Workshop on Perinatal Exposure to Dioxin-like Compounds. VI. Role of Biomarkers

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Studies of perinatal exposures to dioxin-like compounds (DLCs), coplanar polycyclic halogenated aromatics whose prototype is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), have employed a variety of outcome measures to investigate effects on the reproductive/developmental, endocrine, immune, and neurobehavioral systems. The effects include infertility, growth retardation, fetal loss, changed sexual differentiation, reduced cognitive/motor function, dermatologic and other ectodermal effects, and decreased immune response. Significant biomarkers have included sperm count; CD4/CD8 ratio; and levels of testosterone, T4, and dopamine. Using specific dioxin or PCB congeners, these and other markers were used to investigate the mechanisms of the observed effects. The DLCs, which include some PCB congeners, are characterized by high-affinity binding to the Ah receptor; most biological effects are thought to be mediated by the ligand–Ah receptor complex. Other PCB congeners have low affinity for the Ah receptor, and operate by non-Ah receptor mechanisms. The biologic activity of a PCB mixture is the sum of the agonist and antagonist activities of the different constituents in the mixture. Animal studies with specific PCB congeners can help to clarify these activities. With similar approaches, biologic markers of effect can be developed and applied in epidemiologic studies to monitor for, and predict, adverse effects in humans. — Environ Health Perspect 103(Suppl 2):161–167 (1995)

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Introduction

A biologic marker, or biomarker, is generally defined as any functional or biochemical change in a cell or tissue that is indicative of exposure, response or potential susceptibility to a drug or an environmental agent (Figure 1) (1). Generally, biomarkers are laboratory-based measures that are intended to increase the sensitivity, specificity, or power of a study to establish a causal association between an exposure and an outcome, and/or provide useful information about the mechanisms involved in exposures, responses, or susceptibilities. A workshop reviewed the evidence that perinatal exposures to these compounds may lead to adverse health outcomes.

Biomarkers of Exposure

Many of the dioxins, dibenzofurans and PCBs resist biodegradation and, because they are lipophilic, concentrate in adipose tissues. Tissue concentrations of these parent compounds serve as good measures of environmental exposures, and residue levels in body fat or in lipid fractions of breast milk or blood are used to quantify this exposure. A vast literature describes the levels of organochlorine residues found in body fluids or tissues of animals and humans, and documents the increased concentration of these contaminants in the fat of animals at successively higher trophic levels of the food chain.

The residue profile (nature and levels of residues) in tissues is unlikely to be identical to the composition of the original environmental mixture because different individual components of this mixture may be taken up and metabolized at different rates by the organism. For example, octachlorodioxins are absorbed via the gut much more poorly than tetrachlorodioxins, and consequently are under-represented in the residue profile in tissues.

Biomarkers of Effect

The attractiveness of biomarkers of effect as surrogates of disease is that they can provide an indication of increased likelihood of a disease outcome before clinical symptoms appear. Ideally, the application of such markers is simple, quick, and inexpensive and can trigger early preventive measures. Such measures can bring major public health benefits if they are directed to diseases of long latency, particularly those that are either life-threatening (e.g., cancer), cut off productive life years (e.g., reproductive/developmental, neurobehavioral...

| Exposure | Internal dose (tissue levels) | Biologically effective dose (at target) | Early biologic effect | Clinical disease |
|----------|-----------------------------|----------------------------------------|----------------------|----------------|
| DLCs: (TCDD, dioxins, dibenzofurans, some PCBs) | levels in tissues | levels at target | sperm count | infertility |
| | | | T4 level | endocrine? |
| | | | dopamine levels | behavior? |
| | | | CD4/CD8 ratio | immunity? |

Figure 1. Schematic continuum of biologic markers.

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disorders), or have great medical or social costs (e.g., Alzheimer’s disease). Thus, biomarkers of effect
- signal that an adverse clinical effect has the potential to occur;
- identify exposed or responsive individuals;
- help to develop preventive measures by giving more detailed information (e.g., mechanism) on the health problem;
- help to develop regulatory strategies for disease prevention (e.g., regulations based on biomarker end points).

Identifying Biomarkers of Effect and Susceptibility: Understanding Disease Mechanism(s)

A key to identifying a suitable biomarker of effect or susceptibility is having a good understanding of the mechanism(s) by which the adverse health outcome arises. In the medical model, the adverse outcome is disease, and is accompanied by the disease pathway paradigm: predisposing conditions, early warning symptoms, preclinical indicators and, finally, clinical symptoms. Some adverse outcomes (e.g., neurobehavioral) are not easily characterized as a series of stages on a pathway to an abnormal pathology or disease, and may not fit this model; instead they may represent an impaired capacity within what is considered the normal range (e.g., lowered IQ; see “Issues”).

Optimally, a marker of exposure indicates that the agent has access to the causal mechanism; an effect marker is an element of this mechanism; and a marker of susceptibility bears some relationship to this mechanism. Rarely, however, is our knowledge of disease mechanisms sufficient for us to declare that a marker is strongly associated with either disease susceptibility or outcome. Instead, we place a marker on the continuum of biomarkers (exposure, early biological effect, or effect) depending upon the weight of evidence that connects the biologic measure with the disease outcome. The predictive value of any marker of effect depends upon the strength of evidence that links it with the adverse health outcome.

Most markers lack a strong enough association with disease outcome to be classified as markers of effect; consequently, if their response is found to vary proportionally with dose, they are classified as either markers of exposure or as markers of early biologic effect. Chromosomal aberrations, for example, are considered markers of early biologic effect rather than of cancer because our knowledge of their specific role in cancer causation is limited (2). Lowered sperm count, on the other hand, is a marker for infertility because it has a demonstrated relationship with this outcome (3–5).

Mechanism(s) of Action of Dioxin-like Compounds (DLCs): The Ah Receptor

Most cells contain a cytoplasmic protein, called the Ah receptor, which has a high affinity for TCDD (6). Other compounds that bind to the intracellular Ah receptor are termed dioxin-like compounds (DLCs). These include representatives of the coplanar, halogen-substituted multiring structures such as the PCDDs, PCDFs, and some of the coplanar PCB congeners. Most PCB congeners that are noncoplanar are reported to have low affinity for the Ah receptor.

The TCDD–Ah receptor complex is believed to mediate many of TCDD’s observed effects. The DLC–Ah receptor complexes produce a characteristic spectrum of responses in animal models. The potency of DLCs as effectors of teratogenicity, carcinogenicity, immunotoxicity, and biochemical changes (drug-metabolizing enzymes and growth factor pathways) strongly correlates with their binding affinity to the Ah receptor (7–10).

The binding of a molecule of ligand to the cytoplasmic Ah receptor results in activation of the receptor and translocation to the nucleus. This is a complex event in which heat shock proteins dissociate from the receptor and another protein, Ah receptor nuclear translocator (ARNT) associates with the receptor. The ligand–AhR–ARNT complex acts as a transcriptional enhancer: it binds to regulatory regions on DNA, upstream of the CYP1A1 gene and other dioxin-responsive genes, and increases the transcription of these genes (11,12). The altered transcription of genes in the ligand–Ah-responsive gene battery is believed to be the mechanism by which DLCs produce their biological effects (13).

Non-DLCs operate by unknown mechanisms. Some non-DLCs are more active in producing certain biologic responses than related DLCs. For example, a noncoplanar PCB congener, PCB 28, is more active in altering dopamine levels in the forebrain of rats than dioxin-like coplanar PCB congeners (14). This suggests that the neurochemical response is mediated by a non-Ah receptor mechanism, which is consistent with the reported absence of the Ah receptor from neurons (15).

The discussion below summarizes studies that employ a variety of markers of effect to investigate disease outcomes. Only a few are directly linked to outcome (e.g., sperm count and infertility). Most markers described below signal changes in functions but are not yet strongly linked to specific adverse outcomes. For example, CD4/CD8 ratios, or levels of T4 or dopamine, are now considered markers of early biologic effects. Future studies may link them to adverse outcomes such as immune system depression or motor/cognitive deficits. CYP1A1 activity is also considered a marker of early biologic effect (DLC bound to the Ah receptor), lacking a known association with disease.
Biomarkers and Perinatal Studies

Perinatal exposures encompass prenatal (transplacental) as well as postnatal (lactational) exposures. What is unusual about the application of biomarkers to the study of perinatal exposures?

Perinatal exposures are unique in that they impact upon organ systems that are in varying stages of development. The responses exhibited by these developing systems, for example, to perinatal exposures to DLCs are influenced by several factors including: the chemical nature of the agents; the timing and duration of the exposure during the developmental period; and the interrelationships between the developing systems. The broad range of responses that DLCs produce in multiple developing organ systems suggests that DLCs act via a central mechanism (e.g., hormonal) and that the developing systems interact.

In 1968 researchers discovered ectodermal abnormalities and cognitive/motor deficits among children who had accidental perinatal exposure to DLCs and non-DLCs in cooking oil (16–18). Since then, researchers have sought to do the following:

- characterize the full range of clinical effects arising from perinatal exposures to these compounds;
- identify the mechanism(s) of action of these compounds in causing seemingly disparate effects;
- understand the interrelationships of these effects in developing systems;
- investigate the mechanism(s) and the interrelationships between these complex clinical outcomes using biomarkers of exposure, susceptibility and effect.

Animal Studies

As reported in the workshop, perinatal exposures to DLCs in animal studies have provided information on effects in several developing systems, including the reproductive, endocrine, neurobehavioral, and immune systems. Adverse reproductive or developmental effects included reduced fertility in primates (19), endometriosis in primates (20), altered sexual differentiation in male and female rats (21–23), reduced sperm count in rats (24), and growth retardation in primates and rats (19,25). In the endocrine system, the effects included reduced T4 levels in the prefrontal cortex of rats (25), reduced testosterone levels in rats (22,23), and altered dopamine levels in the forebrain of primates and rats (14,26,27). Neurobehavioral effects included spatial learning/memory deficits in primates and rats (27–30).

In the immune system, DLCs act as immunosuppressors and immunoenhancers in rats and mice (31), affecting mainly T-cell responses (31–33), although effects on other cells of the immune system are reported (34–36). There is suggestive evidence that DLCs affect the primary immune response by changing the CD4/CD8 ratio (the ratio of helper T to suppressor T cells) or the ratio of other lymphocyte subpopulations (31–33,37–40).

For several of the Effects seen in animal studies, there appear to be corresponding outcomes in humans: reductions in infant height and primary immune response in Infants whose mothers had breast milk with high PCB levels (41,42); and reductions in cognitive or motor functions in infants whose mothers ingested PCB-containing cooking oil or fish during pregnancy or lactation (16–18,43,44).

Biomarkers and Mechanisms

The wide range of effects seen in different organ systems of animals and humans underlies the current working hypotheses about the mechanisms of action of DLCs and non-DLCs (see Workshop Summary Report, this issue) including the following:

- Biologic effects of DLCs are mediated by their binding to the Ah receptor, while the mechanisms of action of non-DLCs are not well understood;
- DLCs act as "environmental hormones," disrupting normal endocrine functions.
- Pluripotent effects on the reproductive/developmental, endocrine, neurobehavioral, and immune systems reflect the hormone-like activities of DLCs and non-DLCs as well as the highly integrative and interactive nature of these systems.
- DLCs, as hormones, affect the differentiation of cells in various systems in a tissue-, cell-, and stage-specific manner; effects consequently are tissue-, cell-, and stage-dependent; and timing of exposure can be critical to the expressed outcome. For example, a cell may express DLC-sensitive receptors for only a limited period in its life cycle.
- The perinatal period is a sensitive period, a window through which one may examine the effects of DLCs on several developing organ systems.
- As seen in Table 1, a variety of biomarkers have been measured in each biological system (see Lindström et al., this supplement, for their use in specific studies). Results from these studies give insight into mechanisms of action of DLCs during the perinatal period.

Reproductive

Perinatal exposures to TCDD (and presumably DLCs) produce reproductive effects in male offspring that are different from those produced by PCB mixtures (presumably the non-DLC components): PCBs cause infertility in primates without decreasing the sperm count (19); TCDD lowers sperm count in rats without affecting fertility (22–24). The differences in response are attributable either to specific differences or to differing mechanisms of actions of TCDD and PCBs. TCDD acts via the Ah receptor; some PCB congeners may act via non-Ah receptor mechanisms.

Endocrine

Prenatal TCDD in rats causes feminization and demasculinization of male offspring, as manifested by patterns of LH secretion and sexual behavior (21–23). Brain levels of T4 (thyroid hormone) and dopamine (neurotransmitter) were reduced in rats by perinatal exposures to specific noncoplanar (non-DLC) PCB congeners (14,25–27). Dopaminergic effects were, in addition, age-dependent (26). Since T4 crosses the placenta and blood-brain barrier, changes in T4 hormone levels in the fetus may affect normal development in the brain (e.g., branching of apical dendrites). Studies in rats of the effect of specific PCB congeners on T4 and dopamine levels, apical dendrite development, and memory test scores could explore the relationship between these biomarkers and health outcomes.

Immune

Cells within the immune system are continually replenished and are a model for developmental effects and effects on differentiation. The DLCs can act as immuno-suppressors or enhancers in this system, depending upon the cellular target. The effects of DLCs on macrophages suggest an enhancement of inflammatory activity and expression of cytokines, particularly TNF and IL-1 (35,36,45,46). The effects of DLCs on B-cells results in suppression of antibody production and a direct effect on phosphorylation in this cell type (34,47,48). One of the most common observations is for a suppression of the anti-SRBC plaque-forming cell responses in TCDD exposed mice, which may be due to suppression of T-cell responses, although these effects are not observed in rats (31,49).

Changes in cell surface markers on T-cells are apparently a particularly sensitive response to the effects of TCDD in nonhuman primates, and have resulted in exploration of this effect as a biomarker in
Table 1. Biomarkers and their functions.

| Area/biomarker | Function |
|---------------|----------|
| General       |          |
| Ah receptor   | An intracellular binding protein of DLCs which, when attached to a ligand and other binding proteins, is documented to mediate many of the effects of DLCs. At least 27 genes/gene products are controlled by the Ah receptor, including Cyp1A1 and Cyp1A2 (cytochrome P450s); Gst-Ya (glutathione-S-transferase); Nmo-1 (menadione oxidoreductase); T-ALDH (aldehyde dehydrogenase); and P450 (plasminogen activator inhibitor-2) (13). |
| ARNT          | Ah receptor nuclear translocator is a protein that binds to the ligand–Ah receptor complex as the heat shock proteins dissociate from the complex, forming a ligand–Ah receptor–ARNT complex. This new complex is translocated from the cytoplasm to the nucleus, where it acts as a transcriptional enhancer by binding to regulatory regions of the DNA. |
| CYPlA         | Drug-metabolizing enzyme (formerly P450) induced by DLCs through binding to the Ah receptor. |
| Reproduction  |          |
| Birth weight/ | Growth retardation |
| body weight   |          |
| Sperm count   | Risk of infertility |
| Endocrine     |          |
| Sex-dihydrotestosterone | Testosterone metabolite; binds tightly to androgen receptor. |
| Estrogen receptors | Steroid hormone (androgen); needed at critical periods for development of male morphologic and behavioral sexual characteristics. |
| Thyroid       | T3 and T4 are the growth hormones that regulate development and energy metabolism; their synthesis and secretion occurs via secretion of TRH and TSH. Secretion of both TRH and TSH, in turn, is suppressed by thyroid hormone; this feedback inhibition prevents hormone concentration from rising too high. |
| T3            | Triiodothyronine; metabolite of thyroxine; growth hormone similar to T4 but four times as potent. |
| T4            | Thyroxine; unlike T3, passes blood-brain barrier. |
| FT4           | Free, as opposed to bound, T4; synthesis of T3 and T4 occurs within thyroglobulin from tyrosine residues. |
| TT4           | Total T4 (bound + free). |
| TBG           | Thyroxine binding globulin; synthesized in liver; major thyroid hormone binding transport protein for humans; absent in rats. |
| TRH           | TSH (thyrotropin)-releasing hormone produced by hypothalamus (HT), which controls the release of TSH by the anterior pituitary; secreted by HT neuroendocrine cells into blood vessels of pituitary stalk, and travels to pituitary, where it stimulates cells to release TSH into main bloodstream. |
| TSH           | Thyroid-stimulating hormone; produced by anterior pituitary; stimulates cells in thyroid to synthesize and secrete thyroid hormone, which travels in blood to most cells of body; regulates thyroid function. |
| Neurobehavioral |          |
| Bayley        | Measure of cognitive and motor ability (up to age 2). |
| McCarthy      | Measure of cognitive and motor ability (age 3–5 yrs). |
| SDA task      | Spatial delayed alternation task; measures spatial learning/memory impairment in primates and rodents. |
| Immune        |          |
| CD4/CD8       | Cell surface markers on T- (“thymus”) lymphocytes; ratio of “helper” T/”suppressor” T indicates immune function (2 = “normal”; < 1 = compromised). |
| CD4/CD29      | CD4/CD8 are “memory” T-cells subset that have been previously activated by antigens; possible marker of sensitivity or effect to DLC exposures. |
| SRBC PFC      | Sheep red blood cell plaque-forming cell assay for ability of B-cells to produce antibody against antigen; most sensitive assay for effects of DLCs in mice. |
| Stress protein | Family of proteins (includes TNF) synthesized in many cells in response to infections, inflammation, and stress; general effectors on metabolism and homeostasis. |
| TdT/RAG       | Markers for stem cells of lymphocytes in bone marrow; used to demonstrate that DLCs affect development of T-cells. |
| TNF           | Tumor necrosis factor, produced by macrophages, is an immune system hormone (lymphokine) involved in inflammatory response; in animals, DLCs increase TNF and cause toxic effects (weight loss, death). |

Humans (39,50,51). DLC exposures change the ratios of lymphocyte subpopulations in rodents and primates (31–33,37–40), perhaps impairing the primary immune response and accounting for the decreased immunization “take” rates reported for Inuit children (41,42). Changes in the ratio of lymphocyte subpopulations (e.g., changed CD4/CD8 ratio) may mean that DLC and/or non-DLC components of PCB mixtures alter the differentiation pattern of cells of the immune system.

A mechanism for TCDD-induced thymic atrophy is suggested by a study in mice in which the depletion of bone marrow lymphocytes (stem cells) following TCDD exposure was measured using markers that are specific for lymphocytes (terminal deoxynucleotidyl transferase, TdT, and recombinase activating gene, RAG) (32,33). In addition to reducing the number of lymphocytes in the thymus gland, TCDD appears to reduce the number of prolymphocytes in the bone marrow available to repopulate the depleted thymus. In this way, TCDD’s effects on bone marrow are believed to contribute to thymic atrophy.

Neurobehavioral

Perinatal studies with specific PCB congeners and biomarkers suggest a link between behavioral changes in rodents and primates with changes in levels of T4 or dopamine. For example, spatial learning/memory deficits in monkeys and adult rats are produced by perinatal exposures to specific PCB congeners, especially PCB28 (Schantz), a non-DLC ortho-substituted congener. This same congener also alters dopamine levels in monkeys and rats in specific regions of the brain (26).

Issues

For perinatal exposures, are there markers of early events that predict later adverse human health outcomes? Can these markers be used to study delayed effects, i.e., predict adverse consequences in newborns and young children, in adults and possibly second generation children? Are these markers linked to exposure? Do they suggest possible public health interventions?

Two examples from the workshop illustrate the difficulties of establishing associations between biomarkers and disease outcomes. Human data suggest that exposure to DLCs changes the ratio of lymphocyte subpopulations (e.g., CD4/CD8 ratio). These changes fall within the range of variation seen in normal, “unexposed” human
populations. As a consequence, we do not know if they signal an adverse health outcome. If the changes fall outside the normal range, their health significance is still not clear. Are they associated with an adverse outcome? Should we consider a significantly altered lymphocyte subset ratio an adverse health outcome? How big must the change be?

The second example illustrates difficulties with outcome variables that integrate functions, such as IQ. A shift in an individual’s IQ of a few points is not statistically significant and has little impact on the individual or on society. A decrease of two points in population IQ, however, can significantly impact public health. Do we have candidate markers that can predict this shift? Could changes in neurotransmitter levels (e.g., dopamine in forebrain or caudate nucleus) or other effects in the developing or adult brain be used to predict changes in IQ?

Workshop participants emphasized the difficulties in linking chemical exposures with complex responses that have multiple causes (e.g., neurobehavioral end points). For these responses, chemical exposures may not be the primary causes, and their effects may be obscured or confounded by stronger factors. (Alternatively, they may work in concert with other factors to produce an effect.) For example, neurobehavioral studies suggest there is an association between perinatal exposures to DLCs and changes in IQ in children. However, many socioeconomic factors also affect IQ, and these may overwhelm and mask any contribution that is made by a chemical exposure. They act as confounders of chemical exposures. Before chemical linkages can be investigated, neurobehavioral measures are needed that can better control for social/environmental factors.

Finally, neurobehavioral researchers cite drawbacks in the application of biomarkers to their field. First, the medical model of disease, in which there is a pathway from early health impairment (preclinical signs) to frank pathology and disease, may not be applicable to neurobehavioral end points. The various neurobehavioral “biomarkers,” such as IQ, age at development of verbal skills, or short-term memory, are not considered precursors or early signs of disease. They are effects that are less favored. Decrement in these functions do not signal a transition from “normal” to “abnormal,” but rather a shift to a less favorable position within the “normal” range.

The sensitivity and specificity of biomarkers in neurobehavioral studies was also questioned. The most useful cognitive measures are those for which population norms have been established. These measures generally sum the influences of a variety of factors that can affect development. They do not assay the functioning or non-functioning of specific neurobehavioral mechanisms. More specific tests are needed. Importantly, a more complete understanding of the mechanisms involved in the neurobehavioral changes themselves is needed before more specific tests can be developed.

**Epidemiologic Studies in Progress**

Several ongoing epidemiologic studies investigate the effects of perinatal exposures to DLCs and non-DLCs by employing a number of biologic markers. Koopman-Esseboom et al. (32) explore the effects of PCBs and dioxins on thyroid hormone metabolism (TT3, TT4, FT4, and TSH levels) and on neurologic and psychomotor development among children whose mothers had varying levels of coplanar PCBs and TCDD in their breast milk.

Dewailly et al. (this supplement) examine markers of exposure, effect, and susceptibility among Inuit children (n = 200) exposed to PCBs and heavy metals via fish consumption. Levels of PCB congeners, HCB, DDE, dieldrin, PCDD/PCDF, ortho, and coplanar PCBs, and metals (Hg, Pb, and Se) are measured in breast milk, maternal serum, placenta, and cord blood. Biomarkers are measured in placenta and cord blood including, in placenta: enzyme induction (CYP1A1, CYP1A2) and Ah receptor levels (binding assay with 3H TCDD); and in cord blood/lymphocytes: stress proteins (heat-shock proteins HSP90), immunotoxic effects (natural killer cell (NK) activity and CD4/CD8 ratio), sister chromatid exchange (SCE), and endocrine hormone levels (T4, T3, and TSH).

Buck et al. (this supplement) described an ongoing retrospective study of reproductive outcomes among 11,000 New York state angler 18 to 34 years of age. End points examined for subsets of this cohort include fertility rates (NYS live birth and fetal death registries), spontaneous fetal mortality rates (NYS fetal death registries), pregnancy intervals, intrauterine growth retardation, secondary sex ratios, time to conception, intrauterine growth retardation, neurodevelopmental status, and disorders of ectodermal tissue.

Rogan et al. (this supplement) examine the lactational effects of DDE (levels measured in breast milk) in a cohort of 230 mothers in Mexico. Preliminary results suggest that DDE acts as a prolactin antagonist. Rogan et al. continue to follow their cohort of 117 children in Taiwan with regular physical examinations to determine any peripubertal developmental effects from DLC exposures, including stages of puberty (as per Tanner scale). In a similar manner, they are also examining a cohort of 200 children born in Taiwan between 1985 and 1992 for peripubertal developmental effects that may result from DLC-exposures.

Clark et al. (this supplement) examine susceptibility markers in two cohorts, one in Seveso, Italy exposed to high levels of dioxins following a chemical plant explosion in 1976 and the second in Germany, with occupational exposures to dioxins in a chemical plant that produces 2,4,5-trichlorophenol. Within each of these cohorts, certain individuals developed chloracne. Others, in the same cohort with similar exposures, did not. Genetic and biochemical differences between the responding and nonresponding individuals are sought to account for the differences in response. These include Ah receptor levels and the expression of the CYP1A1, TNF or IL-1 beta genes.

**Conclusions**

The potential adverse consequences on the reproductive, endocrine, immune, and neurobehavioral systems of perinatal exposures to dioxins, dibenzofurans, and PCBs are being explored using a variety of biomarkers, including sperm count, CD4/CD8 ratio, and levels of testosterone, T4, and dopamine. In animal studies, exposures to some of the listed compounds produce changes in the levels of these markers, suggesting that the chemicals affect fertility, impact the endocrine system, cause feminization and demasculinization of males, depress the immune system, and produce neuroendocrine and neurobehavioral effects. These and other markers will be employed in future experimental studies to explore the mechanisms of these effects. If validated, these markers can be used in epidemiologic studies to determine the effects in humans that arise from perinatal exposures to dioxins, dibenzofurans, and PCBs.
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