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

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Children differ from adults both physiologically and behaviorally. These differences can affect how and when exposures to xenobiotics occur and the resulting responses. Testing using animal models may be used to predict whether children display novel toxicities not observed in adults or whether children are more or less sensitive to known toxicities. Historically, evaluation of developmental toxicity has focused on gestational exposures and morphological changes resulting from this exposure. Functional consequences of gestational exposure and postnatal exposure have not been as well studied. Difficulties with postnatal toxicity evaluations include divergent differentiation of structure, function and physiology across species, lack of understanding of species differences in functional ontogeny, and lack of common end points and milestones across species. ***Key words:*** **critical periods of development, extrapolation of animal data, hazard identification, regulatory guidelines.** ***Environ Health Perspect*** **112:266–271 (2004). doi:10.1289/ehp.6014 available via *http://dx.doi.org/* [Online 25 November 2003]**

Children and adults are different physiologically and behaviorally. Children eat and drink more (based on size), play and act differently (e.g., very young children engage in more hand-to-mouth activity), are still undergoing development, and may be less or more able to metabolize and excrete certain substances [reviewed by U.S. EPA (U.S. Environmental Protection Agency) (2001)]. Because of these differences, children and adults may differ qualitatively and/or quantitatively in how they are affected by xenobiotic exposure. Effects of xenobiotics in children may be completely different from effects from the same exposure in adults (qualitative difference). On the other hand, the effect of xenobiotic exposure may be similar between children and adults but may occur to a greater or lesser extent in the child (quantitative difference).

Because of the potential differences in response to xenobiotic exposures, recent concerns have arisen in the scientific and regulatory arenas and call for improved safety assessments pertaining to children's health. Children's health risk assessment is the evaluation of the potential for xenobiotic exposures to cause any adverse developmental effect, including growth retardation, malformations, functional deficits, and lethality. Risk assessment includes evaluating available toxicity data (hazard identification) and exposure information (e.g., dose, route, duration, developmental stage of exposure) to determine if a xenobiotic causes potential adverse health effects in humans.

Various initiatives have been undertaken to address the many challenges in conducting children's health risk assessment, including a number of International Life Sciences Institute (ILSI)-sponsored projects. This article was developed to provide a high-level overview of the status of hazard identification as it pertains to children's health. The information was used as background material for an ILSI-sponsored workshop on developing a framework for assessing risks to children from exposure to environmental agents (Daston 2004). Therefore, this article is not intended to be comprehensive but merely provides an initial overview of hazard identification, particularly the use of animal models, as an attempt to identify data gaps in children's health risk assessment.

Critical Periods and Important Milestones in Development

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

Adverse developmental effects can occur during any period of the life span. Although it can be argued that the lifetime of an individual comprises the period of development, the most dramatic manifestations of development occur during the period of maturation to adult status in both humans and animals. This period of life includes both prenatal (preimplantation, embryonic, fetal periods) and postnatal (infancy, childhood, adolescence) development. For purposes of the current effort, the period of pre- and postnatal development is considered childhood and is arbitrarily defined as the period of life encompassing conception to 18 years of age in humans and from conception to sexual maturity in experimental animals. Only recently have scientific and regulatory concerns focused on postnatal development and hazard identification from exposures after birth. Therefore, more information is currently known about the details of prenatal development than those of the postnatal period.

Organ system development, chronology, and physical scaling across species. During development, cells within an organism change from a state of pluripotency (i.e., ability to develop into a great number of different tissue types) to a state of differentiation (i.e., commitment to a particular structural and/or functional role within the body). This concept of increasing cellular/organ differentiation, or specialization, throughout development is depicted in Figure 1 (modified from DeSesso 1997). The trend of increasing differentiation with maturation occurs in all species, but the chronological timetables can be very different from species to species. That is, the developmental time course for species with prolonged gestation periods (e.g., humans) occurs over a greater period of time than that of species with shorter gestation periods (e.g., rats), as illustrated in Figure 2. Humans reach adulthood in 18–25 years (depending on the criteria used to measure adult status), with bursts of developmental activity during both early childhood and puberty. In contrast, rats attain adult status very quickly. The life span of the two species

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can be scaled so that comparable stages of development are congruent, regardless of chronological age (Figure 3)—this is scaling to physiological time. By doing so, it can be noted that, based on developmental stages, birth occurs much earlier in the rat than in the human. Birth is not a maturational landmark; it does not occur at the same developmental stage for each species. Rather, birth is a physiological event that occurs at different developmental stages, depending on the species.

In the 1820s Karl van Baer (1828) made some observations about differentiation among species. Looking at prenatal development, he noted that the more general features of an embryo appeared earlier than the more specialized features, and that as development progressed, different species diverged morphologically. For example, forelimb buds appeared early in development and looked similar across species. Later in development, the forelimb buds of a fish became fins and those of a bird became wings, whereas those of a human became arms.

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

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

In postnatal life some milestones in animal species are denoted by the appearance of particular behaviors or neurological activities (e.g., acoustic startle response, exploratory behavior, rearing, mounting). Unlike with prenatal development, there are few reliable, comparative postnatal milestones in typically used experimental animals and humans. Without a database of normal milestone appearance, derived from many control animals, little information is available about the normal variation in these data. This, in turn, leads to obvious difficulties in interpreting such findings as a delay in the appearance of a particular milestone. Furthermore, interpretation of some behaviors is confounded because they are related to olfaction in macrosmatic species such as rodents and carnivores, and it is not clear

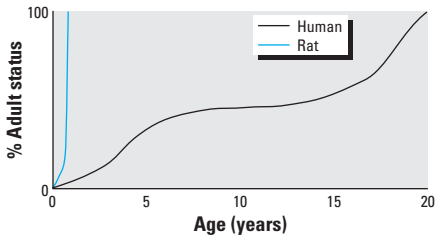

Figure 2. Time to develop adult characteristics.

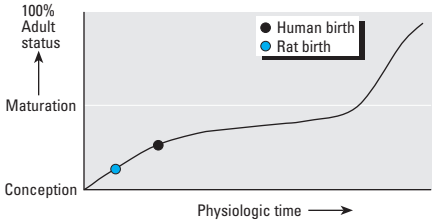

Figure 3. Relationship between extent of maturation and birth in rats and humans.

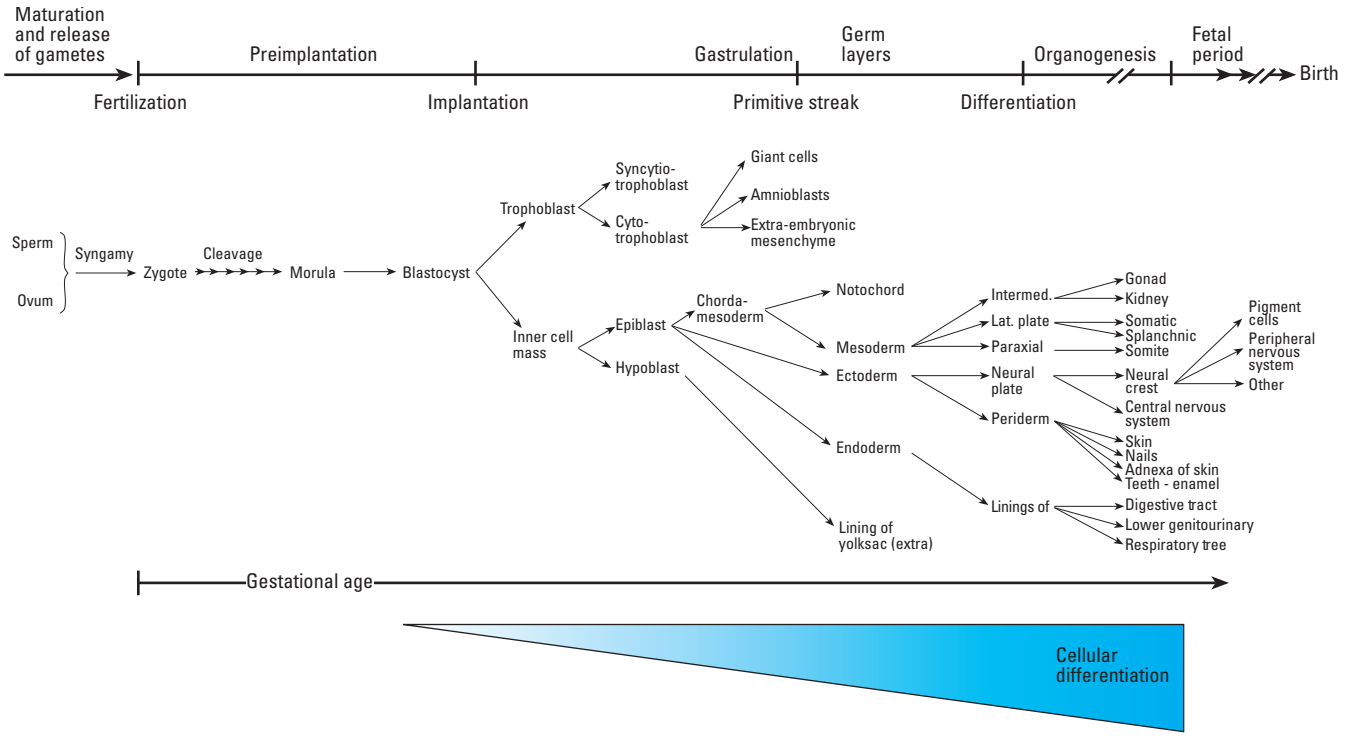

Figure 1. Differentiation increases with age of developing organisms. Modified from DeSesso (1997).

that these behaviors have counterparts in microsmatic species like man. Thus, the relevance of some of these findings will be problematic for use in risk assessment.

Because a generally accepted suite of postnatal developmental milestones in animal species is not yet available, the critical periods for the developmental processes associated with those milestones have generally not yet been defined. Additionally, there may be more than one critical period of vulnerability and end point affected. For example, brain structures have different peak periods of growth. Therefore, depending on the particular time of exposure, compounds could differentially affect the structures undergoing peak development. In a review of animal studies and their clinical implications, Rodier (1980) found that exposures occurring at different stages of brain development had different effects on brain and behavioral functions. For example, based on data from azacytidine-treated mice, hyperactivity seemed to result from insults that occurred during the middle part of neuron production (midprenatal insult), whereas hypoactivity seemed to result from insults that coincided with cerebellum proliferation (early prenatal and early postnatal insults). Additionally, the developmental outcome of methamphetamine exposure in rats depends on the developmental stage at the time of exposure, both prenatal and postnatal. Early prenatal exposure results in anophthalmia, whereas late prenatal exposure results in folded retina (Acuff-Smith et al. 1996). Early postnatal exposure results in persistent decreases in body weights, whereas late postnatal exposure results in impaired spatial learning (Vorhees et al. 1994). Both early and late postnatal exposures result in impaired complex learning, demonstrating that there may be overlap in periods of vulnerability or that some periods of vulnerability may be extended in relation to similar functional development.

Table 1. End point (humans and laboratory animals).

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

Understanding both the fundamental biology and the temporal schedule that underlie the development of whatever milestones are eventually selected is paramount for performing well-grounded, scientifically based risk assessments. Therefore, it is important to begin by defining important pediatric milestones for growth and development, both global and organ system specific. These milestones should be readily assessed by clinicians, reflect normal maturational processes, be liable to disruption when normal maturation is perturbed, and demonstrate susceptibility to pharmacologic perturbation (i.e., display properties of dose response and time action). Subsequently, end points in animal species analogous to these pediatric milestones should be identified accordingly. The first step in identifying such milestones could be to consult pediatricians to determine the key end points monitored in the clinic and then evaluate their relationships to end points used in animal models.

Relevance/Predictability of Extrapolation of Animal Data to Children's Risk Assessment

Animal/human concordance has been reasonably well characterized in two primary areas pertaining to children's health: developmental toxicology and developmental neurotoxicology. Species comparisons of the development of physical structures and organ systems have been made by Otis and Brent (1954), Hoar and Monie (1981), and DeSesso (1997). These comparisons enhance the predictability of the type of damage that may occur in humans after insult at various stages of development. Several studies have assessed the ability of animal models to predict human risk for developmental toxicity (Frankos 1985; Hemminki and Vineis 1985; Holson et al. 1981; Jelovsek et al. 1989; Kimmel et al. 1984; Newman et al. 1993; Nisbet and Karch 1983; Schardein 1995; Schardein and Keller 1989; Schardein et al. 1985). Holson et al. (1981) and Kimmel et al. (1984), in the most thorough analyses, evaluated data only from experimental animal studies and epidemiology studies that met stringent design criteria [see discussion by Holson et al. (2000)]. These investigators concluded that concordance of developmentally adverse effects exists when one considers all of the measures of developmental toxicity (death, malformation, growth effects, functional deficit). Strict anatomical concordance is not always present but is not necessary when one only needs 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 one can compare similar target organ doses at equivalent stages of development. If such detailed knowledge were available, it would be possible to test for the ability of an agent to elicit specific malformations.

Table 1 indicates potential developmental landmarks that have been measured in humans and/or laboratory animals. This list is not all-inclusive and requires input from individuals with pediatric expertise. Tests for acquisition of these landmarks may differ in humans and laboratory species, but the same basic end point is being measured. Representation on this list does not necessarily indicate that these tests are being conducted routinely or that they are validated. Several areas such as hormone measurements, and evaluation of metabolism and pharmacokinetics/pharmacodynamics are listed but have not been well characterized in children and young animals. Evaluation of additional functional areas that develop postnatally, such as the immune and genitourinary systems, has not been extensively explored. Biomarkers for these areas are needed, and data should be evaluated for concordance between humans and laboratory animals. Surrogate markers in laboratory animals might include, for example, pinnae detachment and eye opening. However, the relevance of surrogate markers is questionable and therefore should not necessarily be included in a battery of tests for developmental landmarks. For example, other than as a general indication that development in the animal is proceeding at a normal pace, the clinical significance of eye opening is nil. Furthermore, although the ability to taste and smell can be measured in humans and laboratory animals, its relevance to hazard identification is unknown.

A work group (Adams et al. 2000) specifically charged with addressing questions relevant to risk estimation in developmental neurotoxicology found that, within the context of methods used in regulatory testing batteries, extrapolation appears stronger with

regard to effects on sensory and motor functioning than for cognitive or social functioning. To improve detection of learning and memory deficits, they suggested that the integrity of learning mechanisms should be further challenged through task complexity. Additionally, the workshop suggested the use of more contemporary and sensitive methods for evaluating behavior. These include adding prepulse inhibition to the startle paradigm, improving water maze learning tasks by adding reversal learning, and examining morphological and functional effects in young as well as in aged animals.

Methods for Assessing Potential Hazards to Children

Animal studies. Current animal toxicity assessments include prenatal developmental toxicity studies, fertility and reproduction studies, developmental neurotoxicity studies, and/or perinatal and postnatal studies. Tables 2 and 3 are meant to summarize the overall designs of these protocols; for detailed descriptions the reader is referred to the original guidelines [U.S. EPA 1991, 1996, 1998; U.S. FDA (U.S. Food and Drug Administration) 1994]. Additionally, several reviews discuss and compare the U.S. EPA, U.S. FDA, and the Organisation for Economic and Co-operation and Development guidelines (Collins et al. 1998; Kimmel and Makris 2001).

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

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

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

One design flaw regarding exposure to the offspring is apparent with the multigenerational test and the prenatal and postnatal test and limits their use in determining the potential postnatal toxicity of a compound. Pups are assumed to be exposed to the compound through the mother's milk until weaning. However, exposure to the compound during the lactational period usually is not verified or measured in these studies. As a result, exposure to the offspring is uncertain and not quantified. The compound could be excreted or sequestered by the mother, such that the offspring are not exposed to the extent assumed; alternatively, the compound could be hyperexcreted in the milk, such that the offspring receive a much higher dose than expected.

Another weakness associated with the multigenerational and the prenatal and postnatal testing paradigms is that differences between the metabolic capacity of adults versus offspring and differences among species in terms of organ system development are often not well characterized (and frequently are completely unknown). Neither of these factors should be overlooked, and as discussed below, both should play an important role in the choice of an appropriate animal model for

Table 2. U.S. EPA testing guidelines.

	Prenatal development study	Reproduction and fertility effects (two-generation)	Developmental neurotoxicity study
Dosing period	GDs 6–20 (rat) GDs 6–29 (rabbit)	P: 10 weeks prior to mating through weaning F: weaning through mating, pregnancy and lactation	GD 6–PND 10
Number of animals	20 pregnant females	20 pregnant females	20 litters
Typical end points evaluated	Maternal toxicity Number of implantations and corpora lutea Embryo/fetal mortality Fetal weight and sex Fetal morphology (external, visceral, skeletal)	Estrous cyclicity Semen quality Mating indices Fertility indices Parturition Offspring growth and viability Reproductive landmark development (vaginal opening and preputial separation) Reproductive organ weights and histopathology	Offspring growth and viablity Offspring toxicity Developmental landmarks Motor activity Auditory startle habitation Learning and memory Neuropathology including morphometric analysis

Abbreviations: F, filial; GD, gestational day; P, parental.

Table 3. U.S. FDA testing guidelines.

	Fertility and early embryonic development	Pre- and postnatal development, including maternal function	Embryo–fetal development
Dosing period	2 weeks (females) and 4 weeks (males) prior to mating through GD 6	GD 6–PND 20	GDs 6–17 (rat) GDs 7–19 (rabbit)
Typical end points evaluated	Maternal toxicity Mating indices Fertility indices Number of implantations and corpora lutea Embryo mortality	Growth and viability Maturation and fertility indices Sensory functions and reflexes Behavior (motor activity; learning and memory)	Maternal toxicity Number of implantations and corpora lutea Embryo–fetal mortality Fetal weight and sex Fetal morphology (external, visceral, skeletal)

postnatal toxicity testing. Furthermore, understanding the developmental differences in organ systems across species is critical to study design. If exposure does not occur at the right time (i.e., during critical periods for exposure), then potential adverse outcomes will be missed. Additionally, if the evaluation of the developmental milestone/process does not occur at the right time (i.e., during the critical periods of expression/assessment), then again potential adverse outcomes will be missed.

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

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

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

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

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

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

That said, traditional developmental milestones are considered to be the result of highly integrated processes and do not lend themselves to *in vitro* mechanistic evaluation. As with the assessment of developmental toxicology, the most expedient way to evaluate developmental milestones may be with a standardized screen to identify effects, leaving *in vitro* mechanistic evaluations in the realm of effect characterization on a case-by-case basis. In the future, genomics and biomarkers may be important in the detection of early stages of xenobiotic-induced diseases in children. However, validation is required, and as with *in vivo* screening, much work is needed to understand relevancy and clinical implications.

Data gaps. Data gaps pertaining to hazard identification of children's exposure include the lack of understanding of the relevance of animal models for predicting outcomes in humans, the lack of comparative developmental profiles in animal models and humans (i.e., are the life stages studied in animal tests analogous to human stages of concern), and the lack of testing protocols, both of animals and humans, evaluating functional outcomes at relevant life stages. These data gaps should be investigated by appropriate scientific experimentation. Addressing these data gaps will reduce the range of uncertainties for children's risk assessment.

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

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

Summary

To conduct better risk assessments of children's health, we must first be able to understand the relevance of animal models for predicting outcomes in humans. Children are not adults. They differ by activities and stages of development. These differences in turn can affect how and when exposures to chemicals occur and the resulting responses. Historically, evaluation of developmental toxicity has focused on gestational exposures and morphological changes resulting from this exposure. Current processes for evaluating growth, survival, and morphological change due to gestational exposure are adequate. However, functional consequences of gestational exposure and postnatal exposure are not as well studied. Difficulties with our experience and knowledge base for postnatal toxicity evaluations include divergent differentiation of structure, function, and physiology across species, lack of understanding of species differences in functional ontogeny, and lack of common end points and milestones across species.

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