Utilization of Animal Studies to Determine the Effects and Human Risks of Environmental Toxicants (Drugs, Chemicals, and Physical Agents)

Robert L. Brent, MD, PhD

ABSTRACT. Toxicology studies using animals and in vitro cellular or tissue preparations have been used to study the toxic effects and mechanism of action of drugs and chemicals to determine the effective and safe dose of drugs in humans and the risk of toxicity from chemical exposures. Studies in pregnant animals are used to determine the risk of birth defects and other reproductive effects. There is no question that whole animal teratology studies are helpful in raising concerns about the reproductive effects of drugs and chemicals, but negative animal studies do not guarantee that these agents are free from reproductive effects. There are examples in which drug testing was negative in animals (rat and mouse) but was teratogenic in the human (thalidomide), and there are examples in which a drug was teratogenic in an animal model but not in the human (diflunisal). Testing in animals could be improved if animal dosing using the mg/kg basis were abandoned and drugs and chemicals were administered to achieve pharmacokinetically equivalent serum levels in the animal and the human. Because most human teratogens have been discovered by alert physicians or epidemiology studies, not animal studies, animal studies play a minor role in discovering teratogens. In vitro studies play an even less important role, although they are helpful in describing the cellular or tissue effects of the drugs or chemicals. One cannot determine the magnitude of human risks from these in vitro studies. Performing toxicology studies on adult animals is performed by pharmaceutical companies, chemical companies, the Food and Drug Administration, many laboratories at the National Institutes of Health, and scientific investigators in laboratories throughout the world. Although a vast amount of animal toxicology studies are performed on pregnant animals and numerous toxicology studies are performed on adult animals, there is a paucity of animal studies using newborn, infant, and juvenile animals. This deficiency is compounded by the fact that there are very few toxicology studies performed in children. That is why pregnant women and children are referred to as "therapeutic orphans." When animal studies are performed with newborn and developing animals, the results demonstrate that generalizations are less applicable and less predictable than the toxicology studies in pregnant animals. Although many studies reveal that the infant and the developing animal have difficulty in metabolizing drugs and are more vulnerable to the toxic effects of environmental chemicals, there are exceptions that indicate that infant and developing animals may be less vulnerable and more resilient to some drugs and chemicals. In other words, the generalization indicating that developing animals are always more sensitive to environmental toxicants is not valid. For animal toxicology studies to be useful, animal studies have to use modern concepts of pharmacokinetics and toxicokinetics, as well as method-of-action studies to determine whether animal data can be used for determining human risk. One example is the inability to determine carcinogenic risks in humans for some drugs and chemicals that produce tumors in rodents, because the oncogenesis is the result of peroxisome proliferation, a reaction that is of diminished importance in humans. Scientists can use animal studies to study the toxicokinetic and toxicodynamic aspects of environmental toxicants, but they have to be performed with the highest level of scholarship and objectivity. Threshold exposures, maximum permissible exposures, and toxic effects can be estimated but have to be interpreted with caution when applying them to the human. Well-performed epidemiology studies are still the best method for determining the human risk and the effects of environmental toxicants. Pediatrics 2004;113:984–995; methods of evaluation, environmental toxicology, pharmacokinetics, pharmacodynamics, toxicokinetics, toxicodynamics, MOA (method of action), deterministic, threshold phenomenon, stochastic, biologic plausibility, in vitro systems, in vivo animal studies.

ABBREVIATIONS. MOA, method of action; FDA, Food and Drug Administration; CNS, central nervous system.

This article deals with a complicated and important issue. Can the magnitude and type of environmental risks to the embryo, child, and adolescent be determined from animal studies, and how different are these risks when compared with adults? In many instances, environmental agents will exploit the vulnerabilities and sensitivities of developing organisms. In other instances, there will be no difference between the developing organism and the adult when exposed to toxicants, and in some instances, children and adolescents may even withstand the exposures with less insult. The difficulty that we have at this time is that in many situations, we do not have enough data and/or scholarly techniques to arrive at a conclusion about the relative sensitivity of the developing organism to some environmental agents. Rather than arrive at conclusions.
about environmental agents or exposures for which there are insufficient data, we need to initiate investigative approaches to obtain the necessary data concerning agents and exposures that have not been clarified, so it is important that we initiate and expand quality research in environmental toxicology.

Although chemicals and drugs can be evaluated for their toxic potential by using in vivo animal studies and in vitro systems, it should be recognized that these testing procedures are only 1 component in the process of evaluating the potential toxic risk of drugs and chemicals. The evaluation of the toxicity of drugs and chemicals should include, when possible, data obtained from a number of investigative approaches: 1) epidemiologic studies; 2) secular trend or ecological trend analysis; 3) animal studies; 4) pharmacokinetic, toxicokinetic, pharmacodynamic, and toxicodynamic studies; 5) mechanism of action (MOA) studies; and 6) basic science studies that pertain specifically to the agent, such as MOA studies, which include receptor affinity, cytotoxicity, genotoxicity, organ toxicity, neurotoxicity, etc.

Human studies are expensive and take years to complete. Therefore, scientists have asked whether appropriate animal models are available to evaluate the risks of environmental toxicants to the embryo, infant, child, and adolescent. This is not an easy task.

There are a few toxicologic principles that should precede the specific discussion. Frequently, drugs or chemicals are grouped into categories (pesticides, trihalomethanes, organochlorines, solvents, progestins, heavy metals, chemotherapeutic agents). It is important to note that this type of classification may be useful for some purposes but not for concluding generalizations about the toxic effects of all of the agents in the group. As an example, the Food and Drug Administration (FDA) published a report in the Federal Register disclaiming the term “progestins” to describe a group of drugs with identical effects and toxicity. Second, chemicals may be referred to as “poisons.” This is not a useful label because every known chemical or drug has an exposure that is toxic. Paracelsus stated in the 16th century, “What is there that is not poison? All things are poison and nothing is without poison. Solely, the dose determines that a thing is without poison.”

Three areas of animal testing are discussed: 1) reproductive effects from exposures during embryonic and fetal development; 2) toxic effects of drugs and chemicals administered to animals after birth as newborns, infants, juvenile animals, and adults; and 3) oncogenic effects of environmental toxicants.

**USE OF ANIMAL STUDIES TO DETERMINE REPRODUCTIVE RISKS IN HUMANS (TERATOGENESIS, GROWTH RETARDATION, PREGNANCY LOSS, STILLBIRTH, AND INFERTILITY)**

Pediatricians and other clinicians have little training on how to interpret animal toxicology studies during medical school and residency training. This is probably more true of reproductive toxicology studies than in any other area of animal testing. Unfortunately, for physicians, the most frequent source and contact with animal testing information is in the package insert or the Physician’s Desk Reference. The Physician’s Desk Reference uses the FDA’s classification of reproductive risks, partly based on animal testing. The categories are A, B, C, D, and X. The A category includes drugs that have no risk for reproductive effects. The B, C, and D categories have increasing risks, and the X category includes drugs such as methotrexate, Accutane, and thalidomide that should not be used in pregnant women or women of reproductive age who are not on contraceptives. These categories are misleading more than they are helpful. Teratologists, obstetricians, and other clinicians who counsel pregnant women have been very critical of the FDA classification because the classification ignores the basic principles of teratology and the importance of modern pharmacokinetics when evaluating animal studies. In 1990, a published article indicated that of the 200 most frequently prescribed drugs, none of them represented a significant teratogenic risk, yet only a small proportion of these drugs were placed in category A by the FDA. There are many reasons for these misclassifications, but the most important reason is the misapplication of animal testing results. Let me give you some examples.

When a new drug is marketed or a new environmental toxicant is discovered, frequently the only information that is available is the animal data. Three examples are used to emphasize the difficulties that occur: 1) meclizine produces cleft palate at very high exposures in the rat; 2) leflunomide and its MOA; and 3) radiation produced mental retardation; a deterministic or stochastic effect (Table 1)?

<table>
<thead>
<tr>
<th>Phenomenon</th>
<th>Pathology</th>
<th>Site</th>
<th>Diseases</th>
<th>Risk</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stochastic</td>
<td>Damage to a single cell may result in disease</td>
<td>DNA</td>
<td>Cancer, germ cell mutation</td>
<td>Some risk exists at all dosages; at low doses, risk is less than spontaneous risk</td>
<td>The incidence of the disease increases, but the severity and nature of the disease remain the same</td>
</tr>
<tr>
<td>Threshold</td>
<td>Multicellular injury</td>
<td>Multiple, variable cause, affecting many cell and organ processes</td>
<td>Malformation, growth retardation, death, toxicity, etc</td>
<td>No increased risk below the threshold dose</td>
<td>Both the severity and incidence of the disease increase with dose</td>
</tr>
</tbody>
</table>

Modified from Brent.12
Meclizine Produces Cleft Palate at Very High Exposures in the Rat

Meclizine is an antihistamine with a lengthy history and like most antihistamines has not been demonstrated to have reproductive toxicity in multiple epidemiologic studies, yet its pregnancy category classification is B, primarily because “reproductive studies in rats have shown cleft palates at 25 to 50 times the human dose.” Actually, what the clinician needs to know is what the blood level is in the rat and mouse when teratogenesis is produced and how that blood level compares with the level in patients who receive therapeutic doses of the medication. Without this information, the animal experiments are meaningless. There are hundreds of drugs in categories B and C with animal studies using the arachid mg/kg dose. This same failing has occurred in toxicologic studies with environmental toxicants (lead, mercury, polychlorinated biphenyls, pesticides, fungicides), namely, using mg/kg exposures in rodents or other animals rather than determining serum levels in the animal and the human population for which there was concern. Fortunately, more recent environmental toxicology studies have been using modern toxicokinetic techniques, but serum levels of these toxicants are not always available in humans.

Leflunomide and Its MOA

Leflunomide is a relatively new drug (1998) that is used to treat rheumatoid arthritis. It has a box warning for reproductive effects (teratogenesis) and has been placed in category X. Because there were no human data available at the time of marketing, the label was based on the animal studies: “There are no adequate and well-controlled studies evaluating Arava (leflunomide) in pregnant women. However, based on animal studies, leflunomide may cause fetal death or teratogenic effects when administered to a pregnant woman.”

Leflunomide is a novel isoxazole immunomodulatory agent that inhibits de novo pyrimidine synthesis and has antiproliferative activity. In vitro, after mitogen stimulation, the active metabolite of leflunomide inhibits T-cell proliferation, DNA synthesis, and the expression of certain cell surface and nuclear antigens directly involved in T-cell activation and proliferation. It inhibits mitogen-stimulated proliferation of human peripheral blood mononuclear cells and proliferation in transformed murine and human cell lines in a dose-dependent manner. It has been demonstrated that the active metabolite binds to and is a potent inhibitor of dihydroorotate dehydrogenase, an enzyme in the de novo pyrimidine synthesis pathway important for DNA synthesis. Together, these data suggest that at serum concentrations achievable in patients, leflunomide inhibits de novo pyrimidine synthesis in activated lymphocytes and other rapidly dividing cell populations, resulting in reversible cell cycle arrest.

In oral embryotoxicity and teratogenicity studies in rats and in rabbits, leflunomide was embryotoxic (growth retardation, embryolethality, and teratogenicity) in rats, consisting of malformations of the head, rump, vertebral column, ribs, and limbs; and in rabbits, malformation of the head and bilateral dysplasia of the spine of the scapula. The no-effect level for embryotoxicity and teratogenicity in rats and rabbits was 1 mg/kg body weight, which resulted in serum levels of 3.7 and 4.1 µg/mL, respectively.

The active metabolite of leflunomide, which is the pyrimidine antagonist, is maintained at a blood level of 40 µg/mL in patients being treated. The decision to label leflunomide as having a teratogenic risk was based on the fact that the human serum level was in the range of the teratogenic blood level in the animal models, so the initial labeling was an appropriate precaution to prevent birth defects.

After 4 years of treatment of patients with rheumatoid arthritis and no indication of an increase in teratogenesis in a very small group of pregnant patients who were treated and continued their pregnancy to term, we can reanalyze the animal data as follows. This is referred to as the MOA approach. The potential mechanisms of teratogenicity for leflunomide are as follows:

1. Suppression of DNA synthesis by interfering with pyrimidine synthesis based on the presumption that suppression is equal in the rat, rabbit, and human at the same serum levels of the active metabolite of leflunomide. This was the basis of the X category labeling.
2. The susceptibility of the enzyme to the active leflunomide metabolite that is involved in pyrimidine incorporation into DNA in the human and animal models.
3. The ability of the active metabolite of leflunomide to interfere with cell proliferation in the human and animal models.

If all 3 mechanisms of action were operative to the same degree at the same serum level in the animals and the patients, then there would be concurrence and the human risks would be determined to be identical from studying all 3 mechanisms. In vitro studies of the active metabolite of leflunomide revealed that the rat was 40 times more sensitive to the suppression of dihydroorotate dehydrogenase than the human and that the rat was 328 times more sensitive to the active metabolite of leflunomide than was the human in suppressing cell proliferation.

What this means is that if enzyme suppression or antiproliferative activity is the MOA of teratogenicity in the rat, then the clinical use of leflunomide in pregnant women would probably not be teratogenic, but no one would act on these findings without confirmation from the ongoing epidemiologic surveillance of this drug. This is an example of modern pharmacokinetic studies having improved risk assessment and made epidemiologic studies understandable.

In Utero Effects of Ionizing Radiation on the Risk of Mental Retardation

Here is an example in which animal behavioral studies and concomitant pathology were helpful in resolving an important issue with regard to in utero radiation–induced mental retardation. The main is-


From the perspective of biological plausibility and the results of animal studies, it seems that the data favor the viewpoint that mental retardation is a deterministic effect with a threshold above 0.2 Sv. Histologic examination of the irradiated brain exposed to 0.01 Sv reveals no pathologic consequences that could account for severe mental retardation. That would mean that the pattern of effects produced by ionizing radiation that accounts for mental retardation when the fetus is exposed to 0.5 to 2 Sv does not occur at very low exposures. Furthermore, additional studies by Schull and Otake revealed that these authors were able to quantify the risk of reduced intellect after in utero ionizing radiation exposures. They estimated that there was a reduction in intellect of approximately 30 IQ points per Sv in their studies. Even if there were a linear relationship between the dose and IQ reduction, one could predict that 0.01 Sv could not account for a doubling of the incidence of mental retardation, because a linear extrapolation of Otake and Schull’s data would represent only a maximum reduction of 0.3 of an IQ point at 0.01 Gy. Behavioral studies in animals were unable to demonstrate neurobehavioral effects below 0.02 Gy (Table 2). Although one has to be careful in extrapolating animal data to humans, the lack of neurobehavioral effects from in utero irradiation supports the other findings that indicate that mental retardation is a threshold (deterministic) effect (Tables 1 and 2).

Once a drug, chemical, or other agent is suspected of producing congenital malformations or other reproductive effects, appropriate use of in vitro and in vivo testing can be helpful in evaluating the specific allegation and in determining the mechanism of action of the agent. Whole animal testing, although serving important and useful purposes, can still be improved so that they can be better used to estimate human reproductive risks. These improvements are listed in Table 3.

In vitro tests can be used to study the mechanisms of teratogenesis and embryogenesis and for preliminary screening procedures, but in vitro studies will never be able to be predictive of human teratogenic risks at particular exposures without the benefit of data obtained from whole animal studies (Table 3) and epidemiologic studies. Despite the advances in in vitro and in vivo testing for teratogenicity, human epidemiologic surveillance by various methods is and will be our most powerful tool for discovering human reproductive toxins and teratogens. It may be difficult for experimental teratologists to accept that alert physicians and scientists have been the most prominent contributors to the discovery of the environmental causes of birth defects (Table 4).

### TABLE 2. Effect of In Utero Ionizing Radiation on Developmental and Neurologic Parameters in the Rat

<table>
<thead>
<tr>
<th>Dose of X-ray (Gy)</th>
<th>Embryonic or Fetal Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9th Day</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Effect</td>
<td></td>
</tr>
<tr>
<td>Growth retardation at term</td>
<td>-</td>
</tr>
<tr>
<td>Growth retardation postpartum</td>
<td>-</td>
</tr>
<tr>
<td>Developmental parameters (4)</td>
<td>-</td>
</tr>
<tr>
<td>Reflexes (5)</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 3. A Whole-Animal Teratology-Reproductive Toxicity Protocol Should Include the Following Parameters and Goals

1. Determine the reproductive effects at stages of gestation that may have markedly different endpoints, namely, preimplantation, organogenesis, and early fetal and late fetal stages.
2. The importance of various reproductive endpoints may vary considerably by the gestational stages being evaluated, and exposures at one stage may exaggerate, modify, or eliminate effects that occur at another stage
   1. Teratogenesis
   2. Embryolethality
   3. Growth retardation
   4. Postnatal physiologic, biochemical, developmental, and behavioral effects
3. Determine the no-effect dose for the parameters mentioned in item 2 at various stages of gestation.
4. Determine the ratio of the no-effect dose to the human therapeutic dose, usual exposure dose, or maximal permissible exposure for the parameters mentioned in item 2.
5. Determine the quantitative relationship between the human and animal model pharmacokinetics and toxicokinetics concerning the dose and the blood levels and the metabolism in the animal model and human.
6. Determine the MOA of the environmental toxicant.
7. Determine the ratio of the LD/50 for the mother and the embryo.
EFFECTS OF ENVIRONMENTAL TOXICANTS THAT ARE ADMINISTERED TO ANIMALS AFTER BIRTH AS NEWBORNS, INFANTS, JUVENILE ANIMALS, AND ADULTS FOR DETERMINING HUMAN RISKS

It is obvious that animal experiments cannot be planned to consider all of the variables that occur in the human. In fact, there are situations in animal studies that differentiate the animal species from the human. For example, coprophagy and other behaviors in rodents and other species can markedly alter the dynamics of toxicity studies. Differences in absorption, metabolism, and excretion of drugs and chemicals represent the greatest barrier to applying risks obtained from animal studies directly to the human.

It is hoped that regulatory agencies and toxicologists who deal with issues of developmental toxicity will develop animal models that will predict toxicologic effects in children and adolescents from exposure to drugs and chemicals. Although this is an optimistic view, Done28 pointed out that although the number of drug hazards that have proved to be unique in the infant have proved to be small: “Without exception, recognition of the proved hazard has come about only after widespread use, and then usually when tragic consequences focused attention on the drug.”28

**Animal Toxicology Studies**

Historically, the administration of drugs and chemicals to humans and animals in experimental studies has used the mg/kg exposure method. Even in the 1800s, there was recognition that there was not a proportional relationship between body weight and dose between the infant and the adult human.29 It was apparent that appropriate infant doses would in some cases be toxic in the adult and appropriate adult doses would be inadequate for the infant if the mg/kg approach were used. Animal investigators have long been aware of this dilemma. It became apparent that comparisons of toxicity or therapeutic effects bore a closer relationship to the 0.7 power of body weight, and this figure was closely related to surface area.30,31 Many physiologic functions are proportional to surface area because the extracellular volume in humans is constant on a surface area basis. Therefore, the serum concentrations obtained from administration or exposure to drugs and chemicals on a surface area basis would result in serum concentrations that would be similar. This is more closely related to the 0.73 power of the weight at all ages in humans.32,33 If, however, drugs or chemicals are also distributed in the total body water, then neither the mg/kg nor the surface area model will be accurate, because total body water is not a constant using the surface area constant or the mg/kg relationship. It is obvious that no one method of dose calculation for the young will be satisfactory for evaluating appropriate therapeutic doses or for determining toxic risks. If that is the case for human exposures, then animal toxicology studies that are based on the mg/kg or surface area will not be universally appropriate for determining human risks or proper doses. In fact, the fields of pharmacokinetics and toxicokinetics have demonstrated that animal toxicology experiments have to be performed knowing the serum level of the drug or chemical in the human and using those levels in animal toxicology studies.

We are interested in the usefulness of information obtained from animal toxicology studies, using drugs and chemicals for determining the risks to children and adolescents from these exposures. The largest literature in this field pertains to animal toxicology studies using newborn and infant animal models. Most of these studies are acute toxicology studies and use the mg/kg method of dosing the adult and infant animals. Much of the information is simply the determination of the lethal exposure or the effect on growth. The most important finding in

**TABLE 4. HOW SOME HUMAN TERATOGENS HAVE BEEN DISCOVERED**

<table>
<thead>
<tr>
<th>Agent or Drug</th>
<th>Human Epidemiology Studies</th>
<th>Alert Physician or Scientist, Cluster</th>
<th>Animal Studies</th>
<th>In Vitro Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubella</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminopterin</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydantoins 1963</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethadione 1970</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valproic acid 1982</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A, 1953</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isotretinoin 1983</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etretinate 1984</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCBs 1968</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coumarin 1968</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol 1967</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithium 1970</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethylstilbestrol 1971</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillamine 1971</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Misoprostol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethorpin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chorionic villous sampling</td>
<td></td>
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</tbody>
</table>

PCB indicates polychlorinated biphenyl.
these studies is that newborn and infant animals are not always more sensitive or more deleteriously affected by drugs and chemicals when compared with adults.

Urethane, an anesthetic that is no longer used for that purpose, was unable to anesthetize newborn animals at exposures that anesthetized adults, whereas ether altered reflexes at lower concentration in newborn animals than in adults.\textsuperscript{34} Newborn mice and other animal species have demonstrated a tolerance to hypoxic conditions that is not present in adult animals.\textsuperscript{35–39} Newborn mice continued to breathe for a longer period when exposed to ether than adult mice.\textsuperscript{40} Newborn mice had a prolonged survival when compared with adults that were exposed to asphyxia as a result of exposure to CO, HCN, CO\textsubscript{2}, H\textsubscript{2}, and CH\textsubscript{3}. Longer exposures to strychnine, curare, cyanide injection, strangle-up, hypoxia, or nitrobenzol were necessary to produce respiratory arrest in newborn mice as compared with adult mice.\textsuperscript{38}

In summarizing this information, Done\textsuperscript{28} was cautious, pointing out the multiplicity and variability of experimental details in these studies. He concluded, “Some tentative generalizations and observations may be worth making. First, it is apparent that immaturity does not necessarily entail greater sensitivity. A notable example is thiourea, which is 50 to 400 times as toxic in the adult as in infant rats.”\textsuperscript{41,42} Conversely, the animal experiments with chloramphenicol clearly demonstrated that this drug was more toxic in the infant rat than in the adult. Animal toxicity studies corroborated the toxicity reported in infants.\textsuperscript{43–45}

In Done’s\textsuperscript{28} review of developmental toxicology, he indicated that the newborn or infant animal was more sensitive to many drugs (eg, chloramphenicol, morphine, some other opiates, picrotixin, tetracycline, novobiocin, some organophosphate anticholinesterases, atropine, histamine, sodium salicylate) and less sensitive to others (eg, ethanol, strychnine, metrazol, codeine, acetocycloheximide, thiourea, thyroid hormone). Many other drugs had sensitivities that were similar in the neonate and adult animal, but, of course, most of these data were based on the mg/kg dosage and the endpoints were simplistic, ie, death or cessation of respiration.


Can concerns about developmental problems from exposure to developmental toxicants in children and adolescent be evaluated with appropriately designed animal studies? Seleveran et al\textsuperscript{46} indicated “that little concrete information exists on critical windows for exposure during the postnatal period.”\textsuperscript{47,48} However, a systematic examination has not been done of available data on critical windows of vulnerability during postnatal development. Most available data are focused on prenatal exposures. Prenatal exposures have been examined for only a few agents (eg, lead, pesticides, radiation),\textsuperscript{49–52} and it can be stated that the pesticide analysis is far from definitive. The most important aspects in designing these animal studies are the application of modern toxicokinetics and pharmacokinetics. Exposure levels should include exposures that occur in the environment, and a major effort should be made to determine the no-effect level.

The developmental events that can be affected by environmental exposures to drugs, chemicals, and physical agents include the following developmental events that occur during childhood and adolescent development.

\textbf{Interference With Growth, Epiphyseal Development, and Epiphyseal Closure}

Alterations in growth from exposure to environmental toxicants can result in accelerated growth or growth retardation. Accelerated growth and maturation can result in larger stature or smaller stature. Smaller stature can result from the combination of growth acceleration and earlier epiphyseal closure. Drugs and chemicals that are cytotoxic or interfere with normal hormonal and endocrine relationships have the potential for altering growth and development, but the exposure has to be above the threshold for producing results. Useful information about the effect of environmental toxicants can be obtained by exposing animals during various developmental stages before puberty.

\textbf{Reproductive and Hormonal Effects}

Do exposures during childhood and adolescence from environmental agents having hormonal activity, cytotoxicity, or other effects alter the timing of puberty, alter the maturation of sexual organs including breast development, or affect fertility or the normalcy of spermatogenesis and oogenesis?\textsuperscript{53} Gamete production in both the male and female begins at puberty: spermatogenesis in the male and ovulation in the female. Immature and pubertal rats seem to be more sensitive than adults to testicular toxicity induced by phthalate esters.\textsuperscript{54–56} but the primate does not have the same susceptibility as the rat. The pesticide 1,2-dibromo-3chloropropane affects the immature rat testes more severely than the adult, although the testes of the adult are also affected, as 1,2-dibromo-3chloropropane was banned because occupational exposure in adult males resulted in infertility.\textsuperscript{57,58} Lemasters et al\textsuperscript{59} pointed out that immature animals are not always more sensitive than adults. Fetal Leydig cells are less sensitive than adult Leydig cells to ethane dimethane sulfonate, a known Leydig cell toxicant.\textsuperscript{60} Before the onset of puberty, rats are insensitive to testicular toxicity after exposure to 1,3-dinitrobenzene, a Sertoli cell toxicant, and young adults are less sensitive than mature male adults.\textsuperscript{61} Although spermatogenesis has many similarities among mammalian species, oogenesis varies considerably. Even the number of primordial ova varies in different mammalian species.\textsuperscript{53} Exposure of female rats to 4-vinylcyclohexene diepoxide results in destruction of oocytes in small follicles, and adult rats are less sensitive to the ovotoxicity of this compound.\textsuperscript{62} Would the onset of menopause be affected?
by certain chemical and drug exposures during childhood and adolescence?

Environmental toxicants can affect thyroid development and therefore have a direct impact on neurologic normalcy, because normal thyroid function is crucial for normal central nervous system (CNS) development.63 The most common environmental cause of mental retardation in the world is endemic cretinism as a result of iodine deficiency and is not an environmental toxicity in the usual sense.64,65 Conversely, children’s thyroids have been demonstrated to be more sensitive to the oncogenic effect of external ionizing radiation exposure as well as radioactive iodine localization in the thyroid.66–69

With regard to environmental toxicity, questions have been raised about the effect of organochlorine compounds (polychlorinated biphenyls, dioxins) on thyroid function.70–75 It is difficult to determine the magnitude of the risk of these compounds on thyroid function with the data that are available. The worldwide problem of endemic cretinism from iodine deficiency is without question a real problem. Few studies have evaluated the risk of environmental toxicants on thyroid function and other endocrine organs when exposed during childhood and adolescence.

In the article by Pryor et al53 dealing with reproductive effects, the authors stated, “Although it is the dose that makes the poison, there is no doubt that timing of the exposure may be as important as dose in determining the potential toxicity of a compound to the reproductive system.” This is not a rare statement in the “environmental literature,” but it is not correct. Timing of exposure is important, but it is not important if the actual exposure is below the threshold. If the threshold dose for an effect at any stage of development is not exceeded, then timing is irrelevant.

Do Exposures to Environmental Agents During Childhood and Adolescence Affect the Normalcy of the Adult Immune System?

Although it is true that many chemicals can affect the immune system at high exposures, the question of whether environmental exposures play any role in altering the immune system has not been answered. It has not even been determined whether this is a high priority area to be studied using appropriate animal models. In the review on this subject by Holladay and Smialowicz,76 the authors stated, “The possibility that developmental exposure to immunotoxins may play a role in inducing or exacerbating hypersensitivity or autoimmune responses needs to be investigated in laboratory animals.”

Vulnerability of the Nervous System to Environmental Agents During Childhood and Adolescence

Critical developmental processes during the development of the CNS include 1) the development of the germ layers, 2) neurulation, 3) the closure of the neural tube, 4) neuronal proliferation, 5) neuronal migration, 6) differentiation, 7) synaptogenesis, 8) myelination, and 9) apoptosis. These processes can be studied in animal models.77,78 The first 5 or 6 developmental events have occurred before the period of CNS development during childhood and adolescence. Rice and Barone79 raised the question as to whether schizophrenia, dyslexia, epilepsy, and autism may be caused by environmental influences. Weiss and Landrigan80 speculated that attention-deficit/hyperactivity disorder and Parkinson’s disease may be attributable to exposures that occurred during development. We know that epilepsy can be caused by trauma, infection, and genetic abnormalities and that autism can be produced by an insult to the nervous system very early in embryonic development.81,82 Rice and Barone79 also raise the question as to whether early exposures to toxicants can cause acceleration of age-related decline in CNS function. Some of these questions are amenable to animal studies in both rodents and primates, but these studies are neither easy to perform nor inexpensive, especially in the primate. Two important problems exist with regard to evaluating the risk of neurotoxicity of environmental toxicants at various stages of development using animal models: 1) we do not have precise information that equates various stages of prepartum and postpartum brain development in the human and animal models,83 and 2) we cannot be certain of our ability to identify and recognize the most important neurologic diseases in animal models (eg, attention-deficit/hyperactivity disorder, dyslexia, autism, schizophrenia, Parkinson’s disease).

In the publication by Adams et al,84 a number of important concepts are discussed. The authors indicated, “Inherent in the brain’s protracted period of development is also the phenomenon of neuroplasticity, and the nervous system’s consequent potential for compensation after insult.” This is probably the most difficult area to investigate in both human and animal models. In fact, it is such a difficult area that the authors indicated that it was beyond the scope of their review, but it is an area that could be investigated using animal models. Adams et al85 specifically discussed the topic of the “vulnerability during the adolescent period of development.” They indicated that the brain of the adolescent undergoes “striking” transformations, which is observed in many mammalian species. These regions include areas of remodeling of the prefrontal cortex and other forebrain regions that receive projections of the mesolimbic dopaminergic terminal projections. In addition, there is a decline in the volume of the prefrontal cortex in humans86 and the rat.87 According to Adams et al,84 there is also substantial synapse elimination of presumed glutamnergic excitatory input to the motor cortex,88 whereas dopaminergic input to the prefrontal cortex increases during adolescence to reach levels higher than that seen earlier or later in life.88 Estimates of basal synthesis and turnover of dopamine decline in prefrontal cortex during adolescence in rats, which contrasts with the increase in these measures reported in the nucleus accumbens and striatal dopamine terminal region of adolescent rats.89,90 Maturational events have also been reported in a variety of other areas, including the hippocampus in humans91 and rodents92 and in the hypothalamus.93 Adams et al84 suggested that the adolescent
brain may be especially vulnerable during this period of remodeling and referred to the publications of Salimov et al., who reported the toxic influence of alcohol exposure during this stage of development in the rat. All of these studies are of interest and inform the reader about the developmental processes that may be occurring in the brain of adolescents, but few of these studies reveal whether environmental toxicants have any effects on these developmental processes.

**USE OF ANIMAL STUDIES TO DETERMINE THE ONCOGENIC RISKS OF ENVIRONMENTAL TOXICANTS**

There is a truism in medicine that indicates that children are at greater risk for the induction of cancer than adults from exposure to agents that are mutagenic or have demonstrated oncogenic potential. That is certainly proved for high doses of ionizing radiation and for exposures to radioactive isotopes. Studies of the oncogenic effects of radiation in Hiroshima and Nagasaki demonstrated that children have a higher risk of cancer after whole-body irradiation. However, this increased risk is magnified by the higher proportion of acute lymphocytic leukemia in children and the increased risk of this disease in radiated children. The calculated overall risk of cancer in irradiated children has 95% confidence limits of 1.0 to 1.8.

There are very few cancer studies in animals that expose the animals during a narrow window of time that would be equivalent to childhood or adolescence. Most animal cancer studies using environmental chemicals and drugs involve life-long exposures. The children who were exposed to high doses of ionizing radiation in Hiroshima and Nagasaki did have an increased incidence of leukemia to a greater extent than did the exposed adults. There are studies involving children and adolescents who have been treated for cancer with chemotherapeutic drugs and radiation, and these survivors are at an increased risk of second cancers. However, when they become parents, they do not have offspring with an increased incidence of cancer. Animal studies that would involve only short exposures to proven human carcinogens during the equivalent of childhood or adolescence could be performed. The most appropriate first approach would be to select agents that have been demonstrated to be positive in a life-long animal study or agents that are definitely mutagenic as a first approach to determine the oncogenic sensitivity to environmental toxicants during various developmental stages.

There are extensive reports concerning the oncogenic effect of drugs and chemicals in life-long animal studies. Many of these cancer studies have evaluated environmental chemicals (e.g., organochlorine chemicals, ethylene oxide, pesticides, organic solvents, phthalates, acrylonitriles, trihalomethanes). Most of these cancer studies have used rodents and have also exposed the animals at relatively high exposures.

Most agents that have been demonstrated to be carcinogenic in humans will produce cancer in some laboratory animals but not all laboratory animals, but the converse is not true, namely, that all agents that have been demonstrated to be carcinogenic in animals are carcinogenic in humans. When the MOA of a carcinogenic agent is understood, the relevance of the animal studies can be placed into proper perspective. The following 2 examples are animal studies that indicated a carcinogenic potential, but when the MOA was understood, these agents were determined not to have human carcinogenic potential.

Animal studies have revealed marked differences among species with regard to the oncogenic susceptibility to environmental chemicals and drugs as exemplified by the phthalates. For example, chemicals such as the phthalates induce peroxisome proliferation in the rodent resulting in hepatocarcinogenicity, but there is less responsiveness in primates or human liver cells. There is much discussion and controversy in the literature regarding the mechanism of this carcinogenic effect, namely, the role of increased cell division as the cause of mutation and eventual carcinogenicity. Whether the carcinogenicity is the result of mutation or some other mechanism related to peroxisome proliferation is of interest, but the important aspect of this topic is the marked difference in oncogenic susceptibility in various species. Animal carcinogenicity studies using the phthalates and other chemicals that stimulate the peroxisome proliferation response may not be appropriate models to determine human cancer risks.

The second agent that received much attention is saccharin, which produced bladder cancer when high doses of saccharin were administered to rodents. At high doses, precipitates of saccharin develop in the rodent bladder, producing inflammation and proliferation that ultimately result in bladder tumors. Other experiments indicated that human exposures of saccharin would never result in the situation that occurred in the rodent.

The phthalate and saccharin experiences indicate that when the MOA for carcinogenesis is deterministic (a threshold effect), the risk may not be present at lower exposures and that species differences in metabolism and response may make it difficult to apply animal risks to human risks. Conversely, when the oncogenic effect is related to a mutagenic agent, the theoretical risk for a no-threshold or stochastic effect exists (Table 1).

For determining whether the oncogenic risk for drugs and chemicals is greater during postpartum animal development, protocols would have to be developed during these stages of animal development. Before embarking on the initiation of such testing, it would be important to determine whether these studies would be of benefit for human assessment of oncogenic risks. Pilot studies could be performed using known mutagenic or carcinogenic agents. The increased costs and possible benefits of the new information would have to be evaluated to determine whether we should initiate these developmental oncogenic studies. This is a difficult issue to settle. It might be better to perform research on MOA
of carcinogenic agents and use that information in combination with the usual animal carcinogenicity studies.

CONCLUSION

There are a number of important observations that one could derive from reviewing the literature on using in vivo animal studies for studying the effects of environmental toxicants in developing fetuses and postpartum developing animals. A few federal agencies are requesting protocols to improve animal testing to study the sensitivity of neonatal, infant, and juvenile animals to determine the effects of environmental toxicants. Attempting to expose animals during narrow windows of development is more difficult and more expensive, but because of the differences in animals and humans, infant and juvenile studies have to be designed to correct for these differences. It is true that the multigenerational studies performed in animals are expensive, but they provide information on growth, reproductive capacity, cancer, and lethality, and these studies would have to be performed before embarking on selected targeted studies at various stages of development. There is no question that animal studies can provide valuable information pertaining to human and animal vulnerability to environmental toxicants at different stages of development. If risk estimates and maximum permissible exposures are to be determined, then they have to be based on quality studies in animals and humans using modern pharmacokinetics and toxicokinetic methods, as well as MOA studies. Protocols for such studies are contained in Tables 3 and 5. One useful aspect of animal studies is for corroborating findings reported in epidemiologic studies. Attempts at risk assessment can be made using toxicokinetic data that have been obtained in an animal model and exposure levels of the alleged toxicant and its metabolites that have been determined in the human. Studies determining the mechanism of action in the animal model and whether the same mechanism is functioning in the human would further add to the toxicologist’s ability to estimate human risks. This is not a simple process, and that is why quality epidemiologic studies are so valuable in evaluating human risks and toxicity.

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