

# Workshop to Identify Critical Windows of Exposure for Children's Health: Cardiovascular and Endocrine Work Group Summary

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The work group on cardiovascular and endocrine effects was asked to review the current state of knowledge about children's windows of vulnerability to developmental toxicants and to recommend how that information may be used to improve risk assessment and public health. We considered differences between structural defects, where periods of vulnerability are rather well defined, and functional defects, where periods of vulnerability are quite elusive. *Key words:* developmental toxicity, endocrine, heart, teratogen. — *Environ Health Perspect* 108(suppl 3):569–571 (2000). <http://ehpnet1.niehs.nih.gov/docs/2000/suppl-3/569-571barr/abstract.html>

The work group on cardiovascular and endocrine effects reviewed the current state of knowledge about children's windows of vulnerability to developmental toxicants and made recommendations for how that information may be used to improve risk assessment and public health. The three background papers by Hoet and Reusens (1), Osmond (2), and Sadler (3) form an essential base for our ensuing discussions, and little of the information in those articles will be reiterated here. We cite only a few references in support of particular points.

There are windows of vulnerability for structural alterations; whereas the inception of vulnerability is often poorly defined, the end of vulnerability is usually well defined. The principal reason for uncertainty about the initiation of the vulnerable period is that cascades of gene action and development exist, but their sequences and timing are not yet fully worked out. The closer we look, the more evident it is that often there is not a uniform response within a given "window." There may be peaks and valleys, or various slopes of sensitivity, within the period of vulnerability. Although there is increased recognition of the role of regulatory genes and gene cascades, it is also evident that the same gene has different roles and timing in different tissues.

We believe that the question of who might be susceptible to teratogenesis is largely determined by the genome. Genomic influence on the expression of teratogenesis has been repeatedly reported in laboratory animals (4), and at least one recent study notes the interaction between a teratogen and a specific gene in the mouse (5). There is anecdotal evidence that teratogen–gene interaction occurs in

humans as well (6,7). Drugs and genes may not only interact, but one may mimic the other [e.g., sonic hedgehog and retinoids (3)]. In the future, epidemiological and genetic study combined may identify subgroups of children who are more vulnerable through environment–genome interaction.

For humans, the definition of windows of vulnerability for functional aberrations is more elusive than for structural anomalies. Currently, windows of vulnerability are considered the period of liability that extends from at least the second trimester of gestation, and probably the first trimester, onward. Preconceptional liability remains more speculative.

The term "developmental toxicant" is used rather loosely to encompass true teratogens, embryotoxins, and fetotoxins. Recognized human teratogens cause rather specific syndromes, although it is possible that environmental agents may also act to interfere with development in such a way as to produce single malformations or particular functional deficits. For prenatal exposure, we restricted our consideration to maternal exposure; paternally mediated, environmental effects were considered unsubstantiated. It was noted that additive or synergistic exposure effects are largely unexplored, particularly in humans. There is abundant evidence that nutrition plays a key role in the etiology and prevention of birth defects and in the consequent health of an individual. In the case of the conceptus, the route and dynamics of nutrition change during gestation. Initially by simple diffusion, nutrition is then controlled by the yolk sac (in the rodent) and ultimately by the chorio-allantoic placenta.

## Cardiovascular

We distinguished between structure and function in the cardiovascular system. In this system, a structurally abnormal heart may have abnormal function. For this system, "functional abnormality" refers more specifically to aberrant function or disease of the structurally normal heart.

### Cardiovascular Structure

Each particular defect will have its own critical window of vulnerability. As the details of cardiogenesis become better understood, these periods of vulnerability will be better defined. Further investigation in this area should be supported. More information on possible environmental interactions with gene cascades in cardiogenesis would enhance our ability to detect potential teratogens.

However, we believe there is already enough known about cardiovascular development that it is no longer tenable to lump heart defects together as though they were a single entity. Rather they should be considered individual defects or mechanistically related groups of defects (8). As for the particular mechanisms involved in cardiogenesis, neural crest migration, endocardial cushion development, and mesenchymal–myocardial transformation would seem to be the most important areas on which to concentrate. Alterations of hemodynamics may affect structural cardiovascular development, but associating this with environmental exposure is uncertain. In distinction to other organs such as the brain, apoptosis appears to have a very limited role in normal cardiogenesis (only in the atrial septum). An appropriate

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strategy for epidemiological study would focus on the most common structural lesions as individual entities. The detection of rare lesions associated with rare exposures (e.g., lithium and Ebstein malformation) could be left to the "alert practitioner."

Epidemiological studies to date have largely failed to verify environmental agents as causes of cardiac defects, but they have raised some question about particular exposures and particular defects (9). Several past surveys have encountered problems with sample size, misclassification of exposure, and inappropriate grouping of outcomes. More refined data on actual timing of exposure would be helpful in determining any cause-effect relationships for specific defects. For example, the recurring controversy over a putative link between steroid exposure and cardiac malformation might be resolved by more precise information on the timing of exposure. Nonetheless, we recommend not narrowing the current definition of the window of vulnerability to cardiac malformation (fertilization to 8 weeks, most lesions at 3–5 weeks).

We recommend that patent ductus arteriosus (PDA) should be treated separately from true structural defects of the heart and great vessels. Because prostaglandin inhibition affects ductal closure, we think that PDA is better viewed as a functional defect.

Current protocols, for both laboratory animal and human investigation, largely miss what we believe is important information. Standard developmental toxicology studies should include not only tabulation of early losses (miscarriage and resorption) but a characterization of their morphology or reason for loss. This would involve the examination of conceptuses before their *in utero* death or, in the case of humans, collecting sonographic and fetal autopsy data. An understanding of why a conceptus died would surely enhance our ability to comprehend mechanisms of developmental toxicity.

### Cardiovascular Function

**Acute effects.** Cardioactive drugs can affect cardiac function of the fetus, especially in the third trimester, but it is not yet clear what, if any, long-range effects may result. Studies to collect this information should be supported. Some suggestive lines of evidence for functional impairment of cardiac function from fetal exposure include four that we mention here. First, cortisol inhibits cardiac myocyte replication and simulates the postnatal growth pattern (hypertrophy) of the heart (10). Second, in rats, nicotine affects sinoatrial reactivity to hypoxia without impairing cardiac conduction *per se*. This possibly explains the increased vulnerability of infants of mothers who smoke to sudden infant death syndrome (11). Third, thyroid status

has been linked to muscle development. Thyroid hormone is required for proper development of the adult phenotype of myosin heavy chain of muscle (12). Although the experiment in rats was conducted on skeletal muscle, it may apply to cardiac muscle as well. Fourth, maternal lupus erythematosus is associated with fetal heart block; however, the biological basis for this observation remains obscure (13).

The role of the adrenals and kidneys, through their control of fetal blood pressure, in affecting development (e.g., weight and hemodynamics) is being explored (14), but long-term effects are largely unexplored. Cardiomyopathy may be genetic, infectious, or consequent to structural defects. Whether it might also be consequent to chemical exposure is unknown.

**Delayed effects.** We reviewed with interest the programming hypothesis that early changes lead to late effects (2). Low birth weight has been associated with an increased risk of developing hypertension, coronary artery disease, and diabetes—but why? The principal gap in knowledge is uncertainty about the etiologies and mechanisms of intrauterine growth restriction (IUGR). Morphological and functional studies of the placenta are underutilized in tackling the problem of IUGR. Early protein deprivation also appears to have long-term effects (1), but we need to understand more about basic mechanisms to generate better hypotheses. Maternal birth weight correlates with her infant's birth weight (2)—but again, why? Certainly, we need a better understanding of possible links between lifestyle factors and cardiovascular effects [e.g., smoking and fetal cardiac myohyperplasia (15) or possible liability to later cardiovascular disease; cocaine exposure and possible liability to later cardiovascular disease].

We must distinguish between prematurity and IUGR because the mechanisms involved are quite different. There should also be differentiation between IUGR with and without postnatal catch-up growth because the former is likely to represent interference with late gestational nutrition, whereas the latter may stem from early gestational insult. Assessment of prenatal and postnatal growth should include weight, length (height), and head circumference as a minimum. More extensive growth profiling of human fetuses, including limb and visceral measurements, has shown that reasonably distinct patterns of growth aberration can be correlated with etiologic factors (16).

## Endocrine

### Endocrine Structure

To the best of our knowledge, structural defects of the endocrine glands (e.g., agenesis

of any gland, annular pancreas, pancreatic nesidioblastosis, lingual thyroid, and aberrant adrenal nodules) have not been related to environmental exposures. Goiter, which does have environmental exposure antecedents, is viewed as a functional defect.

### Endocrine Function

**Pancreas.** Low birth weight appears to be associated with an increased risk for later development of diabetes mellitus (2). Why this is so remains a mystery. Maternal diabetes has been associated with a variety of different patterns of defects. We wondered if the particular, and usually nonoverlapping, patterns of defects are determined by interaction between diabetes and the genome or other environmental factors. Hyperglycemia in gestation can increase birth weight (obesity and macrosomia), but brain weight is normal for age (17). Diabetes mellitus may also be associated with IUGR, particularly with the co-occurrence of structural defects. The long-term consequences of these aberrations merit continued study.

**Thyroid.** Goiter has long been associated with dietary iodine deficiency. Thyroid function is amenable to transplacental modification (e.g., treatment of fetal thyrotoxicosis). This signals a warning against nontherapeutic exposure to thyroactive drugs and chemicals. Thyroid function also affects fetal growth and perhaps cardiovascular development. Thyroid status has been linked to skeletal, and perhaps cardiac, muscle development (12).

**Adrenal.** There is extensive literature on the development of the adrenal glands and their cytomorphology. Nonetheless, we need to know more about fetal adrenal function and response to environmental factors, especially stress. There are reports that fetal stress (e.g., hypoxia and chorioamnionitis) is associated with increased bulk of the fetal adrenal cortex (18,19). However, the functional implications of these observations are unknown. Rudolph et al. (10) demonstrated the effect of cortisone on cardiomyocyte replication. Exposure to androgenic steroids affects genital development. The experience with diethylstilbestrol leading to both morphologic alteration and increased risk of tumor formation has raised concern about the deleterious effects of other steroids.

**Pituitary.** Pituitary function is affected in a number of central nervous system malformations. The importance of the complex interaction among hormones (i.e., control and feedback functions) in the evolution of other structural abnormalities has not been adequately studied. Aberrations of pituitary axis function are of considerable importance in the practice of pediatric endocrinology. To what extent these aberrations may be due to

prenatal or postnatal environmental exposure is uncertain.

**Parathyroid.** Calcification abnormalities are difficult to interpret as an endocrinological function, as they may involve mechanisms other than parathyroid function. We know of no suggestion of environmental induction of abnormal parathyroid function, but we did not fully explore the issue.

**Thymus, pineal, and gonads.** We did not discuss the function of these organs, or their discussion was deferred to other workshop sessions.

## Conclusions

Many questions remain as to how early in gestation the various endocrine systems are active and important to the developmental process. The fetal window of vulnerability to endocrine dysfunction appears to be primarily the third trimester, but the second trimester is undoubtedly also important. Damage from first-trimester exposure is more problematic. Endocrine effects from environmental exposure could occur at any postnatal age, and although a number of such effects may be reversible, others may be permanent.

Pharmacological/chemical disruption of endocrine function is possible, but the full range of possibility has yet to be explored. Are effects only during exposure? What is the potential for recovery? Are there long-range effects? Are there permanent effects (e.g., on brain development or function; or on immune or reproductive function)? Screening for endocrine disruptors should include assessment of prenatal development. Diurnal and postcibum variation in hormone levels can complicate the collection and interpretation of data.

High-priority targets for research should include: *a*) thyroid, which is affected by environmental exposures (e.g., polychlorinated biphenyls); *b*) pancreas, especially  $\beta$ -cell function and its interaction with nutrition; *c*) adrenal stress response and cortisol production; and *d*) pituitary and the neuroendocrine axis.

We considered the appropriateness of various animal models for studying endocrine effects. For thyroid, commonly used animal models should be quite useful. For pancreatic function, rats are not appropriate as animal models because they can regenerate  $\beta$ -cells. For other hormones, there is insufficient information to comment, and although it was noted that steroids are teratogenic in certain strains of laboratory animals, similar patterns of teratogenicity have not been confirmed in humans.

We expressed a number of qualifications about extrapolating from laboratory animal data to the human situation. We agreed that uniformity of effects across species carries greater weight for predicting human effects than does abnormality occurring in only one species. When comparing studies in different species, it is essential to match for the comparable stage of development in each species. Animal models should be chosen for as much pharmacokinetic and metabolic similarity to humans as possible.

We noted that there are distinctions to be made among statistical, biological, and social significance of a finding of developmental toxicity. Although the maxim that an ounce of prevention is worth a pound of cure is undoubtedly true, in setting priorities for regulatory action, greater weight should be placed on those agents with proven developmental toxicity as opposed to those with only hypothetical toxicity. It is unlikely that there are currently any undetected potent developmental toxicants; this was thought to apply more certainly for structural aberrations but also for functional aberrations. Rather, the possibility is greater that there are undetected low-level toxicants and agents with delayed effects. We urge support of long-range studies linking relatively uncommon and/or delayed outcomes to exposure; these studies will be important to our understanding of the particular vulnerability of children to developmental toxicants.

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