Workshop to Identify Critical Windows of Exposure for Children's Health: Immune and Respiratory Systems Work Group Summary

Rodney R. Dietert,[1] Ruth A. Etzel,[2] David Chen,[3] Marilyn Halonen,[4] Steven D. Holladay,[5] Annie M. Jarabek,[6] Kenneth Landreth,[7] David B. Peden,[8] Kent Pinkerton,[9] Ralph J. Smialowicz,[10] and Tracey Zoetis[11,*]

[1]Department of Microbiology and Immunology and Institute of Comparative and Environmental Toxicology, Cornell University, Ithaca, New York, USA; [2]Epidemiology and Risk Assessment Division, Food Safety and Inspection Service, Washington, D.C., USA; [3]Office of Children's Health Protection, U.S. Environmental Protection Agency, Washington, D.C., USA; [4]Respiratory Sciences, Arizona Health Sciences Center, University of Arizona, Tucson, Arizona, USA; [5]Department of Biomedical Sciences and Pathobiology, College of Veterinary Medicine, Virginia Polytechnic Institute, Blacksburg, Virginia, USA; [6]National Center for Environmental Assessment, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [7]Department of Microbiology and Immunology, West Virginia University Medical Center, MBR Cancer Center, Morgantown, West Virginia, USA; [8]Center for Environmental Medicine and Lung Biology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [9]Department of Anatomy, Physiology and Cell Biology, University of California at Davis, Davis, California, USA; [10]National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [11]Center for Drug Evaluation and Research, Food and Drug Administration, Rockville, Maryland, USA

Fetuses, infants, and juveniles (preadults) should not be considered simply "small adults" when it comes to toxicological risk. We present specific examples of developmental toxicants that are more toxic to children than to adults, focusing on effects on the immune and respiratory systems. We describe differences in both the pharmacokinetics of the developing immune and respiratory systems as well as changes in target organ sensitivities to toxicants. Differential windows of vulnerability during development are identified in the context of available animal models. We provide specific approaches to directly investigate differential windows of vulnerability. These approaches are based on fundamental developmental biology and the existence of discrete developmental processes within the immune and respiratory systems. The processes are likely to influence differential developmental susceptibility to toxicants, resulting in lifelong toxicological changes. We also provide a template for comparative research. Finally, we discuss the application of these data to risk assessment. *Key words*: children's health, developmental exposure, developmental immunotoxicity, respiratory toxicity, risk assessment, windows of vulnerability. — *Environ Health Perspect* 108(suppl 3):483–490 (2000).
http://ehpnet1.niehs.nih.gov/docs/2000/suppl-3/483–490dietert/abstract.html

A review of the literature to date in virtually any area of health-related toxicology indicates that an overwhelming proportion of previous research and testing has been directed toward exposure of adults as opposed to children. Yet it is suspected that immature (embryonic, fetal, and juvenile) populations are at greater risk for environmentally induced toxicity. Therefore, safety information based solely on adult toxicity data is unlikely to result in effective protection of our most at-risk human populations. This paper represents a combined effort of immunotoxicologists, respiratory toxicologists, allergists, and dosimetry and risk assessment experts; we have considered the challenge of developmentally based differences in toxicological risk to the immune and respiratory systems as relates to research, comparative risk assessment, and, ultimately, effective protection of the human population.

A series of papers (*1–3*) defining the existing literature on developmental toxicology of the immune and respiratory systems served as the background for a workshop on the topic of critical developmental windows of exposure. Participants in the immune and respiratory systems work group considered the fundamental knowledge of immunological and respiratory development, the existing dosimetry and laboratory animal models used for toxicological assessment as pertains to human risk, the existing developmental toxicity data, the need for additional comparisons to identify at-risk populations (e.g., sex comparisons), the need for additional developmentally based pharmacokinetic data, and the opportunities to enhance existing risk assessment paradigms. Despite the relative infancy of the research on the issue of differential developmental risk for the immune and respiratory systems, this work group provided the opportunity to fully consider this important area of research, testing, and assessment. It is the purpose of this paper to establish the merits of pursuing developmental immunotoxicity and respiratory toxicity for improved risk assessment and to provide a strategy to this end. We provide a potential template for the generation of new comparative data and the incorporation of these data into the risk assessment process.

The Immune System

The immune system undergoes a number of dynamic changes during the early stages of development in mammals. These changes include sequential formation and expansion of pluripotent hematopoietic stem cells in different tissue compartments, expansion of lineage-committed stem cells, colonization of postnatal lymphopoietic compartments, and, finally, maturation to immunocompetence (*4*). Clearly the ramification of environmental exposures at early stages of development could be different when cells with different potentials to produce hematopoietic progeny are the targets of embryotoxicity. For example, based on inherited stem cell deficiencies, it is clear that impairment or destruction of pluripotent stem cells would have a more pervasive outcome than that which occurs with more targeted single-lineage changes (*5,6*). However, it is not known whether cells with greater progenitor-producing potential are necessarily of equal or greater sensitivity to chemical toxicity than are single-lineage stem cells. As a result, we cannot necessarily assume that the earliest stages of development are the most sensitive to immunotoxicity. This will depend on a number of factors including the developmentally timed appearance of the most sensitive cellular targets for agent-specific immunotoxicity. However, certain dynamic processes during immune development may place the system at particular

This article is based on a presentation at the Workshop to Identify Critical Windows of Exposure for Children's Health held 14–16 September 1999 in Richmond, Virginia.

Address correspondence to R.R. Dietert, Dept. of Microbiology and Immunology, College of Veterinary Medicine, C5135 Veterinary Medical Center, Tower Rd., Cornell University, Ithaca, NY 14853-6401 USA. Telephone: (607) 253-4015. Fax: (607) 253-3384. E-mail: rrdl@cornell.edu

*Current address: Milestone Biomedical Associates, 15 Worman's Mill Ct., Suite 1, Frederick, MD 21701 USA.

We thank S.G. Selevan, D.R. Mattison, and T. McAdams for developing and supporting the workshop and assisting with the completion of these proceedings. The assistance of D. Wallace in the preparation of the manuscript is greatly appreciated.

The views expressed in this report are the responsibility of the authors and do not necessarily represent the official views of the FDA, FSIS, or EPA. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Received 10 January 2000; accepted 28 March 2000.

vulnerability to modulation by a wide range of environmental factors.

After stem cell formation, the formation and subsequent seeding of lymphoid and myeloid cells are important early processes. These cells, including tissue macrophages and mast cells, are dispersed throughout the body and are important for subsequent tissue surveillance and homeostasis. This also leads to the development of various specialized macrophages such as alveolar macrophages and hepatic Kupffer cells. Specialized forms of immune cells differ not only functionally but also in sensitivity to various toxicants. Similarly, the maturation and selection of thymic-derived (T) lymphocytes and antibody-producing (B) lymphocytes proceed through a series of well-defined maturational compartments that could represent windows of potential vulnerability to toxicant exposure. Hence, it is not surprising that later in development when the T- and B-lymphocyte repertoires are well established, the risk of permanent immunotoxicity might differ from that linked with early exposures; toxicant interference with a dynamic process such as T-lymphocyte education (the positive and negative selection of T lymphocytes in the thymus) could be more devastating than the same toxicant exposure of a fully matured T-lymphocyte population.

An additional consideration is that developmental changes in the pharmacokinetics for specific toxicants could alter the actual exposure of equally sensitive immune target cells during different stages of perinatal development. Possible developmental differences in the uptake, distribution, and metabolism of toxicants provide additional incentives for the direct comparison of vulnerability across immune developmental stages.

Examples from the rodent literature indicate that exposure to certain immunotoxicants during development of the immune system (i.e., pre- and early postnatal exposure) produce persistent (i.e., present in the young and/or adult animal long after early exposure) and potentially dramatic effects on immune function. It is noteworthy that these effects may be either reduced or completely absent in adults after similar exposures. Specific examples of highly persistent effects in rodents after developmental immunotoxicant exposure are described in later sections of this paper as well as in the accompanying background paper (*1*).

Evidence for Developmental Windows of Vulnerability

Although there are many examples of developmental immunotoxicity, the information to date tends to be based on rather broad categories of development (e.g., exposure throughout gestation or exposure during gestation and lactation vs. exposure of the adult). Given the relative paucity of information regarding chemicals and differential risk during more narrow and discrete windows of embryonic and early postnatal development, the first challenge is one of defining logical developmental windows for future comparison. Fortunately, this can be based on known developmental changes occurring within the immune system as well as on selected immunotoxicity data. Clearly, a standardized approach to compare specific periods of embryonic and juvenile periods of development for relative immunotoxic risk would facilitate the comprehensive risk assessment process. We have attempted to identify discrete windows of immune development where differential immunotoxic risk is likely to exist and through which comparative assessment can be pursued (Figure 1). A parallel approach for the respiratory system is shown in Figure 2.

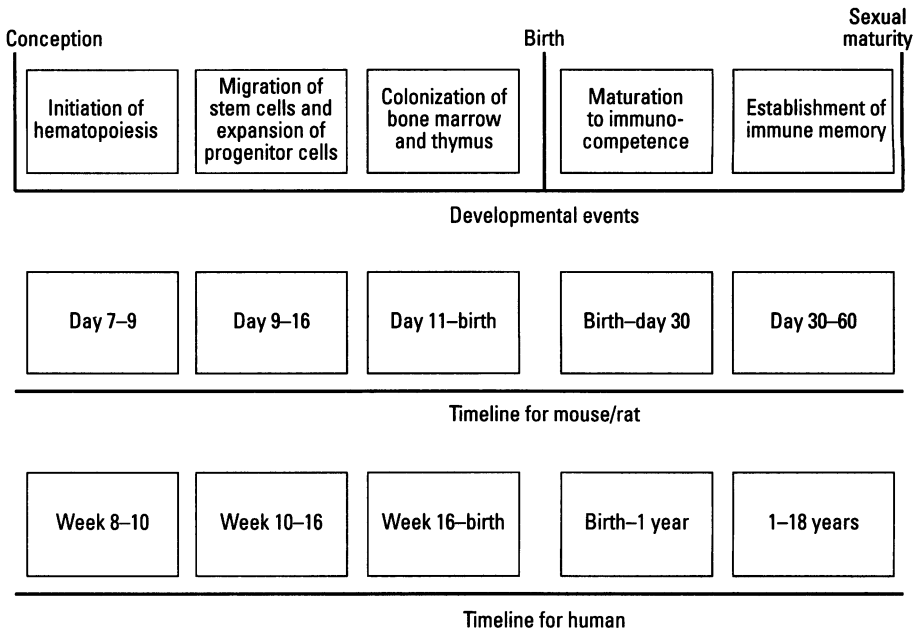

Figure 1. Timeline of critical windows of exposure for immune system development: a comparison of developmental stages for the human, mouse, and rat. The windows represent discrete steps in the formation of the mature immune system and periods in which differential vulnerabilities to immunotoxicants might be expected. Information used to develop these windows from Landreth (*63*), Melchers and Rolink (*64*), Zon (*65*), Muller et al. (*66*), and Benoist and Mathis (*67*).

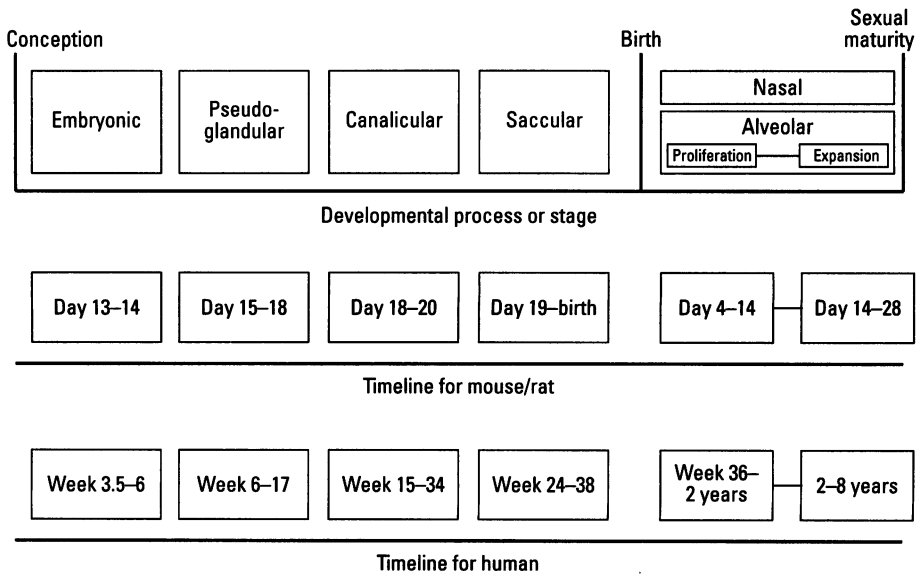

Figure 2. Timeline of critical windows of exposure for respiratory system development, illustrated as discrete maturational windows during pre- and postnatal development. The timeline permits a comparison of similar stages in the human, mouse, and rat. These maturational stages are periods in which differential vulnerabilities to respiratory toxicants would likely occur.

The best examples of known developmental immunotoxicants are chlordane; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); lead; benzo[*a*]pyrene (B[*a*]P), and diethylstilbestrol (DES). It should be emphasized that perinatal exposure to these chemicals produces immunotoxicity at doses that do not affect adults and/or produce persistent changes which do not occur with adult exposure. The relatively small number of such known developmental immunotoxicants reflects the limited number of chemicals and environmental agents examined for developmental immunotoxicity using direct age-related comparisons rather than the established lack of differential developmental risk across broader categories of environmental agents.

Chlordane. Chlordane is an excellent example of a developmental immunotoxicant where progenitor cells appear to be preferentially affected (*1,7*). For example, *in utero* exposure of mice to chlordane results in reduced numbers of granulocyte-macrophage colony-forming units in the spleen. Macrophages are impaired in several functions (*8*). Additionally, there are functional reductions in the 200-day-old adult with both the delayed-type hypersensitivity (DTH) response and the mixed lymphocyte reaction (MLR) depressed after exposure during gestation (*9,10*). As with lead, the persistent developmental immunotoxic effects of chlordane are observed at exposure doses that do not impair the immune system of the mother.

TCDD. TCDD is a potent immunotoxicant with known effect on T-lineage cells and on the thymus itself. Given that some effects have been described on pre-T lymphocytes (*11,12*), it is not surprising that TCDD would exert profound effects on the developing immune system. Exposure of rodents to TCDD during development results in inhibited mitogen response, graft versus host (GVH) reaction, skin graft rejection time, and decreased resistance to challenge with infectious agents or syngeneic tumor cells. TCDD also causes inhibited DTH response at 19 months of age in rats (*13*). Additionally, *in vitro* studies using fetal mouse thymus cultures have suggested that TCDD exposure can disrupt the pattern of thymocyte maturation (*14*).

Lead. Lead is a well-established immunotoxicant, with the majority of studies focused on adult exposures (*15–18*). It was first shown to produce potential developmental immunotoxicity in studies by Luster et al. (*19*) and Faith et al. (*20*). Exposure of rats to lead *in utero* and continuing for 7 weeks postpartum at low levels (25 and 50 ppm) produced altered humoral responses; the effect did not appear to result from altered B-lymphocyte function but rather from altered T-lymphocyte and/or macrophage activity (*19*). In keeping with the likelihood of lead-induced alteration of T-lymphocyte function, Faith et al. (*20*) found that there was a depression in T-lymphocyte mitogenic responses in similarly exposed young Sprague-Dawley strain rats.

More recently, additional information suggests that lead targets both T lymphocytes and macrophages after early exposure. The primary effect on T cells is a shift in the T helper (Th) cell functional balance, with Th1 function depressed and Th2 function elevated (*15*). In a dose–response comparison among pregnant rats and rat female pups exposed during gestation and assessed as young adults, developing fetuses were susceptible to persistent immunotoxic effects of lead at doses that did not affect the pregnant dams (*21,22*). The nature of the immunotoxicity was similar to that reported for exposed adults; rats exposed *in utero* exhibited depressed Th1 function (e.g., reflected by a depressed DTH response) with a concomitant elevation in some Th2-dependent parameters.

The likelihood that different windows of perinatal development have different risk for lead-induced immunotoxicity has recently received support. Preliminary data obtained in chickens suggest that the risk of persistent immunotoxicity from lead exposure is not equal across embryonic development (i.e., windows of differential vulnerability do exist for lead-induced developmental immunotoxicity). For example, early *in ovo* exposure versus late *in ovo* exposure results in a different spectrum of immunotoxic changes in the young animal (*23*).

B[a]P. Polycyclic aromatic hydrocarbons, such as B[*a*]P, depress acquired immunity in adult animals by interfering with effective antigen presentation and the subsequent clonal stimulation of lymphocytes. During embryonic exposure of mice to B[*a*]P and as discussed by Holladay and Smialowicz (*1*), there is a severe reduction in fetal thymocyte number and maturation. Total fetal liver hematopoietic cells were also reduced in these animals, including numbers of both pro-T and pro-B lymphocytes. The end result is severe depression of both cell- and humoral-mediated immune function that is highly persistent (still present at 18 months of age). Early B[*a*]P exposure also causes depressed MLR, GVH response, and increased tumor frequency (*24–26*).

DES. Prenatal exposure of rodents to DES results in long-term impairment of humoral (antibody production), innate [natural killer (NK) cell activity], and cell-mediated (T-lymphocyte mitogen, DTH, and GVH) responses. It also causes increased postnatal susceptibility to viral-induced mammary tumors, transplanted primary tumors, and carcinogen-induced tumors. In humans exposed to DES during gestation, unconfirmed reports suggest possible alterations in T lymphocyte and NK cell function and an increased incidence of autoimmune disease.

Partitioning Immune Development into Testable Windows of Differential Risk

Establishment of the vertebrate immune system requires a sequential series of carefully timed and coordinated developmental events that begin early in life and continue though the early postnatal period. Such development is further characterized by progressive cellular migrations through multiple hematopoietic compartments (i.e., yolk sac, fetal liver, bone marrow, and thymus). It is likely that perturbations or abrogations of critical events in this highly regulated developmental sequence may lead to immune dysfunctions as described for the various chemical agents.

Multiple toxicant-dependent windows of increased vulnerability may exist during development that contribute to postnatal consequences of developmental immunotoxicant exposure. For instance, TCDD induces fetal thymic hypocellularity (at least in part) by selective targeting of progenitor T cells responsible for colonizing the thymus. At the level of the thymus, TCDD also causes a significant inhibition of thymocyte differentiation. The net effect is both fewer and more immature precursor T cells in the TCDD-exposed fetus. Each of these effects may contribute to postnatal immunosuppression; however, it is unclear which effect may contribute more. In addition, TCDD reduces expression of self-antigen-presenting molecules in the thymus, an effect that may alter normal positive and negative selection of T cells (T-cell education) and ultimately affect self-recognition by T cells. In this regard, recent studies in rodents that are genetically predisposed to the development of autoimmune disease indicate that exposure to TCDD during establishment of the immune system causes earlier onset and increased severity of autoimmune disease (*27*).

It is not known if exposure to TCDD before, during, or after establishment of the thymus rudiment may contribute more to thymic involution and postnatal immunosuppression caused by this compound. Similarly, it is not known if exposure to TCDD between establishment of the fetal thymus and birth or early in the postnatal period via lactation may play a greater role in induction or exacerbation of autoimmune disease in genetically predisposed rodents. Although the developmental period is a time of high sensitivity to immunotoxicant exposure, the specific mechanisms by which TCDD targets progenitor or precursor T cells during development are still not clear. Because TCDD inhibits differentiation and proliferation of

developing immune cells, additional research evaluating cell-cycle gene expression (e.g., *bcl-2*, *p53*, and *wee-1*) in both fetal liver and thymus at different stages of gestation would be useful.

In the case of lead, gestational exposure of female rodents at levels that do not affect adults produces both T-cell and macrophage alterations postnatally. There is some suggestion from preliminary studies that targeted exposure during different stages of embryonic development may result in different profiles of postnatal immunotoxicity. For example, exposure of developing chick embryos (21-day incubation time) to lead during the first half of embryonic development (e.g., 5–9 days of incubation) produces persistent changes in macrophage function; however, this occurs without the hallmark decline in T-dependent function (e.g., DTH reaction) that is characteristic of both full gestational and adult exposure to lead. However, exposure of the embryo to lead later in development (12 days of incubation) results in both macrophage and T-dependent functional deficiencies (similar to the effects seen in rodents with full gestational exposure). Given the changes occurring within the thymus during vertebrate embryonic development and the effects of lead on T-cell function, the status of the thymus and T-cell populations might relate to windows of differential developmental vulnerability for lead-induced immunotoxicity. Such observations support the need to define and examine specific developmental windows of increased vulnerability to immunotoxicants.

With the available developmental immunotoxicology information, it is possible to construct a chart of potential windows of vulnerability to immunotoxicants. Figure 1 illustrates the timing for key events of immune maturation from conception to sexual maturity. Comparisons are provided among rodents and humans for the timing of specific immunological maturation events. Immune maturation could be divided using several criteria; Figure 1 identifies five discrete windows of immune maturation based on landmark immunological events (e.g., thymic colonization) and provides a potentially standardized developmental approach to evaluate differential risk of immunotoxicity.

Gaps in Knowledge for the Immune System

As discussed in the background paper (*1*), only limited information is available concerning relative developmental immunotoxic effects as related to discrete windows of early development. Early gestational versus late gestational versus lactational exposures have rarely been examined in studies; evaluation of these exposures and the associated risks would facilitate direct immunological comparisons. Additionally, potential two-generational information is lacking. For example, it is not known whether an environmentally induced alteration in the cytokine and immune metabolite profiles of the gestationally exposed mother might have the potential to influence the course of immune development in the second-generational offspring. Additionally, altered adult immune capabilities could influence reproductive function and the maintenance (e.g., protection from immune attack) of an allogeneic fetus in the mother during pregnancy.

Little information is available concerning the possibility of differential risk based on sex. In the case of lead, preliminary data resulting from *in utero* exposure to a single dose of lead suggest that lead produces different immunotoxic outcomes based on the sex of the fetus (*28*). This suggests that at the very least, sex is likely to influence dose–response sensitivities, if not the spectrum of immunotoxicity resulting from particular developmental exposures.

The Respiratory System

The respiratory system consists of a number of anatomic regions: the extrathoracic region, including the anterior nose and the posterior nasal passages, larynx, pharynx, and mouth; the tracheobronchial region, consisting of the trachea, bronchi, bronchioles, and terminal bronchioles; and the alveolar–interstitial region, consisting of the respiratory bronchioles, the alveolar ducts and sacs with their alveoli, and the interstitial connective tissue. Each region can be distinguished on the basis of structure, size, and function. The characteristics of the air drawn into the respiratory tract and exhaled are greatly influenced by the morphometry of the respiratory tract, which causes numerous changes in pressure, flow rate, direction, and humidity as air moves into and out of the system. Differences in ventilation rates and in the upper respiratory tract structure and in size and branching patterns of the lower respiratory tract between species and among different ages of people result in significantly different patterns of particle deposition and gas transport because of the effect of these geometric variations on air-flow patterns (*29*). Physicochemical characteristics of inhaled particles or gases also influence the particle disposition (encompassing the processes of deposition, absorption, distribution, metabolism, and elimination) and interact with the anatomic and physiologic parameters such as ventilation rate, cardiac output (perfusion), metabolic pathways, tissue volumes, and excretion pathways. The relative contribution of these processes and interactions with the physicochemical characteristics are affected by the exposure concentration and duration.

Timing of the exposure is also important (*30*). There are discrete windows of vulnerability in the development of the respiratory system. To understand these windows of vulnerability, four facts about the development of the respiratory system are important. First, development of the respiratory system is a multievent process that is not restricted to prenatal life. Although the lungs undergo dramatic changes during the embryonic, pseudoglandular, cannicular, and saccular stages, the majority of changes to the lungs continue postnatally. Second, only a limited number of maturational events must be finished at birth for successful survival of the neonate. Third, these developmental events occur in the presence of an increasing mass of total cells. Finally, exposures to smoking, air pollution, and repeated pulmonary infections cause lung alterations that may be difficult to distinguish from changes due to aging alone (*31*).

Growth and development of the human respiratory system is not complete until approximately 18–20 years of age (*31*). As the respiratory system develops, the differences in both airway geometry and respiratory condition are likely to affect its dosimetry and responsiveness (*32*). Development of the human respiratory system involves the differentiation and proliferation of over 40 different cell types as well as the formation of a highly ordered airway branching system with 25,000 distinct terminations giving rise to > 300 million alveoli. The development of the lungs begins with the evaginations of an avascular epithelial bud and subsequent growth into surrounding mesenchymal tissues. After embryogenesis, the fetal lungs in all mammalian species undergo three anatomically distinct stages of growth: pseudoglandular, canalicular, and saccular. Although the lungs have developed sufficiently to sustain life at birth, growth is far from complete. Greater than 80 percent of alveoli in the adult stage arise postnatally. In essence, lung development is a continuum from embryogenesis through early adulthood.

The process of cellular differentiation, branching morphogenesis, and overall lung growth can be affected by exposure to chemicals. The effects of exposure on the respiratory system are likely to be different for each period of development. For example, during embryogenesis and fetal development, cell number, type, and function of the airways and alveoli may be significantly affected by exposure to a diverse number of substances and/or conditions. Both embryogenesis and fetal gestation represent critical periods of cellular differentiation and branching morphogenesis. Because these cells continue to differentiate and divide during the postnatal period, chemical exposure during the postnatal period is

also likely to affect the respiratory system, albeit in a different manner, based on changes in the process of differentiation and morphogenesis. Because growth is largely complete by 20 years of age, exposure to chemicals and other factors are likely to have different consequences in adults than in children.

Differences in airflow in the upper respiratory tract, both between species and for children versus adults, are one of the dominant determinants of lesions in this region for both particles and gases (*33–40*). Likewise, differences in airway geometry and ventilation also result in dosimetry and response differences between children and adults to inhaled agents in the lower respiratory tract (*39–43*). These differences in dosimetry may be important determinants of susceptibility between children and adults. For example, children have higher extrathoracic and total deposition in the tracheobronchial region (*33,43*), which may explain observations of epidemiologic studies suggesting that children have increased morbidity from particulate air pollution.

Peden (*2*) discussed the premise that Th2 responses predominate early in development and that this may be important in the protection of the fetus in the maternal allogeneic environment. However, a lack of development of and/or a blunting of Th1 responses would leave the individual with a predominant Th2 response and could predispose to atopy. We raised the same issue earlier in this paper concerning the effects of lead-induced immunotoxicity where Th1 responses are depressed relative to Th2 responses. Factors identified by Peden (*2*) as potentially important in a consideration of atopy are the polycyclic aromatic hydrocarbon components of diesel exhaust particles, environmental tobacco smoke (ETS), early exposure to allergens, diet, ozone, endotoxin, and the balance of bacterial exposure versus antibiotic use. Pinkerton (*3*) also emphasized the capacity of specific ETS exposures to alter the airway sensitivity of rodents and the possible linkage of similar exposures to the development of airway hyperresponsiveness in humans.

Evidence for Developmental Windows of Vulnerability in the Respiratory System

Although a variety of exposures during development may affect lung function and growth, the timing of exposures during development appears to be critical to the effects observed. Exposure to toxic substances during critical periods of lung development may have effects that would not be seen if the same toxic exposure were to occur during adulthood.

For example, maternal malnutrition during gestation may significantly retard fetal growth and the development of the lungs, leading to compromised lung function throughout life. Other examples include ETS and mycotoxins.

ETS. Exposure to environmental tobacco smoke *in utero* and during the first year of life affects the development of the infant lung (*44*). Children have slower rates of lung development if they are exposed to ETS (*45,46*). Estimates indicate that current maternal smoking reduces forced expiratory volume in 1 sec (FEV_1) by approximately 0.5% per year for each pack of cigarettes smoked per day. Exposure to ETS may actually accelerate the maturation of specific cell types in the fetal lung, but the effects of such a change on lung function are not clear.

Infants are most highly vulnerable to the adverse effects of exposure to ETS before 1 year of age. Numerous studies have documented a consistent association between lower respiratory tract illness and parental smoking; this association appears to be strongest during the first year of an infant's life (*47–50*).

Mycotoxins. The rapidly growing lungs of young infants also appear to be especially vulnerable to the toxins of certain molds. An epidemiologic study in Cleveland, Ohio, found that infants with acute pulmonary hemorrhage were more likely than control infants to live in homes with the toxigenic mold *Stachybotrys chartarum* and other fungi in the indoor air (*51*). Remarkably, whereas infants under 1 year of age developed life-threatening pulmonary hemorrhage, adults and older children living in the same moldy home environments did not develop evidence of pulmonary bleeding. The authors suggested that this may be because young infants' lungs are growing very rapidly (*52*). In infant lungs, protein synthesis of type IV collagen and other endothelial basement membrane components would be particularly sensitive to inhibition by the trichothecene mycotoxins, which are contained in the inhaled mold spores (*53*) and are potent protein synthesis inhibitors. Thus, exposure to these mycotoxins could result in focal areas of capillary fragility in the infant lung, and subsequent exposure to stressors that alter blood flow in the lungs (such as ETS) could lead to areas of increased capillary pressure and subsequent stress hemorrhage of these fragile capillaries.

In mature mice, studies of intranasal administration of *S. chartarum* spores demonstrated severe alveolar and interstitial inflammation with hemorrhagic exudate in the alveoli, but no overt lung bleeding (*54,55*).

Gaps in Knowledge for the Respiratory System

The effects of exposure to environmental toxicants on children's developing immune and respiratory systems cannot be based on studies in adults. In the respiratory system, cellular differentiation, cellular proliferation, and cellular physiological function are continually changing during gestational and postnatal growth. The sensitivity of these cells and their response to environmental exposures are likely to be completely different than that found in the adult. The route of delivery of an environmental toxicant to the respiratory system is completely different during the fetal period compared to the postnatal period. Influences of passage through other organ systems and the vascular and maternal organ systems must be taken into consideration. Our knowledge base regarding perinatal exposure and critical windows is inadequate. Future studies must be designed to address these issues to better understand and provide meaningful data to benefit the health of children during development and into adulthood.

Laboratory Animal Data as a Predictor of Human Response

Susceptibility can be influenced by differences in exposure, pharmacokinetics, pharmacodynamics, and target tissue status among species and among the human population. Exposure differences (e.g., differences in activities such as hand-to-mouth behavior) were beyond the scope of this work group. Because the various species used in laboratory animal experiments and humans may not receive identical doses in comparable internal target tissue compartments, and because the tissue dose of the putative toxic moiety is not always proportional to the applied dose of a compound, emphasis in risk assessment has recently been placed on the need to distinguish clearly between exposure concentration and dose to critical target tissues. The term "exposure-dose–response assessment" has been recommended as more accurate and comprehensive (*56*). This expression refers not only to the determination of the quantitative relationship between exposure concentrations and target tissue dose, but also to the relationship between tissue dose and the observed or expected responses in laboratory animals and humans. The process of determining the exposure-dose–response continuum is achieved by linking the mechanisms or critical biological factors that regulate the occurrence of a particular process and the nature of the interrelationships among these factors. As shown in Figure 3, it is ultimately desirable to have a comprehensive biologically based dose–response model that incorporates the mechanistic determinants of chemical disposition, toxicant–target interactions, and tissue responses integrated into an overall model of pathogenesis (*29*).

Recent guidance from the U.S. Environmental Protection Agency provides an analytical framework for incorporating such

mechanistic information into risk assessment (*57*). Emphasis has been place on characterizing the mode of action, defined as a chemical's influence on molecular, cellular, and physiological functions, and includes elucidation of the mechanistic determinants governing dosimetry and toxicant–target tissue response. Because it is the integration of the biology of the organism and the physiochemical properties of a chemical that leads to an adverse effect, mode of action data will help to identify windows of susceptibility due to pharmacokinetic and pharmacodynamic differences between developmental stages versus those of adults. Mode-of-action concepts echo the basic biological tenets of molecular epidemiology. First, early biologic effects from a toxic effect are more prevalent in the population at risk than the late events of disease (e.g., morbidity and mortality) that were historical outcome measures of interest to risk assessment. Second, the early event may be more specific to the exposure than the end disease outcomes, and third, technological advances allow xenobiotics to be directly or indirectly quantified by identification of some predictable dose-related biologic response (*58,59*). Biomarkers, defined by the National Academy of Sciences (*60*) as cellular or molecular indicators of exposure, dose, or response (or a simultaneous combination of these categories), can provide insights on critical mechanistic aspects that are required to integrate diverse health effects information at various levels of organization (e.g., cellular to population) for risk assessment (*59*). As shown in Figure 4 (*58,59,61*), the biomarker scheme is essentially the same as that used for the exposure-dose–response continuum. Thus, biomarker data based in a mode-of-action framework can essentially provide precursor lesion data and can serve as a basis for a parallelogram approach for extrapolation and determination of human homology for the health effect of interest, as shown in Figure 5 (*59*). This same parallelogram approach could be used to establish the degree of predictiveness and homology of developmental end points versus adult end points.

Because differences in pharmacokinetics and pharmacodynamics underlie the application of uncertainty factors commonly used in risk assessment to account for requisite extrapolations between species and for intrahuman variability, mechanistic research to provide the fundamental dosimetry parameters and on toxicant target interactions will inform the magnitude of these factors as well and increase the accuracy of risk assessments (*62*). Significant progress has been made on modeling respiratory tract dosimetry (*58*) and parallel efforts are underway for other routes of exposure.

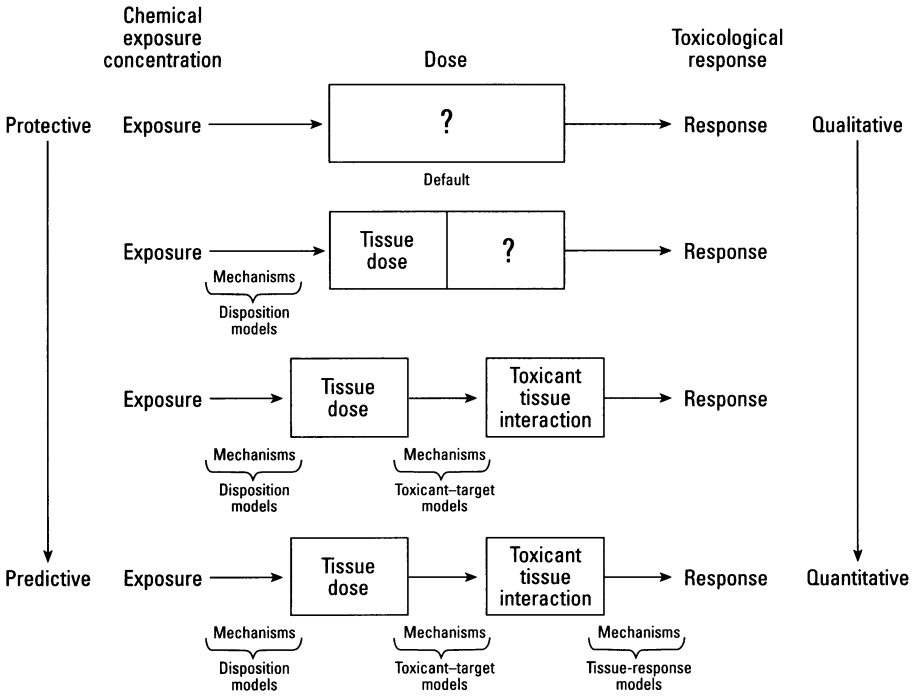

Figure 3. Characterization of the exposure-dose–response continuum in a risk assessment context. The process of determining the continuum is achieved by linking the mechanisms of critical biological factors that regulate the occurrence of a particular process and the nature of the interrelationships among these factors. It is ultimately desirable to have a comprehensive biologically based dose–response model that incorporates the mechanistic determinants of chemical disposition, toxicant–target interactions, and tissue responses into an overall model of pathogenesis. At the rudimentary level, response is related to exposure without consideration of internal dose, and dose–response estimates based at this level of description necessarily incorporate large uncertainty factors to ensure that the estimates are protective in the presence of substantial data gaps. With each progressive level, the "black box" of dose becomes "illuminated," i.e., incorporation and integration of mechanistic determinants allow elucidation of the continuum and, depending on the knowledge of model parameters and fidelity to the biological system, a more accurate characterization of the pathogenesis process. Because of the increase in the accuracy of the characterization with each progressive level, dose–response estimates also progress from more conservative (protective) to factually based (predictive). Reproduced from Jarabek (*29*) and the U.S. Environmental Protection Agency (*58*).

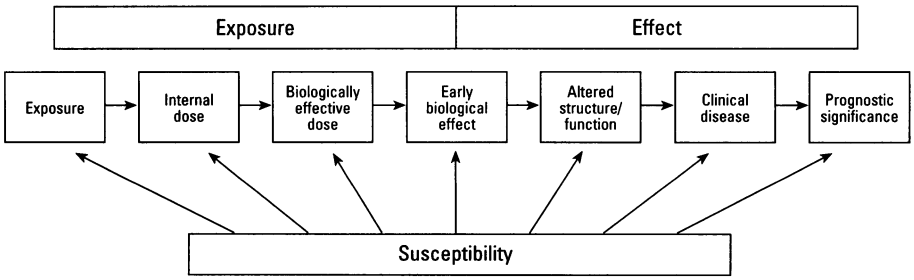

Figure 4. The National Research Council schema for biological markers (*60*), defined as any cellular or molecular indicator of toxic exposure, adverse health effects, or susceptibility, and which represent signals—generally biochemical, molecular, genetic, immunological, or physiological—in a continuum of events between a causal exposure and resultant disease. The schema is based on the tenets of molecular epidemiology, i.e., that early biological effects from a toxic exposure are more prevalent in the population at risk than the late events of disease (e.g., morbidity and mortality) that were historical outcome measures of interest to risk assessment, that the early events may be more specific to the exposure than the end disease outcomes, and that technological advances allow xenobiotics to be directly or indirectly quantified by identification of some predictable, dose-related biologic response. The schema is similar to that depicted in Figure 3 and helps to illustrate how the basis of dose–response estimates on key precursor events can be protective relative to assessment of later stage disease. Reproduced from the U.S. Environmental Protection Agency (*58*), Jarabek (*59*), and Schulte (*61*).

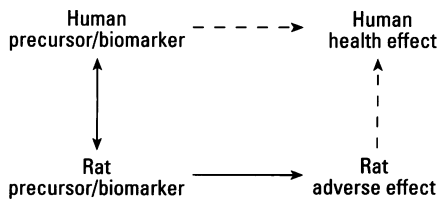

Figure 5. The use of biomarker data in parallelogram extrapolation to human homology. Reproduced from Jarabek (*59*).

Figures 1 and 2 provide schematics that help in the evaluation of how well laboratory animal data predict human response and serve to frame research to establish human homology. For both the immune system and the respiratory system, data on healthy and compromised individuals indicate that the developing organism (prenatal to adolescent) should not be considered a small adult. Because of these qualitative and quantitative differences, scaling practices are inappropriate. There are different critical events (e.g., thymic education in the immune system or morphogenesis and development of metabolizing enzyme systems in the respiratory system) that likely have different governing factors of both dosimetry and toxicant–target interaction (response), i.e., a different mode of action for an agent in the developing organism versus the adult.

In vitro data, biomarkers (or exposure, effects, or susceptibility), or precursor lesions may be useful to extrapolate laboratory animal or human clinical data to predict human health outcomes (disease) from ambient exposure scenarios via this parallelogram approach. There are examples for both end points where this conceptual construct has been used successfully to establish human homology for adults, but this has not been extended to the developmental windows, and it represents a key research area for both systems. Differences in dosimetry may be a key factor to characterize to perform the parallelogram approach properly. Developing more sensitive measures than screening spirometry (e.g., FEV_1) or methacholine challenge is important for evaluating potential perturbations in the respiratory system.

In the immune system, there are three categories of responses to consider: one represents down-regulation of the immune system (immunosuppression) and two represent up-regulation of the immune system (hypersensitivity and autoimmunity). For immunosuppression, inhibition of macrophage function after low-level ozone exposure has been measured *in vitro* and in bronchoalveolar lavage that corresponds to decrements seen in the *in vivo* organism (*Streptococcus* host-resistance model). These same precursor lesions/biomarkers have been observed in human cells exposed to similar experimental conditions. Inhibition of contact sensitivity has similarly been illustrated after the exposure of mice and humans to ultraviolet B radiation.

In the respiratory system, this conceptual framework has been filled for exposure to ETS. Both metabolic functional changes and the cell types affected are analogous between laboratory animal species and humans. Adjustments for dosimetry are required for accurate prediction, especially when extrapolating information from laboratory animals to humans.

Summary

This paper and the accompanying background papers (*1–3*) attempt to provide a framework for the determination of differential toxicological risk to the immunologic and respiratory systems during early and juvenile development. The basic tenet is that children differ significantly from adults in their biological/physiological responses to environmental exposures. Therefore, a systematic and comparative approach to developmental risk assessment is needed. To facilitate this process, we considered immune and respiratory development as a series of discrete windows that represent periods of differential vulnerability to toxicant and other environmental exposures. Although existing toxicological data lend support for this specific approach, it is also clear that the most likely benefit from such designations would come through additional research in which comparative perinatal toxicity data would be collected and applied to assessment. The ultimate benefit of the approach would be the enhanced ability to identify and better protect at-risk populations.

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