and nutritional status may influence vaginal opening</th>
<th>Recommended by EDSTACa to be part of a Tier 1 in vivo screening battery together with the Uterotrophic and the Hershberger assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pubertal male assay</td>
<td>Daily dosing by oral gavage on postnatal days 23-54 At least 2 dose levels plus control N=15</td>
<td>Growth, age at balanopreputial separation, serum T4 and TSH, weight of seminal vesicles, levator ani/bulbocavernosus muscle, and ventral prostate. Thyroid, testis and epididymal weight and histology. +Sensitive to modulators of the HPG-axis and the thyroid +Endpoints the same as in the generations studies -Does not detect all aromatase inhibitors -Growth and nutritional status influence may preputial separation</td>
<td>EDSTACa has also considered an alternative Tier 1 in vivo screening battery, which includes the pubertal male assay and the Uterotrophic assay</td>
</tr>
</tbody>
</table>

**Figure 6.1 Exposure periods in the OECD Test Guidelines**

<table>
<thead>
<tr>
<th>Life stages:</th>
<th>Before conception</th>
<th>Pregnancy</th>
<th>Birth-weaning</th>
<th>Until sexual maturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental toxicity study</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>One-generation study</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Two-generation study</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Developmental neurotoxicity study</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Reproduction/ Developmental toxicity screening test</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>
The prenatal developmental toxicity study was originally designed to investigate malformations and the pregnant animals are sacrificed on the day prior to the expected birth. The background for this is that the dams may eat malformed offspring. As such, the prenatal developmental toxicity study only covers effects induced during prenatal development and visible at term.

**Figure 6.2. Assessment of effects in the OECD Test Guidelines**

<table>
<thead>
<tr>
<th>Life stages:</th>
<th>Pregnancy</th>
<th>Birth-weaning</th>
<th>Until sexual maturation</th>
<th>Adult animals</th>
<th>Aging animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental toxicity study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One-generation study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-generation study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental neurotoxicity study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproduction/ Developmental toxicity screening test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The two-generation study is unique as it is the only study where the animals are exposed during all of the vulnerable time periods. The study requires that growth and survival of the offspring, sexual maturation, fertility, semen quality and oestrus cyclicity be investigated in young adult animals. In the one-generation study, the exposure of the offspring is stopped at weaning and consequently juvenile animals are not exposed. The assessment of effects stops at weaning.

The exposure period in the developmental neurotoxicity study is during gestation and lactation, but not during the juvenile period until sexual maturation. The brain still undergoes development during the juvenile period, but effects induced during this period are not covered in the guideline. The assessment of effects in the study continues after exposure until around the age of 2-4 months.
Table 6.4. Outcomes assessed in offspring in OECD guideline studies.

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>TG 415</th>
<th>TG 416</th>
<th>TG 421 &amp; 422</th>
<th>Draft TG 426</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Survival-perinatal period</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Survival-lactation period</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Survival-adult</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Growth-perinatal period</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth-lactation period</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Growth-adult (adolescence!)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Physical development</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>- sexual maturation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional development</td>
<td>-</td>
<td>(+)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Behaviour</td>
<td>-</td>
<td>(+)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Neuropathology</td>
<td>-</td>
<td>?</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Reproductive functions</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other function (e.g. immune)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ required; (+) optional; - not assessed

The Reproduction/Developmental Toxicity Screening Test is designed to provide initial limited information on reproductive toxicity and the exposure period is from a few weeks before mating, during the pregnancy period, and until a few days after birth (i.e., 3-6 days). Developmental toxicity effects are assessed from birth until around 3-6 days after birth.

In summary, among the current OECD Test Guidelines for reproductive toxicity, all vulnerable periods of development are covered only in the two-generation study design. Late effects are partly covered in young adults, especially in relation to reproductive function and developmental...
neurotoxicity. Effects manifested during aging are not included in any guidelines for reproductive toxicity.

Dosing of foetuses and pups. The dosing of the animals in reproductive toxicity study is mainly orally. Oral dosing by gavage normally includes only the dams (i.e., the foetuses and pups) are assumed indirectly dosed via placenta or maternal milk. Oral gavage may be stressful for the animals especially around the time of birth and consequently the animals are normally not dosed from the day before expected birth until a few days after birth. During the third week of the lactation period, the pups gradually change from maternal milk to their rat diet and the exposure of the pups will therefore decline during this period. This means that although the dosing period in the one- and two-generation study comprises the pregnancy and lactation period, the foetuses and pups are not exposed for some days around the time of birth and the pups are only exposed to a limited extent during the third week of the lactation period. When using oral dosing via diet or drinking water, the dams are normally dosed during the time of birth. When the pups gradually change from maternal milk to rat diet during the third week of lactation they will still be dosed, although the dose level may differ depending on the levels in maternal milk.

For some chemicals, inhalation exposure may be the relevant route. During the postnatal development of the pups, the exposure may be to both the dam and the pups or to the dam only. Direct dosing of pre-weaning animals may be needed to ensure adequate exposure of the offspring during critical stages of development (e.g., when assessing effects on the developing nervous or immune system) and/or to quantify exposure to pups. Details concerning methodologies and design issues in relation to direct exposure of pups are available from the International Life Sciences Institute (ILSI 2003a).

6.2. Growth & Development

6.2.1. Human Studies

The timed and highly interrelated processes underlying human development are important considerations when designing epidemiologic research focusing on growth and developmental
endpoints. The same exposure (or mixture) at varying gestational ages may manifest in different outcomes as discussed above. To this end, exposures need to be considered in relation to life stages relevant for human populations. Prospective pregnancy studies are perhaps the best design for addressing timing and concentration of an exposure along with other attributes (e.g., acute versus chronic; intermittent versus constant) and a spectrum of outcomes at developmentally relevant critical windows. Such information is especially informative when assessing study results with respect to causality between exposure and effect. The short interval between conception and birth and the opportunity to consider a range of exposures within a single design further argues for use of prospective cohort designs.

Growth. By definition, growth and development in humans is a dynamic process involving numerous bodily processes. While some critical windows have been identified for structural birth defects (Wilson 1965) and other health outcomes such as neurodevelopment (Selevan et al., 2000; Morford et al., 2004), critical gaps exist for many other health endpoints relevant for children’s health. Established embryonic critical windows typically begin with early pregnancy or approximately two weeks post-conception. Unfortunately, investigators often are unable to capture exposures during this interval because women may not recognize their pregnancy nor seek medical care. Hence, retrospective collection of information is required assuming women know and can accurately report exposures in the periconception period. The ability to recognize postimplation pregnancy via the hCG biomarker affords investigators an opportunity to define periconception critical windows using prospective cohort designs (Wilcox et al., 1988; Wang et al., 2003). A theoretical description of critical windows during this interval has recently been described (Morford et al., 2004). The periconception critical window is unique in that it is consistent with the couple dependent nature of human reproduction and the potential for maternal and/or parental exposures as described by Chapin et al., 2004. Measuring conception and capturing exposures peri-conception through implantation remains a challenge but field-based technologies are arising and offer promise for future investigations.

Once exposures are identified and measured, it is necessary to determine how best to measure growth and development. If pregnant women comprise the study population, repeated foetal measurements will be needed at standardized intervals. Growth requires a minimum of three
measurements during pregnancy, preferably at established intervals. While this might readily
translate into one measurement per trimester of pregnancy, the study hypothesis should drive the
timing of growth measurement. For example, the peak velocity for length is in the second
trimester and weight in the third trimester (especially the last month of pregnancy). Infants born
short and lean may have had chronic exposure extending throughout pregnancy while infants
born long and lean may reflect acute exposures late in pregnancy. Thus, symmetry of growth
can provide insight into the possible timing and duration of an exposure. Such measurements are
possible with ultrasonography and standardized protocols for assessing numerous indices of
foetal growth are clinically available and adaptable for field research. Birth size measurements
are frequently used to characterize foetal growth. Birth weight is also a frequently used endpoint
when considering in utero exposures and foetal growth. When available, measurements on birth
length, head and abdominal circumference and other anthropometric measurements provide
insight about the rate of foetal growth and possible timing of exposure. While these measures
may be prone to measurement error, none are likely to be biased given the absence of plausible
reasons for systematic differences in the measurement process with respect to many exposures.

For infants and children, a number of well-defined anthropometric methodologies are available
requiring the use of standardized scales and tape measurements by trained personnel along with
skinfold measurements (Dibley et al., 1987; NCHS 1998). Summary measures of adiposity
include the ponderal index for infants and the body mass index for children. Both indices
provide a measure of how lean/fat the infant or child is for his/her birth length or height,
respectively. Simpler methods also have been utilized in the field for measuring growth such as
measuring standing height and arm or leg circumference with standardized strings.

Growth charts can be used as a global assessment of infant or child size for age and are well
suited for temporal surveillance (tracking) and identification of at risk children. Typically,
growth charts are available for weight, height and head circumference so that infants and
children can be ranked in terms of age specific percentiles (NCHS 2003). Growth charts can
also be tailored with regard to infant sex, plurality of birth, and race/ethnicity in recognition of
differences across subgroups of the population. The appropriateness of birth size for gestational
age can be summarized as small (<5th or 10th percentile), large (>5th or 10th percentile) or
appropriate for gestational age. Infants born at the extremes of birth size for gestation may be at risk for mortality or morbidity.

**Development.** The effects of environmental exposures on human development is of global concern (see Chapter 4). Infant and child development can be assessed in relation to major and minor neurodevelopmental impairments. Each type includes a variety of endpoints relevant for studying developmental disabilities (WHO 1980). The key aspect that differentiates developmental disabilities, per se, is that their etiology occurs before birth; developmental disabilities are not acquired after birth, though they can be diagnosed at varying chronologic ages. Major neurodevelopmental impairments include cerebral palsy, mental retardation, blindness, deafness, and multiple impairments. An added dimension to assessing these outcomes is functional status of affected children. Vohr and Msall (1997) noted increasing survival for extremely low birth weight infants (<1,000 grams) without a concomitant increase in major neurodevelopmental impairments. However, an increasing percentage of children may have functional limitations associated with diminished birth weight. For example, approximately 20% of extremely premature infants born were found to have major impairments at age four years, yet all but 5% could function or perform the activities of daily living (i.e., walk unassisted, feed, dress, and toilete oneself). Thus, most investigators encompass neurodevelopmental impairments and function into assessment protocols. Minor neurodevelopmental impairments include deficits (not impairments, per se) in vision, motor, perception and cognition. Typically, these impairments cannot be reliably diagnosed until preschool or school ages. Various options exist for population based research (Msall et al., 1993a; Msall 1996; Krasnegor et al., 1992), though some require administration by trained clinicians. The choice of instrument is largely dependent upon the study hypothesis (exposure and outcome), infant/child’s chronologic age (or mental age for children with cognitive impairments) and whether the instrument needs to be administered by a trained psychologist (e.g., Bayley 1993) or can be self administered by a parent (Bricker et al., 1995) or a teacher (Leviton et al., 1993). Standardized prospective follow-up of children’s growth and health status between birth and age two years as captured with monthly diaries completed by mothers along with in-home assessments of children at 12- and 24-months of age was found to feasible and acceptable to parents (Senn et al., 2004).
Unlike adults (Hutchinson et al., 1992), there is no universally accepted methodology for measuring infant and child development in relation to environmental exposures. The selected approach must be age, gender and culturally appropriate. A number of evaluation tools exist for measuring targeted aspects of infant or child development, especially motor, cognitive or sensory domains. Many instruments can be jointly administered to ensure assessment of various aspects of development.

**Puberty.** Onset and progression of puberty can be used for both boys and girls to measure the influence of exposures on this developmental milestone. This approach includes assessing normal progression and deviations such as early or delayed onset. Puberty can be measured with a variety of instruments such as self-rating schemes, anatomical markers for staging pubertal development performed by highly trained health care practitioners such as the Tanner Scales (Marshall and Tanner 1969), and longitudinal hormonal assessments to capture changes in concentrations. Approaches for assessing puberty (including the applicability to specific subgroups of the population such as adolescents with chronic illness or developmental disabilities) along with promising new biomarkers such as spermarchy in (pre)adolescent boys have been summarized by Rockett et al., 2004a. Hormonally active environmental chemicals (e.g., PBBs, EDCs) can potentially impact precocious puberty (defined as the onset of secondary sex characteristics before 8 and 9 years of age in females and males, respectively) (Bates 1998; Blanck et al., 2000). With regard to pubertal delays, authors have assessed delays in attaining specific pubertal milestones at specific chronologic ages and in terms of the rate of progression between pubertal stages such as Tanner’s stages (Wu et al., 2003; Selevan et al., 2003).

**Birth defects.** Approximately 3-4% of infants are diagnosed with a major birth defect in the first year of life, though birth prevalence varies by many factors including maternal age, race and medical history such as insulin dependent diabetes (Edmonds et al., 1981; Lynberg et al., 1996) (also see Chapter 4). Since many affected conceptuses die before or shortly after implantation, the incidence of birth defects is largely unknown and further complicated in many societies by antenatal testing resulting in termination of pregnancy. Birth defects registries are available for some populations and are a useful tool for etiologic research. Capture of major malformations is more complete when active surveillance of defects is utilized versus passive systems that rely on...
the reportings by physicians or health care providers. In addition, many registries do not include minor malformations (e.g., hypospadia) resulting in an inability to monitor these defects on a population basis. Also, birth defects registries typically do not capture data on relevant study covariates (e.g., maternal diet, lifestyle factors such as cigarette smoking, use of medications or herbs) including the exposure of interest. Additional studies may be needed to capture such data.

Given the rarity of major malformations, the design of choice remains a case control study. The choice of control group is exceedingly important to minimize the risk of over or under-stating an effect. Recall bias, stemming from the systematic difference in reporting exposures between mothers of affected (cases) and unaffected (controls) children is a well recognized threat to validity and needs to be considered when interpreting study findings. The case only design offers promise for assessing gene-environment interactions, though strong assumptions must be made about the independent relation between the environmental factor and genes. A second consideration in designing studies focusing on birth defects is the need to capture exposures during critical windows of human development (Wilson 1965; Selevan et al., 2000).

6.2.2 Animal Studies

**Body weight and postnatal growth.** Foetal body weight is recorded in the prenatal developmental toxicity study (OECD-TG 414), where the foetuses are killed the day before expected birth. Birth weight and postnatal growth are recorded in all other OECD reproductive toxicity guidelines. It is important in the evaluation of the data to consider variations due to different litter sizes or different sex distribution in the litters. A change in offspring body weight is a sensitive indicator of developmental toxicity, in part because it is a continuous variable. In some cases, weight reduction in offspring may be the only indicator of developmental toxicity in a generation study. While uncertainty remains as to whether weight reduction is a permanent or transitory effect, little is known about the long-term consequences of short-term foetal or neonatal weight changes.

**Pre-, peri-, and postnatal death.** Prenatal developmental toxicity studies (e.g., OECD-TG 414) are very suitable for the demonstration of intra-uterine death resorption after implantation. In studies where dosing is started before implantation, preimplantation loss may also be assessed by
comparing the number of corpora lutea in the ovaries of the pregnant animals to the number of implantations. Uteri that appear non-gravid should be further examined.

There are some limitations of generation studies concerning stillbirth and early postnatal death, since the commonly used laboratory animals may eat their dead or seriously malformed pups immediately after birth. An effect may, therefore, only be indicated indirectly by a smaller litter size. If only a few pups are affected, the reduction in litter size will be small compared to the normal variation in litter size and may, therefore, go undetected or not reach statistical significance. Preimplantation losses and resorption are also indicated in an indirect way as a decreased litter size and the sensitivity for these effects may be rather low.

Physical and functional developmental landmarks. The physical development of the offspring is normally monitored by registration of body weight several times during the pre-weaning period and once every second week after weaning. Other physical and functional parameters are monitored by registration of so-called developmental milestones or developmental landmarks. These observations often show “when” rather than “if”, the various landmarks first appear and are used to assess delayed or accelerated developmental time courses for the specific parameters being studied (Lochry, 1985). These tests evaluate the presence or absence of each parameter, usually over a period of successive days, beginning prior to, or approximately on the day of expected development.

Examples of frequently suggested physical developmental landmarks are ear unfolding, first coat, upper and lower incisor eruption, eye opening, full coat and onset of puberty. The reliability of observing six physical landmarks (ear unfolding, first coat, upper incisor eruption, lower incisor eruption, eye opening, and full coat) by different observers was assessed in a study. As a result of this evaluation, the three reliable physical parameters (i.e., ear unfolding, upper incisor eruption, and eye opening) were selected for use. Registration of these physical landmarks is not required in TG 416 or TG 426, but should be considered when appropriate.

Functional development can be assessed for example by registration of the time of emergence of the surface righting reflex, negative geotaxis reflex, auditory startle reflex, and air righting reflex.
The draft TG 426 requires that two measures are to be registered equally spaced over the pre-weaning period. Registration of functional endpoints requires some training in order to ensure that the animals are scored similarly each time. For that reason it is also preferable that the assessment is performed by one person (or a few persons) using similar criteria each time. Early handling during inspection of physical or functional landmark may influence the behaviour of the animals later in life. Therefore, litters from all groups (exposure and control) should be investigated similarly so as to avoid.

The age of the offspring may be decided from the time of birth (i.e., post partum age) or from the time of mating (i.e., gestational age). Studies have shown gestational age to be a better predictor of time of appearance of developmental landmarks in the pre-weaning period than postnatal age, especially if the length of gestation periods differs among groups (Raimondo and Draghetti 1990). Gestational age has been used in several studies of developmental neurotoxic effects (Kelly et al., 1988, Hass et al., 1994b, Hass et al., 1995).

Birth defects, malformations. The prenatal developmental toxicity test (TG 414) is designed to detect malformations. The sensitivity of the test for detection of rare malformations is limited, due to the use of a relatively small number of animals. With the normal group sizes of 20 pregnant rats, it is not possible to identify any increase in major malformations unless high dose levels are administered or the substance studied is highly embryo/foetotoxic (Palmer 1981). To assess the developmental toxicity of a chemical, it is therefore important to include information on other developmental effects such as minor anomalies, skeletal variations, foetal death and growth. In addition, malformations of organs developing also after birth, e.g., the sex organs may not be detected using this guideline (See section 6.3.2). The development of some organs such as the reproductive organs and the brain continues after birth and malformations of these organs may not become manifest until the animal is sexually mature (see sections 6.3.2 and 6.4.2).

The International Federation of Teratology Societies (IFTS) Committee on International Harmonization of Nomenclature in Developmental Toxicology has developed and published a glossary of internationally accepted common nomenclature to use when describing observations of foetal and neonatal morphology (Wise et al., 1997). The purpose of this effort was to advance...
the harmonisation of terminology, and to reduce confusion and ambiguity in the description of
developmental effects, particularly in submissions to regulatory agencies world-wide. Familiarity
with the IFTS terminology for external, visceral, and skeletal observations, and appropriate use
of the terminology in data collection, reporting, and review, is encouraged. It is recognised,
however, that although the common nomenclature developed by this effort has been widely
available and internationally accepted, there is no guarantee that the terminology has been
uniformly used by all laboratories conducting studies for chemical hazard assessment.

6.3. Reproductive Development and Function

6.3.1. Human Studies

Study of human reproductive health necessitates appreciation of the highly interrelated and timed
series of events underlying successful human reproduction (Johnson, 2006). In assessing the
reproductive toxicity of an environmental agent, two broad categories of outcomes can be
studied – fecundity and fertility. Fecundity refers to the biologic capacity of men and women for
reproduction while fertility refers to the ability of a woman to give birth to a live born infant or a
man to have fathered a live born infant (Wood 1994). Fecundity can only be “proved” when the
couple has a live born infant. A live birth is the biomarker of fertility.

The ability to identify structural abnormalities or deficiencies (e.g., cryptorchidism, hypospadia,
endometriosis) in the reproductive organs of children, men and women often requires purposeful
clinical examination and invasive diagnostic procedures such as pelvic laparoscopy in women.
Often this is done in response to health concerns or signs/symptoms indicative of underlying
disease. Epidemiologic studies that incorporate clinical populations frequently are limited with
regard to both internal and external validity making it difficult to systematically evaluate organ
specific abnormalities. Choice of comparison group or the selectedness of individuals who seek
medical care potentially impact study findings. For example, exposure to dioxins and PCBs have
been lined with an increased risk of endometriosis in a few studies. However, diagnosis requires
pelvic laparoscopy or laparotomy to affirm the presence or absence of disease. Choice of
sampling framework to explore environmental causes is difficult because study participants
cannot be asked to undergo laparoscopy in the absence of signs/symptoms for research purposes only. To this end, women with pelvic pain or infertility for whom a laparoscopy is medically indicated are often selected as the comparison group as are women undergoing tubal sterilization procedures. Studies on the former groups of women may fail to reflect an association between an exposure and disease if the exposure affects a spectrum of gynecologic effects (not just endometriosis). Conversely, use of fertile women may exaggerate an association if exposure concentrations have been affected by pregnancy or lactation patterns.

Fecundity can be estimated or approximated by assessing reproductive hormonal profiles of men and women, semen quality in men, or menstruation in women, or by measuring time-to-pregnancy (TTP) for couples interested in becoming pregnant. TTP can be measured in calendar months or menstrual cycles depending upon the study population, and has been shown to be a reasonable estimate of cycle specific probability of conception when comparing exposed and unexposed women or couples (Baird et al., 1986). TTP can be dichotomized as conception delay defined as requiring >6 menstrual cycles for conception or as infertility or the absence of pregnancy despite 12 or more months of regular unprotected intercourse. Women with conception delays are reported to be at increased risk of delivering a preterm or low birth weight infant (Olsen et al., 1983; Williams et al., 1991; Joffe and Li 1994; Henriksen et al., 1999), although no effect was seen in another study after controlling for potential confounders (Cooney et al., 2006).

Fecundity endpoints are not necessarily adverse health states and may also include puberty, sexual libido, gynecologic or urologic disorders (e.g., endometriosis and erectile dysfunction), fecundity impairments (e.g., early pregnancy loss), and premature reproductive senescence. The need to consider fecundity endpoints is supported by a possible relation between PCB exposure and endometriosis (Mayani et al., 1997; Buck Louis et al., 2004) and consumption of PCB contaminated fish and diminished fecundability (Axom et al., 2000; Buck et al., 2000). Male fecundity impairments have been associated with both birth defects and chronic disease. For example, cryptorchidism has been associated with impairments in male fecundity and testicular cancer. This constellation of ecologic patterns (declining semen quality, rising rates of cryptorchid testes and testicular cancer) has been referred to as the testicular dysgenesis
hypothesis for which an environmental origin has been hypothesized (Skakkebaek et al., 2001) [see also Chapter 4).

Use of fertility monitors such as The Clearblue Easy® monitor offers promise for capturing information about hormonal patterns during the menstrual cycle including ovulation and more precisely information about time required for conception. This monitor tracks changes in estrone-3-glucuronide (E3G), a metabolite of estradiol, and luteinizing hormone (LH), using urine collected via special test sticks. Upon urinating on the test stick, two blue lines appear in the test stick window; the monitor optically measures the intensity of these lines to track changes in E3G and LH. The monitor is reported to be highly accurate (99%) in detecting the LH surge and in predicting peak fertility (91%) in comparison to ultrasonography (Behre et al., 2000).

After five minutes, the monitor displays the woman’s fertility status for the current day (e.g., low, high or peak fertility) and can store up to 40 days of detailed information and up to six months of summary data that can be downloaded to a personal computer with the help of card reader. The accuracy of the Clearblue Easy® monitor has been evaluated and supports the WHO criteria for detecting the LH surge or impending ovulation (WHO, 1983).

Pregnancy loss. Pregnancy loss comprises three groups: losses occurring prior to implantation of the conceptus in the endometrium, following implantation and identified with hCG testing, or after clinical recognition of pregnancy. hCG pregnancies are referred to as early or clinically unrecognized pregnancies, in that most women, will not be aware they are pregnant unless measuring hCG such as with home pregnancy test kits. The incidence of early pregnancy loss has been estimated in only a few prospective cohort designs where couples have been enrolled prior to attempting pregnancy with active monitoring of hCG. Of these studies, approximately one-third of all hCG confirmed pregnancies were spontaneously lost, especially in the first few weeks following implantation (Wilcox et al., 1988; Wang et al., 2003). Specifically, two-thirds of these pregnancy losses were hCG detected pregnancies and the remaining one-third were clinically recognized pregnancies. These figures underscore the importance of capturing hCG and not just clinically recognized pregnancies to avoid underascertaining pregnancy losses. An important point to keep in mind is that studies starting with pregnant women or newborns are not designed to capture hCG pregnancy loss necessitating the need to consider other developmental
outcomes. As such, an exposure that causes embryonic death may not be linked to deficits in children. This notion is referred to as competing risk and is important to keep in mind when assessing developmental outcomes and interpreting study results that rely only on clinically recognized pregnancies. The design of choice for assessing embryonic and foetal loss is a prospective pregnancy study design to estimate as accurately as possible the number of conceptions. Losses occurring later in pregnancy include foetal deaths, spontaneous terminations of pregnancy, losses occurring during the foetal period, and stillbirths that typically occur in the few weeks prior to delivery or any time following gestational ages deemed viable. Foetal death registries exist in many countries and can be used for ascertaining cases for defined populations with respect to time. However, a number of methodological issues face use of vital registries such as varying criteria for defining foetal losses (e.g., by gestation, birth weight or both) and quality of data including cause of death is reported to be poor (Kirby 1993; Lammer et al., 1989).

Fertility endpoints include live births, plurality of birth and secondary sex ratios of the ratio of male to female live births. Xenobiotics that selectively impact the x or y chromosome may result in decrements of the sex ratio possibly due to differences in rates of conception or pregnancy loss. In fact, investigators have use this ratio as a sentinel marker of male fecundity (Davis et al., 1998), with declines reported in some populations (Allan et al., 1997; Scialli et al., 1997) and increases in other (Astolfi and Zonta 1999). WHO has established protocols for the evaluation of semen quality (male fecundity) and infertile couples.

6.3.2 Animal Studies

Malformations of reproductive organs. Examination of structural changes of the reproductive organs is included in all OECD guideline studies except TG 426. Malformations of reproductive organs include for example persistent nipples, hypospadias and cryptorchism in male offspring. The OECD 414 is designed especially to investigate major malformations and the pregnant animals are killed prior to the expected day of delivery (gestation day 21 in the rat). The guideline specifies that investigations of the foetuses should pay particular attention to the
reproductive tract. These techniques may not be sensitive for detecting all malformations among organs which are not fully developed at birth.

In studies performed according to the OECD TG 414 Teratology Study before the guideline was updated, the animals were dosed only during the major organogenesis (i.e., gestation days 6-15 in the rats). As important parts of the development of the reproductive organs happen after gestation day 15, the potential for effects on the development of the reproductive organs cannot be assessed in such studies. In the updated OECD TG 414, prenatal developmental toxicity study, the exposure period is extended until a few days before birth. However, the development of the reproductive organs continues after birth and some effects may not become manifest until the animal is sexually mature. Consequently, the OECD 414 is not suitable for detection of some important malformations of the reproductive organs.

Malformations of the reproductive organs such as hypospadias and cryptorchidism can potentially be identified in the generation studies, since the offspring is investigated until young adulthood. However, only 1/sex/litter is selected at weaning for the next generation in contrast to the assessment of malformations using the OECD TG 414, where all foetuses are investigated. As malformations normally are rare effects, the generation studies have a limited sensitivity for detecting such effects, and therefore, malformations in the reproductive organs occurring at low rates may not reach statistical significance. The short-term studies (i.e., TG 421 and 422) include a limited number of litters and the animals are immature when investigated on postnatal day 3 or 4. Therefore, the potential for malformations of reproductive organs can only be assessed to a very limited extent in these studies.

Anogenital distance (AGD). Visual inspection of the AGD in foetuses and newborn pups is used for deciding the sex of the animals, as the AGD normally is twice as long in males compared to females. In the two-generation study, AGD should be measured at postnatal day 0 in the pups in the second generation, if triggered by alterations in the sex ratio or timing of sexual maturation in the first generation. There is a relation between the AGD and the size of the pup and, therefore, the body weight of the pup is normally used as a covariate in the analysis of the data. However, if body weight is significantly influenced by exposure this may actually mask effects on
anogenital distance. In general, a statistically significant change in the AGD (adjusting for size of the pups) would be considered adverse. Several studies have shown that hormones and exposure to EDCs may change the AGD. This is especially true for antiandrogens, where decreased AGD has been shown for some phthalates, procymidone and vinclozolin (Mylchrest et al., 1999, Ostby et al., 1999, Gray et al., 1999a).

Nipple/areola retention. Assessment of nipple or areola (dark area around the nipple bud) retention in male offspring is not included at present in any OECD TG. In female offspring, 12 areolas normally become visible around postnatal day thirteen, while very few or none are visible in male offspring. As the development of fur in the animals makes it difficult (impossible) to see the areolas a few days later it is important to establish the correct time for the assessment in the animals used for the study. Often, only the presence or absence is registered in the males, however, the number of areolas in each male may provide a more sensitive assessment. In general, a statistically significant increase in the number of nipples/areolas in male pups would be considered adverse. This is especially the case if it is shown that some of these nipples are persistent (i.e., they are also present in the adult males).

Sexual maturation and puberty. Assessment of the timing of sexual maturation is included in TG 416 and as an optional endpoint in TG 426. Assessment of the onset of puberty in females is done by inspection of the vaginal opening. In rats, this occurs around postnatal day 30-35. In males, testicular descent or balano-preputial separation, which occurs around postnatal day 21-30 and 42-48, respectively, may be used as indications of puberty. Observation of testicular descent relies on how the animal is handled during inspection and is rather difficult to assess. Balano-preputial separation corresponds to puberty in male rats (Korenbrot et al., 1977) and is the endpoint included in the developmental neurotoxicity study and the two-generation study. Both the age and the body weight of the animal at sexual maturation need to be recorded, as there is a relationship between these end points. In general, significant changes in the timing of sexual maturation that is not explained by body weight effects should be considered to indicate a potential adverse effect in humans.
Fertility. Assessment of fertility is included in generation studies and in the reproductive screening tests. Assessment of fertility in exposed offspring is only possible in the two-generation study. Fertility is generally expressed as indices that are ratios derived from the data collected. For example, the mating index is used as a measure of the male’s or female’s ability to mate and is defined as the number of animals with confirmed mating in the total number of animals cohabitating. The fertility index is a measure of the ability to achieve pregnancy and is defined as the number of males impregnating a female or number of pregnant females per the total number of animals cohabitating. The effects on fertility may be related either to effects induced before mating or thereafter, but before implantation (i.e., pre-implantation losses). The data will not necessarily be similar for studies in which there is more than one mating per generation or more that one mating generation. The interpretation of fertility data should consider the duration of treatment and the number of animals investigated. The males are not dosed during the total of the spermatogenic cycle in the reproductive screening tests and the studies use a limited number of animals. Therefore, it is unlikely that reproductive effects are manifested on the fertility data.

Reduced fertility has been found for a number of chemicals, but often at relatively high exposure levels. However, it has to be considered that the rat is the most commonly used experimental animal and that a male rat can generally still produce normal progeny after having its sperm production reduced to 10% of the normal level (Aafjes et al 1980). Thus, fertility data from rat studies alone can be a rather insensitive endpoint. Human males may not have a similar sperm reserve capacity as rodents and therefore the two-generation study has recently been updated to include assessment of sperm quality. It is unknown whether female rats (compared to human females) also are less sensitive to effects on fertility, but the two-generation study has recently also been updated in that aspect by the inclusion of assessment of oestrus cyclicity.

The assessment of sperm quality includes measures of sperm number, anomalies and motility, but the ability of the sperm to fertilize the ovum is not assessed. In order to increase the sensitivity for detection of fertility effects, investigation of in vitro fertilization could be considered. Another potentially more sensitive option may be the continuous breeding protocol where the animals produce several litters instead of only one litter per pair.
Effects manifesting during the aging process are not included in any guideline for reproductive toxicity. For example, the reproductive span of females is limited from the time of sexual maturation to reproductive senescence. The time of sexual maturation may be assessed in the two-generation study, but reproductive senescence is not assessed. Consequently, effects on the reproductive span of females are not covered in the reproductive toxicity guidelines.

Histopathology of reproductive organs. Histological examination of reproductive organs is included in the generation studies and the in vivo reproductive toxicity screening studies (i.e., OECD 421 and 422). The two-generation study that specifies dosing of the male for the entire spermatogenic cycle combined with analysis of sperm quality requires less extensive histopathological examination than the studies where the dosing regime is shorter and no sperm analyses are conducted (e.g., OECD 421 and 422).

The postlactational ovary should contain primordial and growing follicles as well as the large corpora lutea of lactation. In the two-generation study, the histopathological examination includes assessment of qualitative depletion of the primordial follicle population in the parental animals. A quantitative evaluation of primordial follicles should be conducted for F1 females; the number of animals, ovarian section selection, and section sample size should be statistically appropriate for the evaluation procedure used. Examination should include enumeration of the number of primordial follicles, which can be combined with small growing follicles, for comparison of treated and control ovaries.

Histopathological findings are generally classified according to qualitative criteria and the data are presented as the number of animals affected within a dose group. There may not be an obvious relation between histopathological findings and fertility. For short-term studies (i.e., OECD 421 and 422) in which the animals are treated for less than the duration of spermatogenic cycle, an effect on spermatogenesis may not have had adequate time to become evident as reduced sperm counts. In general, any dose-related significant histopathological finding would be considered to indicate a potential adverse effect in humans.
Sperm quality and oestrus cyclicity. These endpoints are included in the revised two-generation study, but at present not in the one-generation study. However, the one-generation study could be updated to include assessment in the parental animals without significant changes in the design. Assessment of these endpoints in exposed offspring is only possible in the two-generation study design. However, it would be possible to include the endpoints in the one-generation study if the study period were extended to around postnatal day 90 instead of postnatal day 21.

The endpoints included for assessment of sperm quality are sperm number, sperm morphology, and sperm motility. Testicular lesions of sufficient magnitude can impact sperm quality, but normal sperm quality is dependent on a number of other factors. Therefore, changes in sperm endpoints should not be discounted in the absence of histological lesions. For example, sperm changes could be due to effects on the epididymis. In general, a statistically significant change in sperm parameters would be interpreted as indicating a potential effect on human fertility.

Vaginal cytology is evaluated to determine the length and normality of the oestrous cycle in P and F1 females in the two-generation study. The data can provide information on cycle length, persistence of oestrus or dioestrus, and incidence of pseudopregnancy. An effect on oestrous cycle can affect reproductive performance, but this will depend on the nature and the magnitude of the effect. In general, a statistically significant change in the length of the cycle or prolonged oestrus or dioestrus would be considered potentially adverse.

6.4. Neurological and Behavioral Effects (see Chapter 4)

6.4.1. Human Studies

Environmental agents have been associated with neurotoxic effects in infants and children (IPCS 1986a, 2001a). Given the ability of agents to impact various target sites or pathways (e.g., autonomic, peripheral or central nervous system), a diverse range of outcomes should be considered. To this end, clinical assessment coupled with a battery of standardized assessment tools is likely to be needed. With respect to behaviour, gender specific tools should be considered if not implemented. Both DES and PCB exposure has been associated with alterations
in gender specific behaviour (Collaer and Hines, 1995; Guo et al., 1995; Longnecker et al.,
2003). Standardized clinical assessments are available for newborns and pediatric populations,
which can be tailored to meet particular research goals taking into account the characteristics or
physical/biological properties of the exposure under investigation.

6.4.2. Animal Studies

A guideline for a developmental neurotoxicity study was issued by USEPA in 1991 and a revised
US guideline was proposed in 1995 (USEPA 1998a). During recent years, an OECD draft TG 426
Developmental Neurotoxicity Study has been developed based on the US guideline (OECD,
2003a). Developmental neurotoxicity studies are designed to develop data on the potential
functional and morphological hazards to the nervous system arising in the offspring from exposure
of the mother during pregnancy and lactation. The OECD draft guideline is designed as a separate
study, but the observations and measurements can also be incorporated into a two-generation
study. The neurological evaluation consists of assessment of reflex ontogeny, motor activity,
motor and sensory function, and learning and memory; and evaluation of brain weights and
neuropathology during postnatal development and adulthood. The behavioural testing includes
assessment of the individual animal for a number of relevant behavioural functions, but none of the
tests assess two or more animals together. This means that some behavioural endpoints of potential
relevance (e.g., sexual behaviour, play behaviour, social interaction among animals and
aggression) are not assessed using the current Test Guidelines.

Motor activity. According to the draft TG 426, motor activity should be monitored using
automated recording apparatus at least once for each of the pre-weaning, post-weaning, and young
adult periods. The open field and the hole-board have generally been used to measure short term
activity, while automated devices (photo cells) such as the figure-8 maze, radial arm maze, and
cages similar to home cages have been used for measuring activity over longer time periods. The
recommendations given in the draft TG 426 relate mainly to the testing of longer-term activity
(e.g., 30 minutes), and should provide data for evaluating the potential for effects on motor activity
and habituation.
Motor and sensory functions. According to the draft TG 426, motor and sensory function should be examined in detail at least once for the adolescent period and once during the young adult period. Neuromotor abilities are often evaluated in regard to the ontogeny of particular reflexes or coordinated movements. Measures of reflex and motor development are the most widespread of all functional endpoints assessed in behavioral teratology studies (Adams 1986; Buelke-Sam and Kimmel 1979). The procedures for most of the commonly used measurements have been described in several reviews (Barlow and Sullivan 1975; Adams 1986). Until recently, measures of sensory function in developmental toxicity studies have been quite gross; measuring the presence or absence of response rather than the magnitude of the response. During the last decade, however, more sophisticated automated behavioral techniques allowing quantitative assessment of function have been developed.

Learning and memory. In the draft TG 426, testing of learning and memory is required post-weaning and for young animals. The use of different tests or different animals is recommended because repeated testing in the same test animal may decrease the sensitivity of the test. The time of post-weaning testing could be shortly after the end of exposure (i.e., at days 24-28) and just before termination of the study around days 60-80. The test for cognitive function (i.e., learning and memory) should be based on associative learning and should include the possibility to assess changes across repeated learning trials as well as memory function.

A number of different tests have been used for assessing effects on learning and memory. Many of these are based on the animals moving and using their senses; therefore, an impaired performance in a learning test may reflect behavioral effects other than learning abilities. The radial arm maze and the Morris maze have been used to demonstrate the effects of several positive control substances. Schedule controlled operant conditioning in Skinner boxes may detect very subtle effects, but may also be too time-consuming for the initial testing of learning and memory.

Evaluation of effects. Developmental neurotoxicity can be indicated by behavioral changes or morphological changes in the brain. The severity and nature of the effect should be considered. Generally, a pattern of effects (e.g., impaired learning during several consecutive trials) is more persuasive evidence of developmental neurotoxicity than one or a few
unrelated changes. In the standard developmental neurotoxicity study design, treatment is stopped following postnatal day (PND) 10 or PND 21, while neurobehavioral testing is conducted around the time of weaning, during the time of puberty, and again just before termination at approximately PND 60. Apparent reversibility in adult offspring of effects observed early in life may be related to compensatory developmental or behavioural processes and not represent a true recovery, per se. Likewise, findings observed in adult offspring that had not been previously observed in young test subjects should not be discounted for lack of concordance, since they may represent the latent expression of early alterations in neurological development. Effects observed during the beginning of a learning task, but not at the end should not be interpreted as a reversible effect. Rather, the results may indicate that the speed of learning is decreased.

The experience of offspring especially during infancy may affect their later behaviour. For example, frequent handling of rats during infancy may alter the physiological response to stress and behaviour in tests for emotionality and learning. In order to control for environmental experiences, the conditions under which the offspring are reared should be standardised within experiments with respect to variables such as noise level, handling and cage cleaning. The performance of the animals during behavioural testing may also be influenced by factors such as time of day and the stress level of the animals. The most reliable data are obtained in studies where control and treated animals are tested alternately and environmental conditions are standardised.

Most developmental neurotoxicity studies have focussed on general impairment of behaviour, but some studies have also found evidence for effects on sexual dimorphic behaviour. Hormones play a central role in central nervous system development including the sexual differentiation of the brain. Studies on hormones and various EDCs (particularly those with oestrogenic or antiandrogenic effects) have shown that the developing brain may be susceptible to disturbances in sexual behaviour. Therefore, effects on one gender but not the other should not be dismissed, but must be evaluated in the context of effects on sexual differentiation of the brain.
Of critical concern is the possibility that developmental exposure may result in an acceleration of age-related decline in function. Animal studies have demonstrated that developmental exposure to neurotoxicants such as methyl mercury, methylazoxymethanol and ethanol may cause few or no neurotoxic effects in young animals, but marked effects in aging animals. Investigations of effects in aging animals are not included in regulatory guidelines.

6.5. Cancer (see Chapter 4)

6.5.1. Human Studies

Given the relative rarity of childhood cancers, case control study designs are primarily utilized for assessing environmental carcinogens. For many countries, cancer registries will be used for ascertaining cases for a defined population in time. Selection of controls may include the use of siblings, neighbors, schoolmates, or children seeking medical care from clinics, hospitals, or private physician offices. The need for matching on known confounders may exist or at the very least capture covariate data on known and potential confounders.

Linkage studies are sometimes used for assessing possible new exposures. For example, a recent linkage study combined live birth and cancer registries to assess whether children born from assisted reproductive technologies were at risk for childhood cancer in comparison to children born without such technologies (Brinton et al., 2004). This linkage study was a population-based attempt to further explore an earlier report linking in utero hormonal exposure to neuroblastoma (Michalek et al., 1996; Olshan et al., 1999). Linkage studies may be limited by the completeness of data on the exposure (e.g., specific type of ART) and other etiologic factors impacting the couple’s fecundity. The methodologic challenges underlying the separation of an ART effect from underlying couple infecundity have recently been reviewed (Buck Louis et al., 2004).

6.5.2 Animal Studies

Studies for assessing the carcinogenicity of chemicals after developmental exposure are not included in any regulatory test guidelines. Transplacental carcinogenesis has been demonstrated
in rats, mice, hamster, rabbits, pigs, dogs and monkeys for around 60 chemicals (EHC 30). All
the transplacental carcinogens that have been studied were already known to be carcinogenic in
adult animals. It appears that the action of chemical carcinogens may be stronger or weaker after
passing through the placenta. Based on the current knowledge, it is not possible generally to
estimate if peri-pubertal exposure is more or less important than adult exposure. Negative results
in testing for transplacental or perinatal carcinogenic effects should be evaluated with caution,
because the period of development in experimental animals is relatively short compared to
human development and some chemicals may require more prolonged exposure in order to
induce tumours.

6.6. Immune (see Chapter 4)

6.6.1. Human Studies

Environmental agents including chemicals have been shown to affect the immune system in a
number of ways (IPCS 1999a). However, there are no universally established guidelines for
assessing immune function in children in relation to environmental agents. Diagnostic
approaches used by clinicians can be adapted for research purposes and may include alterations
in B and T lymphocytes or T helper/T suppressor cell ratios. Age appropriate assessments
should recognize the ongoing development of the immune system during foetal development and
childhood. Holladay and Smialowicz (2000) describe five stages leading to a mature immune
system each of which has its own set of vulnerabilities (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).

6.6.2. Animal Studies

Studies of immunotoxicity after developmental exposure are not included in any regulatory test
guidelines. Organogenesis of the immune system occurs during the prenatal and early postnatal
period (Holladay and Smialowicz 2000). Consequently, the perinatal period is a time of high
vulnerability to immunotoxicants that can cross the placenta or expose the neonates via lactation.
Postnatal immunotoxic effects from exposure during the initial establishment of the immune organs may be both more severe and more persistent than those that occur in adult animals exposed at similar levels. Chemical agents that induce developmental immunotoxicity in rodents are diverse and include halogenated aromatic hydrocarbons, polycyclic aromatic hydrocarbons, hormonal substances, therapeutic agents, heavy metals and mycotoxins (Holladay and Smialowicz, 2000; Luebke et al., 2006).

6.7. Respiratory

6.7.1. Human Studies

The mature respiratory system evolves throughout foetal life, childhood and early adulthood. Environmental chemicals have been shown that a number of respiratory processes such as cellular differentiation and lung growth underscoring the need to select health endpoints appropriate for age of infants or children (Smiley-Jewell et al., 1998; Fanucchi et al., 1997). There are no universally established guidelines for assessing respiratory function in children following environmental exposures necessitating the need for clinical assessment and capture of acute endpoints (e.g., respiratory infections in a specific period of time) or chronic endpoints (e.g., bronchitis, asthma). Symptom inventories also can be used (e.g., wheezing, cough, phlegm) along with field based spirometry to assess lung function (American Thoracic Society, 1987). Diagnostic approaches used by clinicians can be adapted for research purposes.

6.7.2. Animal Studies

Studies of effects on the respiratory system after developmental exposure are not included in any regulatory test guidelines. Exposure during critical periods of lung development may have effects that would not be seen if the same exposure were to occur in adulthood (Dietert et al., 2000).
6.8. Hemopoeitic/Cardiovascular, Hepatic/Renal, Skin/Musculoskeletal, Metabolic/Endocrine
(see Chapter 4)

6.8.1. Human Studies

There are no established universally accepted guidelines or study protocols for assessing organ
specific health endpoints included in this section. Examples of commonly used clinical tools or
tests that may be appropriate for children’s health issues are summarized in Table 6.5 along with
methods for assessing immunologic and respiratory endpoints. This table underscores the utility
in evaluating children’s overall health status rather than restricted focus on specific organ
systems except for circumstances when a particular exposure or health concern exists for a
defined study population.

6.8.2. Animal Studies

Studies of these specific organ system outcomes are not included in any regulatory test
guidelines.

6.9. Conclusions

Child health status can be measured very narrowly by focusing on one or two related endpoints
or globally by being inclusive of all organ systems recognizing the highly interrelated processes
underlying the dynamic state of children and childhood development. To date, there is no
universally defined or accepted methodology for assessing children’s overall health status in
relation to a multitude of environmental factors, though efforts are currently underway to address
this gap. For many aspects of child health and development such as growth and development, a
number of assessment tools are available and many can be implemented in field based research
while none exist for other organ systems.
Table 6.5. Examples of Global Clinical Assessment Tools by Organ System Amenable for Epidemiologic Investigator of Environmental Influences on Children’s Health

<table>
<thead>
<tr>
<th>Organ System</th>
<th>Clinical Assessment Tool</th>
</tr>
</thead>
</table>
| Immune                | • Blood tests: Antibody response to immunization (humoral immunity); serum concentrations of immunoglobulin subtypes (humoral immunity)  
                       | • Skin testing for common allergens (cell mediated immunity)                                                 |
|                       | • Frequency & duration of common infections                                                                   |
| Respiratory           | • Lung function: Spirometry and pulmonary function testing (measurements of lung volumes and flow rates); peak expiratory flow rate measurements (can be done in field with inexpensive handheld meters)  
                       | • Diffusing capacity for carbon monoxide (measurement of alveolar gas exchange)                            |
| Hematopoietic         | • Blood tests: Complete blood count (red and white blood cell indices and platelets); measurement of clotting factors, prothrombin time, partial thromboplastin time |
| Cardiovascular        | • Blood tests: Serum lipids (cholesterol, triglycerides)                                                    |
|                       | • EKGs: heart rate and rhythm                                                                               |
|                       | • Anthropometric: Body mass index (weight in kg/height in meters squared); skin fold thickness or other methods |
|                       | • Blood pressure: individual or continuous monitoring of blood pressure using portable monitors.             |
| Liver                 | • Blood tests: Liver enzymes (alanine aminotransferase, aspartate amino transferase, alkaline phosphatase, gamma-glutamyl transferase, bilirubin, lactate dehydrogenase) |
|                       | • Ultrasonography (portable units for field studies)                                                        |
| Kidney                | • Blood tests: Serum creatinine, blood urea nitrogen                                                        |
|                       | • Urine collection: 24-hour or spot for protein, glucose, creatinine clearance                              |
| Musculoskeletal       | • Strength testing, flexibility testing using standardized instruments or clinical examination                |
| Endocrine and Metabolic | • Blood tests: Serum concentrations of pituitary hormones (thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone, adrenocorticotropic hormone, growth hormone, prolactin, vasopressin); other hormones (insulin parathyroid hormone, glucagon, calcitonin, Vitamin D); and serum electrolyte concentrations (sodium, potassium, calcium, magnesium).  
                       | • Challenge tests: Releasing hormone challenge tests to test pituitary responsiveness to hypothalamic hormones (gonadotropin releasing hormone, corticotropin releasing hormone, thyroid releasing hormone);  
                       | • Oral glucose tolerance test.  |
| Neurodevelopment      | • At birth, newborn assessments (e.g., suckling, Babinski and startle reflexes) and screening (e.g., )  
                       | • At and after birth, growth trajectories (e.g., weight, length, head, abdominal circumference)             |
|                       | • After birth, standardized assessment tools (either by trained professionals, parental or other raters) for growth and development inclusive of cognition, language, learning, vision, auditory, behavior |

NOTE: Some of above clinical assessment tools are age and/or gender dependent.
Given that children, by definition are in a state of continual growth and development, methodologies for the assessment of child health should be responsive to all organ systems. Prospective studies are particularly relevant since they permit the capture of time varying exposures and other relevant covariates for children’s health. The short interval between many exposures and outcomes (e.g., in utero exposures and infant birth size) further supports the use of prospective studies.

Challenges remain regarding the impact of environmental children’s health during development. One such challenge is the need to identify critical windows including those before, during or shortly after conception for the spectrum of health endpoints relevant for child health. Recruitment of couples prior to first attempting pregnancy offers promise for identifying new critical windows and the ability to assess maternally, paternally and parentally mediated effects on child health. Use of home fertility monitors, in light of our inability to measure conception, may help to time conception and, hence, exposures in relation to conception and gestation. While standardized methodologies exist for measuring some aspects of children’s health status such as growth and development, for many other organ specific endpoints few standardized approaches exist. Methodologies that can build upon clinical assessment of children as a part of well-baby or well-child visits with health providers may offer utility and feasibility for capturing exposures in relation to acute health effects (e.g., air pollution and asthma attacks or upper respiratory infections) while ensuring methodologies for tracking children’s health trajectory in a standardized fashion. The absence of registries for assessing most health outcomes facing children other than cancer or birth defects makes it difficult to assess secular or regional trends in children’s health. Use of in- and out-patient discharge diagnoses may crudely provide information on receipt of care for medical diagnoses. However, the absence of identifying information in such registries makes it impossible to remove clustering of outcomes stemming from our inability to remove repeated diagnoses to the same person.

Methodologies can be tailored to the unique characteristics of the study exposure(s) or other population/host factors that might impact study conclusions. Efforts aimed at identifying a minimal data set essential for the analysis of most environmental exposures and health outcomes
would be informative for investigators and allow for regional comparisons. All new methodologies will need to be sensitive to cultural, gender and racial/ethnicity related issues.

Reproductive toxicity studies in animals provide important information for evaluating the potential developmental toxicity in children. Developmental toxicity effects assessed in reproductive toxicity studies include foetal growth retardation, malformations, foetal loss, decreases in peri- and postnatal growth and survival, retarded postnatal physical and functional development and effects on reproductive organs and the brain. Among the current OECD Test Guidelines for reproductive toxicity, all vulnerable periods of development are covered only in the two-generation study design. Late effects are partly covered in young adults, especially in relation to reproductive function and developmental neurotoxicity. Potentially important areas of concern for children, such as immunotoxicity and functional effects on the respiratory system, are not assessed in regulatory test guidelines.

Potential developmental toxicity effects of endocrine disruptering chemicals is an area of concern. In most cases such effects can only be revealed in the two-generation study. A number of in vivo assays for detecting endocrine effects are currently being developed. In addition, clear effects in these assays give strong indication for developmental toxicity of the chemical. Further development of the in vivo assays for detecting endocrine effects with the aim of using the results for regulatory purposes could reduce the need for performing the relatively long-lasting and expensive two-generation studies. A major data gap in test guidelines is the inability to monitor effects of early exposures that do not appear until at older ages.
CHAPTER 7 – IMPLICATIONS AND STRATEGIES FOR RISK ASSESSMENT FOR
CHILDREN

7.1 Introduction

The preceding chapters have provided ample evidence that children may have different
susceptibilities at different developmental stages, with respect to both exposure and health
outcomes. Poor, neglected, and malnourished children often living in parts of the world where
there is increased environmental pollution and environmental degradation are at greatest risk.

This chapter will address the implications of the data presented in previous chapters for assessing
the risks from environmental exposures. WHO/IPCS has defined risk assessment as an
empirically based paradigm that estimates the risk of adverse effect(s) from exposure of an
individual or population to a chemical, physical, or biologic agent. As shown in Figure 7.1 it
includes the components of hazard identification (Is there an adverse effect?), dose-response
Problem Formulation

Problem formulation is the first step in any risk assessment, including those carried out for the
purpose of determining the potential risk from childhood exposures (US EPA, 1998; Olin and
Sonawane, 2003; Suter et al., 2005). This step brings together risk assessors and risk managers
to define the problem to be addressed in the risk assessment. The problem formulation step also
establishes the goals, breadth and focus of the assessment, and identifies the major factors and
regulatory/policy context to be considered. Although the major components of problem
formulation are no different for assessments of childhood or adult exposures, some of the
specific considerations will be different. Problem formulation is an interactive process that
provides the foundation for the technical approach to be used in the assessment. A risk
assessment on the health effects of chemical exposures to children should focus on the
identification of life stages, the timing and response to environmental exposures, and the integral
relationship among them. Children’s health risk assessment can bring together a number of
interests and areas of expertise, and these need to be considered in the problem formulation
phase. For example, the potential increased susceptibility of children and the possibility of their
unique exposure pathways must be addressed. Historically, assessments of health hazard and exposure are generally carried out independently in a risk assessment by individuals with different expertise. However, the timing and level of exposure are important factors in both hazard assessment and exposure assessment, and thus, needs to be integrated into a common framework. Problem formulation should serve as a qualitative screen to identify the exposure scenario(s) that need to be considered (including settings unique to children), and whether or not there is a potential for higher exposures or greater susceptibility in children.

**Figure 7.1**

*Risk Assessment Paradigm for Human Health*
The identification of exposure scenario(s) can help pinpoint specific populations of interest (e.g., school children, pregnant women, children in developing countries), as well as exposure characteristics with respect to medium (e.g., air, water, soil), route, duration, frequency, and life stage.

From the risk management perspective, there may be regulatory, judicial, economic and societal considerations that may influence the timing and breadth of the assessment. For example, a specific regulatory requirement, a community need, a health crisis, or some other factor may drive the risk assessment. The reason the assessment is being performed, as well as factors that may influence risk management options and the schedule for developing the assessment, must be clear to all concerned, including the public. Consequently, from the risk communication perspective, the interaction of the risk assessment and risk management groups with input from all interested parties during the problem formulation phase is critical.

Problem formulation should result in a conceptual model, based on the qualitative characterization of hazard and exposure (Olin and Sonawane, 2003; Daston et al., 2004; USEPA, 2005a). The conceptual model should identify key components critical to the overall risk assessment, including exposure scenarios, exposed life stage groups, and the chemical and toxicological characteristics of the exposure that may contribute to an increased risk in children. Table 7.1 provides guidance on the type of considerations that should be made during problem formulation and carried through the analytical and characterization phases of the risk assessment.

7.2 Hazard Identification

After the problem formulation stage, comes hazard identification. Hazard identification as defined by IPCS is the identification of the inherent capability of a substance to cause adverse effects when an organism, system, or (sub)population is exposed to that substance (see Figure 7.1). The challenge in life stage risk assessment is not only to identify the hazard but to determine, in the later stage of risk characterization, whether any adverse effects place a disproportionate risk of harm on potentially susceptible sub-populations such as the developing
child. Children may differ both qualitatively and quantitatively in how they are affected by exposure to xenobiotics. Effects from exposures during specific periods of development can be observed at anytime in the life of the exposed individual, and may even cross generations. In addition to the potential for harm during critical periods of development, the long term consequences of early exposure as precursors for later onset of adult disease must be considered.
**Table 7.1 Considerations in Children’s Risk Assessment**

*Characterization of the Overall Risk Assessment*

- Define the purpose of the risk assessment, including the regulatory and/or public health need.
- Consider the historical perspective and whether other assessments of the same or comparable exposures have been carried out.
- Define the life stages of interest.

*Characterization of the Health Hazard*

- Characterize the entire data base that provides information on the potential for health concerns in children. Specifically, describe:
  - The quantity and quality of the data
  - Whether the data are from human or laboratory animal studies (single or multiple species)
  - The appropriateness of the life stages studied and how inclusive the endpoints are with respect to defining alterations in development for a given life stage
  - The potential for not only immediate, but also delayed effects following an exposure
- With specific reference to the available human data, describe:
  - The types of data used (e.g., ecologic, case-control or cohort studies; or case reports or series).
  - The degree to which developmental stages are addressed.
  - The degree to which exposures are detailed.
  - The degree to which confounding/modifying factors are accounted for.
  - The degree to which other causal factors are excluded.
- Characterize the dose-response nature of the effects of an exposure, including:
  - The data used
  - Any model(s) used to develop the dose-response curve(s) and the rationale and chemical-specific information supporting the choice(s)
- Describe the assumptions and uncertainty factors used for the qualitative and quantitative aspects of the assessment, including the impact of extrapolation from the observed data to environmental exposures.
- Describe the route, level, stage and duration of exposure as compared to expected human exposures, including available toxicokinetic data used to extrapolate across route of exposure.
- Describe what is known about the mechanism of action/toxicity of the exposure, and any toxicokinetic considerations that may influence the toxicity of the exposure at specific life stages.

*Characterization of Exposure*

---

1 Adapted from Daston et al. (2004), International Programme on Chemical Safety (2001) and US Environmental Protection Agency (1996).
• Characterize the sources, duration and pattern of exposures at the appropriate life stages.

  Describe:
  • The most significant sources of environmental exposure
  • The relative contribution of different sources of exposure
  • The most significant environmental pathways for exposure

• Describe the populations assessed, including:
  • Children in general, highly exposed groups, and highly susceptible groups
  • Whether all ages or only specific life stages will be at risk for exposure

• Describe the basis for the exposure assessment, including:
  • Any monitoring, modeling, or other analyses of exposure distributions
  • The range of exposures to the "average" child, and subgroups of children (e.g., ethnic, racial, or socioeconomic subgroups)
  • The factors and/or methods used in developing the central tendency estimate; the high-end estimate.
  • The results of different approaches, i.e. modeling, monitoring, probability distributions, including the limitations of each and the range of most reasonable values.
  • The potential for cumulative or aggregate exposures

In evaluating the data base for hazard identification, a number of assumptions are applied when data are not available or are incomplete (USEPA, 1991; Vermeire et al., 1999; IPCS, 2001c, Kimmel et al., 2005). These include uncertainties about toxicokinetics, mechanism of action, low-dose response relationships, and human exposure patterns. Each of these assumptions is supported to some extent by the scientific literature. The following assumptions are generally accepted in risk assessment strategies:

• It is assumed that an agent that produces an adverse effect following exposure during development in experimental animals will potentially pose a hazard to humans following sufficient exposure during childhood.

• It is assumed that all four manifestations of developmental toxicity (death, structural abnormalities, growth alterations, and functional deficits) are of concern.

• It is assumed that the types of effects seen in animal studies are not necessarily the same as those that may be produced in humans.

• It is assumed that in the absence of adequate human data or data from an identified “most appropriate” animal species, the most sensitive species is appropriate for use.
It is assumed that for health effects other than cancer, a threshold or non-linear dose-response relationship exists. This is based on known compensatory and adaptive mechanisms that protect against the toxic effects of childhood exposures, as well as on repair mechanisms at the molecular, cellular and tissue level. (However, for a number of chemicals (e.g., EDCs) the existence of thresholds for certain non-cancer health effects is beginning to be challenged).

7.3.1 Endpoints and Critical Periods of Exposure

For the purpose of this current document, childhood is considered to encompass the life-stages summarized in prenatal and postnatal periods from conception through adolescence. Within each of these stages and the comparable stages of laboratory animal development, there are many endpoints that can used to assess manifestations of childhood exposures. A critical period is a specific phase during which a developing system is particularly vulnerable. Exposure during a critical period can lead to an immediate effect on the developing system or may not result in an observable effect until much later in life. There are many critical periods within each of the stages of development, as described in Chapters 3 and 4, and in Selevan and Kimmel (2000).

Since not all systems are at the same point in their maturity at any particular time in development, the critical period for one organ system will not necessarily coincide with the critical periods of other developing systems, even within the same developmental stage.

From a risk assessment perspective, the tests that are used to define the potential toxicity of an exposure must cover a wide range of developmental endpoints and critical periods of susceptibility. Chapter 6 summarizes the test protocols and the outcomes that are currently covered by the different OECD test guidelines. However, there are gaps in coverage not only of certain life stages, but also in the evaluation of certain endpoints (e.g., the cardiovascular and immune systems) (USEPA, 2002b; OECD, 2004). Consequently, it is important to consider the points noted in Table 7.1 regarding the appropriateness of the life stages and inclusiveness of the endpoints with respect to defining alterations in development within a given life stage. Moreover, the potential for not only immediate, but also delayed effects following an exposure must be considered.
7.3.2 Human Studies

Human data are preferred for determining the potential health effects of exposure. However, human studies are often limited by ethical issues in collecting human data and by their complexity in establishing exposure conditions and associated effects. Consequently, it is important to understand the various human study designs and their strengths and limitations (see Chapter 6).

The application of human data in risk assessment for children has been detailed in a number of publications (USEPA, 1991; IPCS, 2001c; Richter-Reichhelm et al., 2002; Kimmel et al, 2006). In general, the risk assessor should evaluate each human study for its power and potential bias. The power of the study is the study’s ability to detect an effect. It is dependent on the size of the study population, the frequency of the effect or the exposure in the population, and the level of risk to be identified. The greater the population size and the effect or exposure frequency, the greater the power of the study. In studies of low power, it is generally not possible to establish the lack of an association between an exposure and an effect; and even positive findings may be difficult to support. Meta-analysis, which combines populations from different studies, may increase the power of the overall data base, but the potential for the combination of dissimilar populations must be considered in any risk assessment.

Study bias may be selection bias or information bias. Selection bias may occur in the choice of subjects for the study (e.g., exclusion of individuals who are not fluent in a particular language). Selection bias may also result from an individual’s reluctance to participate in a study due to concerns over a perceived exposure, resultant health effect, or educational and socio-economic status of the participants. Parents who perceive that an exposure in their child’s environment may have resulted in an adverse health effect may feel responsible for not “protecting” their child. Information bias may result from inappropriate classification of the individual study participants or from the information provided. For example, interview bias may result when an interviewer is not “blind” to the exposure or of the test population. Recall bias may result when
participants with specific exposures or effects respond differently than those without the specific exposures or effects.

7.3.3 Relevance of Animal Studies for Assessing Potential Hazards to Children.

While human data are preferable for risk assessment, most assessments rely primarily on data from controlled experimental animal studies. Chapter 6 details the various animal tests that are employed in defining the potential for adverse health effects from environmental exposures. During development, all mammalian organisms move from a state of pluripotency (ability to develop into many different tissue types) to one of differentiation (particular structural and functional modes of operation) (Morford et al., 2004). Table 7.2 shows the approximate ages that correspond to specific events or life stages in laboratory animals, compared with those in humans (USEPA, 2002b). Because of a much compressed developmental period and different rates of maturation of specific functional systems in experimental animals, it is often difficult to conduct temporal extrapolations between developing humans and developing experimental animals.

Nevertheless, toxicity testing in experimental animals plays a key role in identifying and characterizing developmental hazards for children (LaRonda et al., 2004). Many studies of animal/human concordance in developmental toxicology and neurotoxicology have been carried out over the past 50 years and for most chemicals known to cause developmental effects in humans, at least one animal species has been found to exhibit similar effects.

Morford et al. (2004) has summarized much of the work that has been carried out on the ability of animal models to predict human risk for developmental toxicity. In one particular comparison of experimental animal studies and epidemiology studies that met stringent design criteria, it was concluded that concordance of developmentally adverse effects exists when all of the measures of developmental toxicity (i.e., death, structural alterations, growth alterations, and functional deficits) are considered (Holson et al., 1981; Kimmel et al., 1984; Holson et al., 2000).
Table 7.2 Approximate age at equivalent life stages in several species
Taken from U.S. Environmental Protection Agency (2002).

<table>
<thead>
<tr>
<th>Life stage</th>
<th>Rat</th>
<th>Mouse</th>
<th>Rabbit</th>
<th>Beagle dog</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Age</td>
<td>Age</td>
<td>Age</td>
<td>Age</td>
</tr>
<tr>
<td>Embryonic</td>
<td>GD 0–16</td>
<td>Embryonic</td>
<td>GD 0–15</td>
<td>Embryonic</td>
<td>GD 0–30?</td>
</tr>
<tr>
<td>Fetal&lt;sup&gt;a&lt;/sup&gt;</td>
<td>GD 16–22 (22–23 days)</td>
<td>Fetal</td>
<td>GD 15–20 (18–22 days)</td>
<td>Fetal</td>
<td>GD 19–32 (30–32 days)</td>
</tr>
<tr>
<td>Neonate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>PND 0–14</td>
<td>Neonate</td>
<td>PND 0–14</td>
<td>Neonate</td>
<td>PND 0–21?</td>
</tr>
<tr>
<td>Young</td>
<td>PND 22–35</td>
<td>Young</td>
<td>PND 21–35</td>
<td>Young</td>
<td>PND 42–?</td>
</tr>
<tr>
<td>Puberty</td>
<td>PND 35–60</td>
<td>Puberty</td>
<td>PND 35–?</td>
<td>Puberty</td>
<td>3–8 mos</td>
</tr>
<tr>
<td>Sexual maturity</td>
<td>2.5–3 mos</td>
<td>Breeding age</td>
<td>1.5–2 mos</td>
<td>Breeding age</td>
<td>6–9 mos</td>
</tr>
<tr>
<td>Old adult</td>
<td>18 mos–2 yrs+</td>
<td>Old adult</td>
<td>Old adult</td>
<td>Old adult</td>
<td>~15 yrs</td>
</tr>
</tbody>
</table>

<sup>a</sup> Range of gestation length in parentheses.
<sup>b</sup> Some neonatal events in rodents occur in utero in humans.
<sup>c</sup> Range of weaning ages in parentheses.

GD = gestation day
PND = postnatal day
These authors noted that although strict anatomical concordance is not always present, an alteration is a signal that development may be perturbed. Testing for concordance of identical anatomical aberrations requires detailed knowledge of comparative stages of organ system development and toxicokinetic information so that similar target organ doses at equivalent stages of development can be compared. If such detailed knowledge were available, it would be possible to test for the ability of an agent to elicit specific malformations. In a report on the relevance of developmental neurotoxicology endpoints, Adams et al. (2000) noted that extrapolation of animal data from standard regulatory test batteries was stronger for effects on sensory and motor functioning than for cognitive or social functioning. They suggested that the testing paradigms and their concordance could be improved by the use of more contemporary and sensitive methods for evaluating behavior and cognitive function.

Testing protocols for reproductive and developmental effects in laboratory animals are well established and include exposure at various time periods and assessment of a number of different outcomes. As with human study designs, it is important that the risk assessor understand the various experimental animal study designs; their strengths and limitations. An advantage of experimental animal studies is that they can be carried out under controlled conditions. In evaluating any study, the risk assessor should confirm that appropriate exposure groups and sufficient numbers of animals were used. As an example, it is highly unlikely that all females will become pregnant in a routine rodent developmental toxicity study. Consequently, the study design should initially incorporate more females than will be needed to assess the effect of an exposure at the end of the study. With regard to exposure groups, the risk assessor will need to consider the route of exposure, the timing and levels of exposure (often based on dose-range finding studies), and the randomization of the experimental animals among the different exposure groups.

During the prenatal and early postnatal period (through weaning), it is important to evaluate the maternal animal. Changes in such endpoints as maternal body weight and weight change, gestation length, food/water consumption, and clinical signs help characterize the effects of the exposure. As examples, changes in weight gain during the exposure period in a prenatal rodent study can be an indicator of general toxicity. Changes in weight gain (corrected for gravid uterine weight) can give an indication of whether a reduced weight gain is a
consequence of intrauterine effects (e.g., reduced pup weights, reduced litter size), or result
primarily from effects on the maternal animal, or a combination of the two.

Chapter 6 has reviewed the endpoints used in assessing potential developmental effects in
animal studies. Other sources also give detailed guidance on how the risk assessor should
consider data on specific endpoints in the overall risk assessment (IPCS, 1984, 1986b, 1999,
2001b; USEPA, 1991; Schwenk et al., 2003). Ideally, all of the manifestations of
development (viability, growth, and structural and functional integrity) should be assessed.
However, this is seldom the case and consequently, the strength of the overall data base may
be limited by the absence of certain data. This is especially true of functional integrity as this
outcome is not routinely evaluated. As discussed below, a general sense of the dose-
response relationship is important for the characterization of the health-related data base.

7.3.4 Reversibility and Latency

Reversible effects are those that appear in response to an exposure, and return to normal
when the exposure ceases. Reversibility is observed after an exposure is resolved following
cessation of exposure. With regard to risk assessment for children, it is important to
understand that many “reversible” effects are related to or are precursors of other adverse
effects. For example, low birth weight may be “reversible” through catch-up growth
postnatally, but it also may be related to developmental delays or other health outcomes that
result from prenatal growth reduction/retardation. Conversely, an agent may produce
relatively mild and reversible neurological effects in adults but produce permanent behavioral
impairment following in utero exposure. Latency or the latency period is the time between
exposure to an agent and manifestation or detection of a health effect of concern. Exposures
during childhood will often result in latent effects. A classic example of latency is the
appearance of clear cell adenocarcinomas in women who had been exposed in utero to
diethylstilbestrol (Chapter 4). Reversibility and latency are only rarely evaluated directly.
Yet, either event could have a major impact on the hazard identification. Additional studies
from less-than-lifetime exposures to evaluate latency to effect and reversibility of effect is a
critical research need (USEPA, 2002a; Damstra et al., 2002).
7.3.5 Characterization of the Health-Related Data Base

Following the review of the toxicity data in both humans and animals, the health-related data base is characterized as being sufficient or insufficient to proceed further in the risk assessment process. The process for characterization of this data base as well as a description of what constitutes sufficient/insufficient evidence is reviewed in several publications (Kimmel et al., 2006; USEPA, 2005a, d). Table 7.3 shows the criteria for characterization used by the USEPA for developmental toxicity (USEPA, 1991). In general, the characterization of hazard considers the context of exposure (e.g., dose, route, duration, and timing), relative to the life stage(s) during which exposure occurred. The strengths and weaknesses, as well as the uncertainties, of the data are described. It is important that all data, whether indicative of a hazard potential or not, are considered in this characterization. This process requires a great deal of scientific judgment, and a multidisciplinary team of experts in specific areas of developmental and reproductive toxicity. When the database is considered sufficient, the risk assessment process continues with the dose-response evaluation.

7.4 Dose Response Assessment

Identifying dose-response relationships is an important component of any risk assessment. This process establishes the exposure levels which produce effects, as well as those that produce no effect. As noted in Table 7.1, it is important to characterize what data were used, what model(s) was employed to develop the dose-response curve(s), and whether chemical-specific information is available to support the observed dose-response relationship. While the risk assessment paradigm shown in Figure 7.1 separates hazard identification and dose-response assessment, in reality these two components cannot be totally separated from one another. The potential for an exposure to result in an adverse effect is dependent not only on the agent to which a child is exposed, but the dose, route, timing and duration as well. The timing of exposure is particularly important in determining the nature and severity of health outcomes resulting from exposure during critical developmental periods.
Table 7.3 Categorization of the health-related database

**Sufficient Evidence**

The Sufficient Evidence category includes data that collectively provide enough information to judge whether or not a human developmental hazard could exist within the context of dose, duration, timing, and route of exposure. This category may include both human and experimental animal evidence.

**Sufficient Human Evidence**

This category includes data from epidemiologic studies (e.g., case control and cohort) to provide convincing evidence for the scientific community to judge that a causal relationship is or is not supported. A case series in conjunction with strong supporting evidence may also be used. Supporting animal data may or may not be available.

**Sufficient Experimental Animal Evidence — Limited Human Data**

This category includes agents for which there is sufficient evidence from experimental animal studies and/or limited human data that provide convincing evidence for the scientific community to judge if the potential for developmental toxicity exists. The minimum evidence necessary to determine if a potential hazard exists would be data demonstrating an adverse developmental effect in a single appropriate, well-conducted study in a single experimental animal species. The minimum evidence needed to judge that a potential hazard does not exist would include data from appropriate, well-conducted laboratory animal studies in several species (at least two) which evaluated a variety of the potential manifestations of developmental toxicity, and showed no developmental effects at doses that were minimally toxic to the adult.

**Insufficient Evidence**

This category includes agents for which there is less than the minimum sufficient evidence necessary for assessing the potential for developmental toxicity, such as when no data are available on developmental toxicity, as well as for data bases from studies in animals or humans that have a limited study design (e.g., small numbers, inappropriate dose selection/exposure information, other uncontrolled factors), or data from a single species reported to have no adverse developmental effects, or data bases limited to information on structure/activity relationships, short-term tests, pharmacokinetics, or metabolic precursors.

7.4.1 Application of Health Outcome Data

As noted previously, human data are preferable for determining the potential health effects of exposures in children. In order to characterize dose-response relationships, it is important to characterize the type of epidemiologic study design, the range and detail of exposures measured, and the specific outcomes and populations monitored. Human studies are often limited in their power to establish an association between an outcome and the range of exposures (especially individual exposures) that have been measured. Moreover, childhood exposures may be greater during certain critical periods of development, and even within a critical period certain children may be more sensitive to certain exposures than others. A specific population that is monitored in a study may or may not be applicable to a particular risk assessment if the critical developmental periods of interest are not included. Thus, establishing a dose-response relationship based solely on human data is even more difficult than identifying a potential hazard using such data. As with the hazard assessment, experimental animal studies offer the advantage of being carried out under controlled conditions. This includes controlled exposure conditions of dose, route, duration, and timing of exposure.

The risk assessor should be sensitive to certain dose-response patterns that are often encountered in studies on developmental toxicity. For example, the lowest effective doses in adults and young are often similar or may be the same, but the type of effects may be very different and the effects on the developing child may be permanent (or lead to latent effects) while the effect on the adult may be transient. Moreover, the difference between the maternal toxic dose and the developmental toxic dose may at times be related to the relative thoroughness with which endpoints are evaluated. Other dose-response components that need to be defined are the variability and level of severity within a particular endpoint, since both of these endpoints can have a significant effect on the power of the study and the ability to establish an effect level. Approach to carrying out dose-response assessments are described below.

7.4.2 Quantitative evaluation

The quantitative evaluation of the dose-response nature of a chemical exposure has evolved over the past twenty years, and current methodology has been reviewed in a number of
Traditionally, a threshold has been assumed for health outcomes (with the exception of cancer) resulting from childhood exposures (USEPA, 1991). In this context, a threshold is a level of exposure below which an adverse effect will not be observed. This assumption has been based on the known capacity of the developing organism to compensate for or repair damage at various levels of biological complexity. While this is beginning to be challenged as an across-the-board assumption, current quantitative methods described below continue to recognize this assumption.

7.4.2.1 Tolerable daily intake (TDI) and reference dose (RfD)/reference concentration (RfC) approaches

The dose-response evaluation of either human or animal data has traditionally been based on developing health-based guidance values such as a tolerable daily intake (TDI) or reference dose (RfD)/reference concentration (RfC). These values are derived by dividing the lowest observed adverse effect level (LOAEL) or the highest exposure level at which no adverse effects are observed (no observed adverse effect level, NOAEL) by uncertainty factors. More recently, the use of chemical-specific adjustment factors has been introduced to provide a method for the incorporation of quantitative data on interspecies differences or human variability into the risk assessment process (Meek et al., 2002; IPCS, 2005). These approaches are generally applied to lifetime exposures and are not focused on exposures during specific life stages (Groeneveld et al., 2004). Modifications that account for less-than-lifetime exposures include developing acute and short-term reference exposure values and acute dietary levels (Renwick, 2000; Solecki et al., 2005). Specific life-stage dose-response methodology is limited, but there are approaches for developing drinking water health advisories and for assessing the incidental non-dietary pesticide ingestion in toddlers that are thought to be protective of children (US EPA 2002b).

Uncertainty factors (UFs) are intended to account for animal-to-human extrapolation, variability within the human population, use of a LOAEL where a NOAEL is not available, and database deficiencies. Lack of reproductive and developmental toxicity data is often used as a basis for including a database factor. The default value for any one uncertainty factor is 10, but this may be reduced depending on the confidence in the data or information...
that provides assurance of reduced intra- or interspecies variability (Renwick et al., 2000).

As noted above, chemical-specific data on toxicokinetics and toxicodynamics may be used to replace part or all of these UFs, and this strategy has been used by WHO/IPCS (IPCS, 1994, 2001c, 2004, 2005).

When such data are available, life stage considerations can be included in two general ways, i.e., intraspecies adjustments or interspecies extrapolation (US EPA 2005a). In general, qualitative predictions of the relative difference in toxicokinetic processes between children and adults can be made using adult/child ratios for a toxicant that is metabolized by the same pathway. Quantitatively, adjustments to adult physiologically-based toxicokinetic models can be used to develop an appropriate dose metric for a specific life-stage in children. In an evaluation of child/adult pharmacokinetic differences based on the therapeutic drug literature, Ginsberg et al. (2002) reported that half-lives of drugs are 3-9 times longer in neonates than in adults, the difference disappearing by 2-6 months of age. This range of longer half-lives exceeds the 3.16 uncertainty factor that is applied to account for interindividual pharmacokinetic variability. Consequently, the traditional uncertainty factor may be inadequate at this life stage. Additional data will have to be derived from animal studies. Unfortunately, the comparability of the developmental stages in test animals and humans is not always straightforward and will have to be determined (Zoetis et al., 2003; Zoetis and Hurtt, 2003a; Zoetis and Hurtt, 2003b; Marty et al., 2003; Beckman and Feuston, 2003; Walthall et al., 2005). Moreover, quantitative comparisons will need to account for interspecies toxicokinetic differences at equivalent developmental stages. Obviously, the application of appropriate toxicokinetic and toxicodynamic data can increase the confidence in a life-stage specific risk assessment. However, the risk assessor must be cognizant of the models that are used and their uncertainties, so as not to substitute one uncertainty for another. Additional toxicokinetic considerations are noted in Section 7.4.2.5.

The lowest effective doses in adults and young are often similar. However, the type and severity of effects from an exposure may be very different. This becomes an important consideration, especially in evaluating prenatal animal studies. Because the developing embryo/foetus is exposed in the maternal animal, it has been argued that if maternal toxicity is observed, any developmental toxicity could be due to the compromised maternal system. However, several issues should be considered. The difference between the lowest maternally
toxic dose and the developmentally toxic dose may at times be related to the relative thoroughness with which endpoints are evaluated in dams and offspring, as well as to the sensitivity of the endpoints. Moreover, the severity of the effects must be considered; the developmental effects may be permanent while the maternal effects may be reversible. From a risk assessment perspective, developmental toxicity in the presence of maternal toxicity cannot be simply considered “secondary to maternal toxicity” and discounted (USEPA, 1991).

As noted above, an assumption that has generally been made in the dose-response evaluation of endpoints other than cancer is that there is a threshold relationship at low-exposure levels. The TDI and RfD/RfC approaches described above are based on this assumption. However, as more becomes known about the cellular/molecular mechanisms of toxicity, the dichotomy in the approaches to cancer and all other endpoints is coming into question. This has led to a move toward harmonization of risk assessment approaches (see http://www.who.int/ipcs/methods/harmonization/) (Bogdanffy et al., 2001). With regard to cancer, the concern for early life susceptibility to environmental agents has led to the development of guidance for age-dependent adjustment factors for toxicants acting through a mutagenic mechanism of action (USEPA, 2005d). This guidance is based on limited data comparing adult with early life exposures and the subsequent risk of carcinogenesis. With regard to endpoints other than cancer, a recent major review of the RfD and RfC approaches recognized the continued need for a default approach like the RfD/RfC approach, but stated that this approach “…can and should be improved upon or replaced when more specific data on toxicokinetics and mode of action are available to allow the development of a chemical-specific or a biologically based dose-response model …” (USEPA, 2002b).

7.4.2.2 Benchmark dose (BMD) – benchmark concentration (BMC) approach

When sufficient data are available, use of the benchmark approach is preferable to the traditional health-based guidance value approaches (IPCS, 1999b, 2001c, 2004; USEPA, 2000a; Sonich-Mullin et al., 2001; Slob et al., 2005). The BMD (or BMC) is the lower confidence limit on a dose that produces a particular level of response or change from the control mean (e.g., 10% response rate for quantal responses; 1 standard deviation from the
control mean for a continuous response) and can be used in place of the NOAEL. The
BMD/BMC approach provides several advantages for dose-response evaluation: 1) the model
fits all of the available data and takes into account the slope of the dose-response curve, 2) it
accounts for variability in the data, and 3) the BMD/BMC is not limited to one experimental
exposure level and the model can extrapolate outside of the experimental range.

Briefly, a mathematical model is selected, based on the data that are being analyzed and the
characteristics of the response. Generally, the more limited the data base, the more simple
the model. Data bases with larger numbers of dose groups and a greater experimental
complexity will be better suited for more complex models. The model is applied with the
appropriate considerations for statistical linkage, parameter estimation, and response. As
noted in IPCS (2004), this approach will generate a BMD/BMC, but will also provide dose-
response functions and/or extrapolated risk estimates. The BMD method includes the
determination of the response at a given dose, the dose at a given response, and their
confidence limits. Using extrapolation of the dose-response model below the biologically
observable dose range, the response at specified (lower) dose levels as well as the dose
corresponding to a specific response level can be estimated. For more detailed description
and examples of this approach see USEPA (2000a) and IPCS (2004).

7.4.2.3 Biologically-based dose-response models

Biologically-based dose response models are considered a major advance for evaluating
dose-response relationships (Shuey et al., 1994; IPCS 2000). Although considerable work
remains in developing such models, they should provide information on the potential for
chemicals to alter critical signaling pathways, define the toxicokinetic and toxicodynamic
similarities and differences between animal models and humans, and provide a more accurate
estimation of low-dose risk to humans.

7.4.2.4 Duration adjustment

Approaches to duration adjustment are reviewed in Kimmel et al. (2006). Prior to derivation
of NOAELs, LOAELs, or BMDs, the toxicity data are adjusted to a continuous exposure
scenario. For oral studies, a daily exposure adjustment is made (e.g., a 5-d/week exposure is
converted to 7 d/week). For inhalation exposures, a concentration × time (c × t) adjustment is
made. Traditionally, the inhalation exposure adjustment has not been done, because of
concerns about peak versus integrated exposure and the likelihood of a threshold for effects.
However, a review of the reference dose and reference concentration processes, by the US
EPA, recommended that inhalation developmental toxicity studies be adjusted in the same
way as for other endpoints (USEPA, 2002b). Derivation of a human equivalent concentration
(HEC) for inhalation exposures is intended to account for pharmacokinetic differences
between humans and animals.

A number of approaches have been developed for establishing short-duration (less than
lifetime) exposure limits that are applied in specific exposure scenarios (Kimmel, 1995;
Jarabek, 1995). A common feature of most of these approaches is the assumption that
Haber’s Law applies (i.e., the response depends on the cumulative exposure, the product of
exposure concentration and duration). Recent reviews of this approach have begun to
question its general applicability over wide ranges of concentration and duration (Pierano et
al., 1995; Eastern Research Group, 1998). Evidence is accumulating that demonstrates that for
several environmental exposures, short, high level exposures have a greater effect on
development than cumulative equivalent long, low level exposures (Tzimas et al., 1997;
Weller et al., 2002; Kimmel et al., 2002).

7.4.2.5 Toxicokinetics

Toxicokinetic data provide information on the absorption, metabolism, distribution (including
placental transfer), and/or excretion (including via breast milk) of an agent. Chapter 3
(section 3.5) reviews many of the issues that must be considered in relation to toxicokinetics
and child development. When available, toxicokinetic data can provide estimates of internal
dose, as well as the level and duration of an exposure at the target site (e.g., peak
concentration). This can be useful for interspecies extrapolation, as well as for indicating the
range of intraspecies variability (Gundert-Remy et al., 2002; deZwart et al., 2004). From an
exposure perspective, toxicokinetic information can help define similarities and differences
among routes of exposure.

Daston et al. (2004) reviewed the considerations that should be made in analyzing
toxicokinetic data and factoring it into an assessment of children’s health risks from exposure
to environmental agents. Data that are particularly important include: the absorption rate for
the relevant exposure pathways, the distribution from the exposure sites and systemic
distribution to the metabolizing or target organs, the type of storage and body compartments
of interest (in utero, this would include maternal, placental, and embryo/fetal compartments),
and the metabolism rates for both activation and detoxification pathways. To apply this in a
life-stage specific assessment, chemical-specific data are needed to identify the main
pathways of chemical activation, detoxification, and clearance as seen in adults or animal
models, and then age-specific data are needed to adjust these factors for the particular
childhood period of interest.

7.5 Exposure assessment

The exposure assessment characterizes the pathways, magnitude, frequency and duration of
human exposures from various sources. Chapter 5 provides an overview of these
components and addresses the principles of exposure assessment in children. General
principles of exposure assessment have been reviewed in a number of publications (IPCS,
1999b, 2000; Needham et al., 2005; USEPA, 1992a, 2005a). This chapter will focus on the
considerations that are important when applying the exposure data to a children’s health risk
assessment (see Table 7.1).

7.5.1 Age-specific exposures

A child’s anatomy, physiology and metabolism change as well as their behavior and
interaction with their environment changes over time. The age/developmental stage of the
child must be a primary consideration when conducting an exposure assessment. A
breakdown of age/developmental stages and corresponding behavior, physiological and
exposure characteristics are shown in Tables 5.1 and 5.2. All of these characteristics must be
considered in trying to establish an estimated exposure to children during specific
developmental stages. Socio-economic, cultural, and physical conditions can also influence
exposure levels.

The pathway and physical-chemical characteristics of a particular environmental exposure
can provide useful information on the likelihood of childhood exposure (Chapter 5).
Examples of relevant age-specific exposure pathways include placental transfer, breast milk,
toys, soil, indoor air/dust, child care centers, schools and occupational settings. Exposure to
persistent environmental chemicals are of special concern and should be measured whenever possible, and related to the critical stage(s) of. Exposure to persistent chemicals may continue after the initial exposure has ceased and may result in a level sufficient to cause effects during critical developmental periods.

Exposure of either parent may affect the germ cells that will form the child. Prenatal and postnatal (via the breast milk) exposure occurs through the maternal system; the mother is exposed directly and her developing child is generally exposed indirectly. The exposure assessment will have to consider maternal absorption, distribution, metabolism, and excretion, as well as placental/lactational metabolism and transfer. In utero, transit time will be influenced by the ability of the child to metabolize and/or excrete the chemical. Most chemical agents are able to reach the child in utero, and there may be accumulation in the embryo/fetus if there is conjugation and reduced embryo/fetal excretion. Assessment of exposure via breast milk should consider such physical-chemical characteristics as fat solubility, since breast milk and maternal fat form a “sink” for fat soluble compounds. The mother’s exposure alone cannot be assumed to be a surrogate for the prenatal or nursing child. The exposure of the in utero/nursing child may not be the same as for the pregnant or lactating mother, and measurement of the agent in cord blood and in breast milk may give a better estimate of exposure.

At birth, the child is exposed to the environment directly. At this time, the child’s respiration rate is rapid, food and water consumption is high, and the skin surface area/body weight ratio is larger. Children’s metabolic pathways, especially in fetal life and in the first months after birth, are immature. If the child breast feeds, the exposure of the mother continues to be a source of exposure.

The infant/toddler is small in stature, crawling or just beginning to walk, and prone to be in contact with rugs, floors, lawns and compounds that layer at low levels. The limited variety of their diets may shield them from many exposures, but may make them particularly susceptible to others. This is especially true if the exposure pathway of an agent is through particular foods that comprise a large portion of the diet, such as fruits and milk/milk products. This is also the stage at which hand-to-mouth behaviours may result in exposure.
By adolescence, the child has become increasingly independent. Physically, adolescents go through a new growth spurt accompanied by an increase in food and water consumption. Their environments are more varied, including home, school, and expanded social and occupational settings. Consequently, they will be exposed to a greater variety of chemicals and physical agents. As adolescents begin to take control of their own life decisions, their limited life experience and willingness to take risks may result in a greater disregard for exposures that may be harmful.

Default values have been published for use in estimating exposures, e.g., from food and water consumption in adults and children, soil ingestion in children, and respiration rates in children and adults (USEPA, 1990). The Child-Specific Exposure Factors Handbook summarizes data on human behavior and characteristics that affect children’s exposure to environmental agents and recommends values to use for these factors (USEPA, 2002a).

7.5.2 Assessment Methods

Guidelines for exposure assessment (IPCS, 2000; OECD, 1999; USEPA, 1992a) and a handbook of child-specific exposure factors (USEPA, 2002a) have been published. Both list a number of references that are applicable to the quantitative estimate of exposure. Generally, the methods used for the quantitative estimate of exposure are not different for children and adults. The magnitude of exposure is a product of the exposure concentration as a function of time. Other IPCS documents (IPCS 1999b, 2000) have reviewed this quantitative approach, and discuss the integration of exposures for a given population and the determination of the applied dose. Doses are often presented as dose rates (i.e., the dose per unit time). For biological processes that are described in terms of lifetime probabilities (e.g., cancer) lifetime average doses are often presented.

The risk assessor should understand the type of methods and models used to determine exposure (i.e., direct, biomarkers, and modeling). Direct methods of assessment measure the contact of the child with the agent and can identify exposure concentrations in a particular medium over an identifiable period of time. In pregnancy, the measurement is not direct (except in cases of physical exposures, e.g., heat, radiation) and the maternal exposure generally serves as a surrogate for embryo/fetal exposure. The actual embryo/fetal exposure will depend on maternal absorption, distribution, metabolism and excretion, and on the
placental transfer of the agent or its metabolites. Direct methods offer the advantage that the
exposure is made at the point of contact and are likely to provide the most accurate estimate
of exposure of the defined time period. When data from a large number of individuals is
combined, the individual variability within the population can be estimated. Limitations that
should be considered in the application of direct method data are 1) the accuracy and
variability of the measurement devices and techniques used; 2) any assumptions that are
made concerning the relationship between short-term sampling and long-term exposures; and
3) the fact that this method is not source specific (IPCS, 1999b).

Biomarkers of exposure are an indicator of absorbed dose, and may present unique
advantages in exposure assessment. Biomarkers demonstrate that internal exposure has
occurred and can be used to estimate chemical uptake over time and help establish the
relationship of exposure and effect (see Chapter 5). They are apical in nature in that they
account for and integrate over all sources of exposure. Specifically in relation to potentially
sensitive subpopulations like children, biomarkers may be able to identify increased
absorption or biological response in comparison to the general population. Biomarker data
alone cannot be used to establish source and route of exposure, and are limited in providing
information on frequency, duration and intensity. Moreover, metabolism of any chemical
biomarker necessitates a clear understanding of the properties of the metabolites.

Mathematical models can be used to quantify the processes leading to exposure (and internal
dose). The general models that are used for estimating exposure are shown in Figure 5-1 and
examples of specific models are referenced in Table 5-2.

7.6 Risk Characterization

The final phase of the risk assessment process is risk characterization. Risk characterization
involves the synthesis of critically evaluated information and data from exposure assessment,
hazard identification and dose-response considerations into an overall evaluation of the
assessment that can be communicated to risk managers and public health officials, and should
be based on the purpose for the risk assessment that was defined in the problem formulation
stage. The risk characterization should incorporate all life stages that were identified in the
problem formulation stage, and if part of a larger risk assessment, it should place the
vulnerability of the child in perspective with the other populations being considered.
It is important that both the qualitative and quantitative characterization be clearly communicated to the risk manager. The qualitative characterization includes the quality of the data base, along with strengths and weaknesses, for both health and exposure evaluations; the relevancy of the data base to humans; the assumptions and judgments that were made in the evaluation; and the level of confidence in the overall characterization. The quantitative characterization also includes information on the range of effective exposure levels, dose-response estimates (including the uncertainty factors applied), and the population exposure estimates. Kimmel et al. (2006) have reviewed many of the components of the risk characterization for reproductive and developmental effects and have provided a comprehensive list of issues to be considered for each of the components of the risk assessment.

In general, integration of the health and exposure assessments should include statements regarding the relevance of the route, timing and duration of exposure modeled from the experimental data to the expected human exposure modeled from the exposure assessment. The dose-response patterns (shape and slope), the method for dose-response analysis, and where possible, the relevant toxicokinetics should be characterized. In addition to the route, timing and duration of exposure, the size and characteristics of the exposed populations, and the pattern of exposure and how it can influence the target end point(s) should be described. Because of differences in vulnerability during critical windows of susceptibility, the timing and sequence of exposure should be characterized whenever possible. If similar effects can be expected in adults, the risk characterization should indicate this and whether the effects will occur at different exposure levels than in adults.

The uncertainties and variability of the data base, along with the judgments and assumptions that were made during the assessment should be clear. The description should include the major strengths and weaknesses of the data base and the limits of understanding of particular mechanisms of toxicity that may be involved in the effect(s). Whenever alternative views can be supported by the data base, these should be addressed in the risk characterization. If the assessment favors one view over others, the rationale for choosing that view should be stated.

Three types of descriptors of human risk are especially useful and important (Kimmel et al., 2006). The first of these is related to interindividual variability, i.e., the range of variability
in population response to an agent and the potential for highly sensitive or susceptible subpopulations. The second is related to highly exposed individuals, i.e., individuals who are more highly exposed because of occupation, residential location, behavior, or other factors. The third descriptor that is sometimes used to characterize risk is the margin of exposure (MOE), i.e., the ratio of the NOAEL (or BMDL) from the most appropriate or sensitive species to the estimated human exposure level from all potential sources. This means that the lower MOE, the greater the risk. The MOE can be used to prioritize different contaminants, providing that a consistent approach has been adopted. The acceptability of an MOE depends on its magnitude and is ultimately a risk management decision (IPCS, 2004). To aid that decision, the risk assessor should provide information on the nature and magnitude of uncertainties in both the toxicological and exposure data. Although the risk assessor should not provide an assessment of the acceptability of the MOE, guidance should be given on it adequacy taking into account the inherent uncertainties and variability (IPCS, 1994; Kimmel et al, 2006).

Ultimately, the risk characterization results in a statement(s) of the potential susceptibility of children for specific effects from specific exposures to environmental agents. This statement(s) forms the basis, together with other considerations, on which regulatory or management decisions will be made. Often, the risk manager is not a specialist in children’s health, and thus, it is imperative that the risk characterization be clear, definitive, and unencumbered by scientific terminology that may be misunderstood or misinterpreted. The risk assessor must effectively communicate what is known, what is not known, and what is questionable, in order for the risk assessment to be appropriately factored into the overall risk management process.

7.7 Conclusions

This chapter has reviewed the major scientific principles underlying the assessment of health risks from exposure to environmental chemicals in children during critical stages of development from conception to adolescence. This chapter and the monograph as a whole should be a useful tool for public health officials, research and regulatory scientists, and risk managers in addressing the major scientific principles underlying the assessment of health risks from exposure to environmental chemicals in children during critical stages of development. Considerable progress has been made in developing risk assessment
approaches that address the special, developmental stage-specific vulnerabilities of children. Focused research has led to improvements in data collection and the breadth and depth of the overall data base. There is an increased understanding of normal and abnormal development, and the influence of age-specific conditions on a child’s susceptibility. Nevertheless, life-stage specific risk assessments are only beginning to be incorporated into the overall risk assessment process, and many gaps in knowledge and in the appropriate application of data into child protective risk assessment policies need to be addressed. These include:

- The development of new conceptual frameworks with a particular focus on the uniqueness of early life stages should be harmonized in order to focus the risk assessor on the critical elements of the risk assessment.
- Life-stage specific risk assessments are likely to require modification of the current toxicity testing and human health assessment paradigms.
- Considerable effort will be required to develop approaches to incorporating data from molecular studies and generated from new technologies (e.g., genomic) into a meaningful framework for children’s health risk assessments.
- The addition of the problem formulation step to the risk assessment paradigm will assist in focusing the purpose for determining the potential risk from specific childhood exposures, and foster an increased interaction between scientists, risk assessors, public health officials and the public.
- A greater sensitivity to effective communication among individuals of varying backgrounds and points of view will be required.
- Risk assessors must be particularly sensitive to the potential for significantly higher exposures in areas of the world where environmental exposures are not sufficiently controlled. Compounding factors such as poverty, inadequate nutrition, and compromised health status must also be considered in problem formulation throughout the risk assessment process.
- Since risk assessments generally rely on test animal data, comparative studies into the toxicokinetic and toxicodynamic of animals and humans are important for extrapolating test animal data to the human situation. Moreover, in the case of children’s risk assessment, it is important to define the comparative adult/child toxicokinetics and toxicodynamics at different life stages.
- Research on the impact of environmental factors on children’s health has most often focused on an exposure to specific chemicals or particular organ systems or
endpoints. Additional emphasis should be placed prospective longitudinal studies capturing multiple exposures over various life stages.

- Childhood exposures may lead to immediate effects, or there may also be a long latency period between exposure and effects. Considerably more information from less-than-lifetime exposures needs to be collected in order to evaluate issues of latency and reversibility of effect.

- There is a continuing need for validated biomarkers of exposure that provide information on frequency, duration and intensity of an exposure, as well as a better understanding of distribution, metabolism and excretion within the individual.
References Master List


http://www.cdc.gov/exposurerreport/3rd/


Fanucchi MV, Buckpitt AR, Murphy ME, Plopper CG (1997). Naphthalene cytotoxicity of


thyroid deficiency during pregnancy and subsequent neuropsychological development of the


Regulation of Adrenocortical Development. Endocrinology 146(3): 1018-1024.

Hanley, N. A., Hagan, D. M., Clement-Jones, M., Ball, S. G., Strachan, T., Salas-Cortes, L.,
McElreavey, K., Lindsay, S., Robson, S., Bullen, P., Ostrer, H. & Wilson, D. I. (2000) SRY,
SOX9, and DAX1 expression patterns during human sex determination and gonadal

Hanrahan JP, Tager IB, Segal MR, Tosteson TD, Castile RG, Van Vunakis H, Weiss ST, Speizer


meningiomas: Experience at the Mount Sinai Hospital and review of the literature. J. Neurosurg.
75: 564-574.

and Assessment of Endocrine Disrupters as a basis for regulation of substances with endocrine
disrupting properties. Tema Nord 555: 1-100.

postnatal development and behavior in rats. Neurotoxicol Teratol 16: 241-249.

postnatal development and behavior in rats. Neurotoxicol Teratol 17: 341-349.


adducts in spontaneously aborted fetal tissue. Carcinogenesis. 11(9): 1673-1675.

Differences in pharmacokinetics between children and adults-II. Children’s variability in drug
elimination half-lives and in some parameters needed for physiologically-based pharmacokinetic

Health Canada (2003). Canadian Arctic Contaminants Assessment Report II. Minister of Indian
Affairs and Northern Development. Ottawa.


271
Lowrey GH (1973) Growth and development of children. Sixth edition, Yearbook medical
Publishers, Chicago, IL.

developing immune system to xenobiotics: evidence supporting the concept of developmental
immunotoxicity testing guidelines. Report to the Environmental Protection Agency.

immunotoxicity of five selected compounds following developmental or adult exposure. Journal

Luster M, Dean J & Germolec D (2003) Consensus workshop on methods to evaluate

Action: CDC’s Public Health Surveillance for Women, Infants, and Children. LS Wilcox and

racial/ethnic minority groups, United States, 1983. MMWR CDC Surveill Summ. 39:1-12.


of vinyl chloride monomer: A model of risk assessment on an experimental basis. Environ
Health Perspecti 41: 3-29.

Mancheste r, D.K., Weston, A., Choi, J.S., Trivers, G.E., Fennessey, P.V., Quintana, E., Farmer,

(Fr.).

Markwald R, Trusk T, Gittenberger-De Groot A, Poelmann R. Cardiac morphogenesis:
Formation and septation of the primary heart tube. In: Kavlock RJ, Daston GP, eds. Drug toxicity
in embryonic development I. Advances in Understanding Mechanisms of Birth Defects:

exposure to hazardous wastes and risk of central nervous system and musculoskeletal birth

Child 45:13-23.


Msall ME, Rogers BT, Buck GM, Mallen S, Catanzaro NL, Duffy LC (1993a) Functional Status of


Olshan AF, Smith J, Cook MN, Grufferman S, Pollock BH, Stram DO, Seeger RC, LookAT, Cohn SL, Castleberry RP, Bondy ML (1999). Hormone and fertility drug use and the risk of...


Ozanne, S.E. (2001) Metabolic programming in animals. British Medical Bulletin 60: 143-152


Skakkebaek NE, Rajpert-De Meyts E, Main KM (2001) Testicular dysgenesis syndrome: an increasingly common development disorder with environmental aspects. Hum Reprod 16:972-978.


http://www.unhcr.ch/cgi-bin/texis/vtx/statistics


http://www.unicef.org/protection/index_childlabour.html


http://www.unicef.org/evaldatabase/ZIM_01-805.pdf


http://www.epa.gov/history/topics/justice/01.htm


USEPA (2004d) Background Information on Mercury Sources and Regulations U.S. Environmental Protection Agency http://www.epa.gov/gtrlakes/bnsdocs/mercsrce


WHO (2004b) Burden of Disease Attributable to Selected Environmental Factors and Injuries Among Europe’s Children and Adolescents. EUR/04/5046267/BD/10. World Health Organization/Regional Office for Europe, Copenhagen, Denmark/


