The Concern for Developmental Neurotoxicology: Is It Justified and What Is Being Done about It?

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In general, it is believed that the possibility of an adverse developmental outcome following conception is relatively high. In most cases, the cause of the defect is not clear, although exposure to chemical agents at a critical period during development has been proposed to play a significant role. Consequently, regulatory agencies such as the U.S. Environmental Protection Agency (U.S. EPA) have promulgated testing guidelines for assessing developmental neurotoxicity of chemicals in animal testing protocols. Concerns have been expressed about the use of behavioral tests to evaluate chemicals for developmental neurotoxicity, since some investigators believe that they lack predictive validity for human developmental neurotoxicity. Other investigators have indicated that results from such studies are difficult to interpret because of a lack of standardization and sensitivity of the tests. Furthermore, it has been argued that the developing organism is not especially sensitive to chemicals or, if effects are observed, the develop-ing organism is capable of compensating for the deficit. Recent research, however, has adequately demonstrated that developing organisms are especially vulnerable to chemical agents if the exposure occurs at a critical period during development, while other studies have supported the assumption that functional or behavioral effects observed in animal models can be extrapolated to humans. These findings support the routine assessment of chemicals for developmental neurotoxicity using functional end points and suggest that currently available methods could be used to determine more precisely the mechanism of chemical-induced developmental defects. — Environ Health Perspect 103(Suppl 6):147–151 (1995)

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Developmental Neurotoxicology: Scope of the Problem

Adverse effects produced during pregnancy and childhood, or developmental toxicity, are an obvious societal and public health concern. Approximately 50% of all conceptions may result in spontaneous abortion (1), while over a third of postimplantation pregnancies end in embryonic or fetal loss (2). Of the number of live births, approximately 3% have one or more congenital malformations at birth, while another 3% have serious developmental defects by their first birthday (3). Of the children in hospital wards, it has been estimated that approximately 50% have some prenatally acquired malformation (4).

Developmental toxicity, which includes death, growth retardation, and structural or functional deficits (5), can be detected at any time during the lifespan of the organism as a result of perturbation(s) dur-ing gestation or after birth up to sexual maturity. Many times, there is an associa-tion between morphological defects or con-genital malformations and functional alterations. Functional deficits include severe and mild mental retardation, cere-bral palsy, psychoses, epilepsy and abnor-mal neurological development, learning and memory deficits, sensory dysfunction, changes in motor activity, and disrupted maturational milestones.

It has been estimated that about 20% of developmental defects are related to genetic causes, while another 10% appear to be associated with known exogenous factors such as drugs, infections, ionizing radiation, or environmental factors (6). Thus, about 70% of developmental defects have no known cause. It is possible that some of these defects may be related to exposure to environmental agents or to a combination of genetic factors, nutritional deficiencies, drug abuse, tobacco, and/or therapeutic agents. Generally recognized human chemical developmental neurotoxi-cants include drugs of abuse (ethanol, cocaine, heroin, methadone), therapeutic agents (diphenylhydantoin), environmen-tal agents (methylmercury, lead, polychlo-rinated biphenyls), and physical factors (X-ray) (7,8).

Regulation of Developmental Neurotoxicants

The possibility that developmental expo-sure to environmental agents might result in developmental neurotoxicity has led to promulgation of regulations and testing guidelines. Since 1975, Japan and Great Britain have required behavioral testing in animal studies on new drugs (9). Proposed testing guidelines for developmental neuro-toxic effects of drugs and other chemicals have been published by the World Health Organization (WHO) (10). Recently, the U.S. Environmental Protection Agency (U.S. EPA) published revised testing guide-lines for developmental neurotoxicity assessments of toxic substances and pesti-cides (11). U.S. EPA's testing guidelines provide direction for experimental design and dosing as well as information concern-ing the types of assessments that should be performed. Also included in U.S. EPA's testing guidelines are measures of maternal toxicity, growth and physical development of the offspring, developmental landmarks, motor activity, acoustic startle reactivity, learning and memory, and neuropathology.

Although testing guidelines provide direction as to the design and execution of developmental neurotoxicity studies, they do not specify how results should be interpreted or how risk is estimated. The U.S. EPA recently published guidelines for developmental toxicity risk assessment that include guidance for the interpretation of functional deficits following developmental exposure to chemicals (12). Draft neurotoxicology risk assessment guidelines by the U.S. EPA also provide direction for the interpretation of structural and functional changes following developmental exposure to chemicals.

Critique of Developmental Neurotoxicology

The general concern that developmental exposure to chemicals could have adverse effects on the structure or function of the nervous system is based largely on the assumption that the developing nervous system is differentially vulnerable to perturbation. In 1975, Joan Spyker wrote that "it is now fairly well accepted that an individual is more vulnerable to certain adverse factors during the period of development than at any other time in life" (13). Spyker suggested that differences in metabolizing enzymes, rates of excretion, lack of a protective blood-brain barrier, binding of serum and tissue proteins, and toxicodynamic factors could explain increased sensitivity of developing organisms.

More recently, the possibility that the developing organism is differentially sensitive to environmental agents was underscored in a report by the National Research Council (NRC) on pesticides in the diets of infants and children (14). This report repeated a fundamental maxim of pediatric medicine that children are not "little adults" because they have quantitatively different pharmaco- and toxicodynamic capacities relative to adults. Furthermore, the NRC report concluded that there are both quantitative and occasionally qualitative differences in the toxicities of pesticides between children and adults. Additional research to address specific differences in neural, immune, and endocrine system responsiveness of adult and immature animals to several representative classes of pesticides was recommended in the NRC report. The NRC report also recommended that the U.S. EPA continue to revise its developmental neurotoxicity testing guidelines as data are collected from ongoing research and the pesticide registration process.

The routine assessment of chemicals for potential developmental neurotoxicity has, however, been criticized on several grounds. Dews (15), for example, suggested that the assumption that the developing nervous system is generally more sensitive than that of adults to environmental factors is not true. Dews acknowledged that the developing nervous system may be quite sensitive to "a few agents," but the developing nervous system also is quite adaptable to insult. Matson (16) expresses this point by indicating that young children have a vast capacity to compensate for or adapt to early perturbations. Furthermore, in animal studies, it is not uncommon to find that behavioral effects observed during early stages of development dissipate with age. The lack of a persistent effect following developmental exposure to chemicals suggests that no permanent adverse effect was produced and/or that the early effects may have been related to pharmacologic activity of the chemical present at the time of testing.

Another criticism of routine testing for developmental neurotoxicity is that current testing guidelines rely heavily on behavioral assessments of sensory, motor, and cognitive function. Such tests are regarded by some as labor intensive, expensive to perform, and difficult to interpret (17). Furthermore, there is often a lack of standardization of testing procedures across laboratories, making comparison of results difficult. Lochry (17) also indicated that there is little evidence to suggest that behavioral tests yield information about developmental neurotoxicity that could not be obtained from other end points of developmental toxicity, including embryo/fetal death, fetal malformation, and delayed development. Furthermore, Lochry suggested that behavioral effects would probably not occur at doses that are not also maternally toxic.

Dews (15) also expressed concern that the predictive value of rodent screens for developmental toxicants in humans has not been adequately demonstrated. The NRC report also acknowledged that extrapolation of toxicity data from adult and adolescent laboratory animals to young humans may be imprecise and careful attention to interspecies differences is necessary (14). Dews (15) suggested that basic research on the mechanisms and control of development is needed before routine testing is required. Dews also suggested that assessment of developmental toxicity should be postponed until more is known about the mechanism of action of the chemical under investigation. Based on knowledge derived from such mechanistic studies, more appropriate and focused end points could be selected for subsequent developmental assessments.

To Test or Not to Test

There is concern that inappropriate toxicological assessments of chemicals using behavioral end points might unfairly impede the development of an otherwise useful therapeutic, industrial, or environmental agent (17). Given the expense of such assessments and the potential loss of a useful product to society, such concerns must be considered. In this section, the points concerning the difficulties of developmental neurotoxicity testing raised by Dews (15) and Lochry (17) are discussed in greater detail. These areas of concern are that a) developing organisms are not especially vulnerable, b) developing organisms are resilient to perturbation, c) behavioral tests provide no advantage, d) maternal toxicity is a confounding variable, and e) poor predictive value. The objective of this discussion is to clarify important testing and data interpretation issues and emphasize certain concepts that underlie the application of behavioral testing in developmental studies.

Vulnerability of Developing Organisms

Dews (15) asserted that it is not true that the developing nervous system is more sensitive to all aspects of the environment. As stated, Dews (15) is correct, i.e., the developing nervous system is not always more sensitive than the mature nervous system to environmental perturbation. A case in point is acrylamide, which produces a peripheral neuropathy in a wide range of species including humans (18). Edwards (19), however, found that acrylamide given to pregnant rats either in a single dose or in the diet had no adverse effect on the offspring, even at doses that produced neuropathy in the mothers. Acrylamide was found to pass the placental barrier, suggesting that the developing rat is not especially vulnerable to the toxic effects of acrylamide during the early period of growth and development. Although Agrawal and Squibb (20) found that prenatal exposure to acrylamide had a transient effect on the number of dopamine binding sites in the striatum, unpublished behavioral studies found no associated functional deficits in acrylamide-exposed rats.

The insensitivity of the developing organism to chemicals such as acrylamide, however, appears the exception rather than the rule. That the developing organism is sensitive to a wide range of chemical agents has been clearly documented. One of the most comprehensive reviews of this area can be found in a book edited by Riley and Vorhees (7). It is clear that tests of behavioral function are sufficient to detect effects following developmental exposure to major classes of drugs, including alcohols, analgesics, stimulants, neuroleptics, antimitotics, anxiolytics, antidepressants, barbiturates, and drugs of abuse; environmental chemicals, including metals, pesticides, and industrial solvents; physical factors, including X-rays; and physiological conditions that may result directly or indirectly from chemical or environmental factors, including hypoxia. There are numerous cases where exposure to chemicals during development have significant neurotoxicity, whereas the same chemicals produce little or no neurotoxicity in adults. Lead, for example, produces significant neurotoxicity if exposure occurs during development but little if any effect in adults (7).

Although the literature supports the conclusion that the developing organism is not always more sensitive to environmental factors than adults, there is clear evidence that the developing organism is differentially sensitive to environmental factors relative to the adult. One reason for this differential sensitivity is that the developing nervous system undergoes defined periods of neurogenesis and migration, synaptogenesis, gliogenesis, and myelination, events that have reached a relative steady-state in the adult organism. Rodier (21,22) was among the first to indicate that there are specific times during maturation of the central nervous system when chemical-induced perturbation can cause neurotoxicity. Exposure to a chemical during a critical period of development will produce neurotoxicity, while exposure to the same agent at another less critical period of development will have little or no effect. A recent example of this principle can be found in a paper by Balduini and colleagues (23), who exposed rats to methylazomethanol (MAM) at different times during gestation. In this study, pregnant rats were injected with MAM on gestational days 14, 15, 16, 17, 18, or 19 and the offspring tested behaviorally at various ages. These investigators found that gestational exposure to MAM caused selective deficits in learning and motor activity that

were dependent on the time of administration. If MAM was given on day 18 or 19 of gestation, learning deficits were observed; injection of MAM on any other day gestationally had no significant effect on learning. With regard to motor activity, the presence and direction of effect (increased or decreased activity) was dependent on the day of gestational exposure. Adults exposed to single doses of MAM do not display persistent behavioral effects. The study by Balduini et al. (23) indicates that effects following developmental exposures cannot with any degree of certainty be predicted from experiments done with adults and that the presence of a neurotoxicological effect can depend on the day that exposure occurs. Testing guidelines for developmental neurotoxicity (11) provide for both pre- and postnatal exposure to ensure that a critical period of development is not missed.

Resilience of Developing Organisms

The second issue, raised by Dews (15) and Matson (16), concerned the resiliency of the developing organism to environmental insult. It is sometimes observed in developmental neurotoxicological studies that effects observed at an early age can dissipate with age, suggesting that no long-term, persistent effect has been produced. It has been observed, however, that in cases where recovery of function appears to occur with age, there is still some evidence of a residual deficit that can be determined with subsequent testing or following pharmacological challenge. Harry and Tilson (24) dosed rats with triethyl tin (TET) on day 5 postpartum and assessed developmental neurotoxicity using behavioral tests for up to 90 days of age. Rats exposed to TET were hyperactive at 21, 28, and 60 days of age. However, TET-exposed animals tended to be hypoactive relative to controls at 90 days of age. One interpretation of these data is that the motor deficit seen at earlier ages was not permanent and that adult animals exposed developmentally to TET had recovered. In a subsequent experiment, however, Harry and Tilson (25) challenged adult rats exposed to TET developmentally with apomorphine, a chemical that stimulates motor activity by activating dopamine receptors directly, and found that TET-exposed animals were significantly more sensitive to the stimulant effects of apomorphine than controls. Subsequent neurochemical experiments found a persistent change in dopamine binding in the striatum of

TET-exposed animals. These studies indicate that apparent recovery of function that occurs with age should be interpreted cautiously in developmental studies. Long-term, persistent deficits in nervous system function cannot be ruled out in studies where apparent recovery is observed.

Another point that should be considered is that developmental exposure to neurotoxicants might interact with the aging process. Barone and colleagues (26) exposed rats to triethyl tin and found that the effects on learning in younger animals were exacerbated as the animals aged. In this study, rats dosed with triethyl tin showed marginal deficits at 3 months of age, no deficits at 12 months of age, and significant cognitive impairment relative to age-matched controls at 24 months of age. Delayed onset neurotoxicity has also been reported in monkeys exposed developmentally to methylmercury (27).

Advantage of Traditional Behavioral Tests

Lochry (17) argued that the behavioral measures used in developmental neurotoxicity studies are not more sensitive than other, more traditional indicators of toxicity. Faber and O'Donoghue (28), for example, identified 41 developmental neurotoxicants from Shepard's catalogue of teratogenic agents (4) and found that 37 showed positive effects in the Chernoff/Kavlock assay, which measures the number of pups per litter, the birth weight, and viability of pups up to 3 or 4 days postnatally. These findings suggested that the Chernoff/Kavlock assay may be sufficient to detect potential developmental neurotoxicants and that more costly behavioral tests may not be necessary for screening. In a followup to the Faber and O'Donoghue report, Goldey et al. (29) evaluated studies in which 126 compounds were assessed for developmental neurotoxicity. Studies in which all or part of the Chernoff/Kavlock screen had been used and behavioral testing had been performed on the same compound were selected for further analysis. Goldey et al. (29) found that of the 126 agents evaluated, 110 were found to be developmental neurotoxicants using behavioral tests. Of those 110 compounds, 72 had been tested on one of the measures in the Chernoff/Kavlock assay; more than a third of them had been found to be negative. The developmental neurotoxicants that were negative in the Chernoff/Kavlock assay included several drugs, food additives, and solvents. These observations

indicate that behavioral tests afford a level of sensitivity needed in developmental neurotoxicology studies.

Lochry (*17*) also suggested that the interpretation of behavioral results may be problematic in studies involving the presence of maternal toxicity. Adverse developmental neurotoxic effects can often result at doses that cause minimal maternal toxicity (e.g., < 20% reduction in weight gain during gestation and lactation), and such effects should not be discounted as secondary to maternal toxicity. Effects seen at doses causing excessive maternal toxicity (> 20% reduction in weight gain) are problematic and are difficult to interpret. At present, it cannot be assumed that developmental effects observed at doses that cause minimal maternal toxicity result only from maternal toxicity. Instead, it may be that the mother and developing organism are equally sensitive to that dose. Furthermore, whether or not developmental neurotoxic effects are secondary to maternal toxicity, the maternal effects may be reversible, while effects on the offspring may be persistent.

Predictive Value of Animal Behavioral Tests

One major concern about the use of behavioral tests in animal studies is that they may have little relationship to what is measured in humans. This issue was specifically addressed in a workshop on the qualitative and quantitative comparability of human and animal developmental neurotoxicity (*30*). One of the working groups at that meeting assessed the comparability of measures of developmental neurotoxicity produced by several known human developmental neurotoxicants (*31*). The working group identified at least four criteria for animal models of human developmental neurotoxicity including *a*) the developmental profile of the behavior or function in animals should resemble that observed in humans, *b*) the neural substrate underlying the function in animals should resemble that found in humans, *c*) there should be operational similarities in the assessment of the function in animals and humans, and *d*) treatments that affect function in animals should affect similar functions in humans. Based on these criteria, the working group identified five functional domains that serve as the basis for comparability of effects between humans and animals, including sensory, motivational/ arousal, cognitive, motor, and social functions.

The effects of several known developmental neurotoxicants in humans and animals were then compared based on a review of the existing literature. A good degree of qualitative correspondence was found in humans and animals for the developmental neurotoxicity of methylmercury, anticonvulsants such as phenytoin, lead, ethanol, polychlorinated biphenyls, and ionizing radiation. The working group also observed that the degree of quantitative comparability, however, was not as good, possibly because of limited dose–response comparisons, incomplete or missing information concerning the actual internal dose relative to the administered dose, and insufficient information concerning the most sensitive end points. One conclusion from developmental neurotoxicity studies using animal models is that the degree of cross-species comparability is facilitated by close attention to using end points for which there are comparable developmental profiles and common underlying neural substrates. Good quantitative comparisons between animal models and humans will be improved following development of mathematical and physiologically based dose–response models.

Summary and Conclusions

The area of developmental neurotoxicology has evolved rapidly over the last several years. Initially it was shown that developmental exposure to environmental agents can affect behavioral measures in the offspring. Subsequent experiments have confirmed that the developing organism is sensitive to a wide range of chemical factors. The potential threat of developmental neurotoxicity following exposure to chemicals has led to the promulgation of regulatory guidelines for the preapproval of chemicals. The potential expense of such tests, however, has elicited concerns about the general sensitivity of the developing organism, sensitivity of functional tests, and interpretation of data generated in behavioral developmental studies.

Research, however, has shown that the developing organism can be highly sensitive to a wide range of environmental factors if exposure occurs at a critical period of nervous system development. Furthermore, standardized behavioral tests are now available to assess the developmental neurotoxicity of chemicals and such tests have been successful in detecting and quantifying the effects of human developmental neurotoxicants in animal models.

Concerns about the cost effectiveness of developmental neurotoxicity studies have been raised in recent years. Advances in developmental neurobiology and experience with standardized testing protocols in neurotoxicology, however, suggest that well-designed studies selecting appropriate behavioral tests can provide useful information about potential neurotoxicity of chemical agents. In addition, behavioral measures in conjunction with appropriate neurochemical and anatomical end points can be used to address hypothesis-driven questions concerning the site and mechanism of action of developmental neurotoxicants.

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