Workshop on Perinatal Exposure to Dioxin-like Compounds. III. Endocrine Effects

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Studies involving endocrine effects in humans and experimental animals resulting from the exposure to dioxin-like (non-ortho-substituted PCBs, PCDDs/PCDFs) and nondioxin-like (PCBs, OC pesticides) compounds (DLCs and NDLCs) were presented. A variety of reproductive and hormonal parameters, including androgen status, sexual differentiation, and thyroid functionality, were discussed. As in utero and lactational exposure of the human fetus/neonate to these environmental contaminants is inevitable, continued research to identify sensitive biomarkers of effect and susceptibility, as well as to define dose–response relationships, is required. — Environ Health Perspect 103(Suppl 2):147–150 (1995)

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Introduction

In opening remarks, Dr. Dewally stated that the endocrine session would present data from experimental studies on the effects of dioxin-like compounds (DLCs) on sex hormone and thyroid functions, and indicate ways in which this information is applied in the development of biomarkers of effects or susceptibility for use in human epidemiologic studies.

Two questions were raised concerning the development of endocrine biomarkers:

- Are changes in thyroid function responsible for the neurobehavioral effects that are observed in experimental animals/humans?
- In the development of endocrine biomarkers from animal data, which of the species differences (rodents vs humans) are significant (e.g., differences in thyroid histology; and lack of thyroid-binding globulin, shorter T1/2 for thyroxine and increased serum TSH levels in rodents)?

Animal Studies

Dioxin and dioxin-like compounds (DLCs) have been shown to cause overt toxicity in the male reproductive system of experimental animals. Recent studies of occupational cohorts exposed to TCDD suggest that some of the adverse effects of TCDD that are observed in rodents (reduced libido, decreased testicular size, gonadotropin/androgen alterations) may occur in humans.

Dr. Moore described the effects of perinatal exposures to TCDD on the sexual differentiation of male rats. For a phenotypic male to develop from a genotypic male, sufficient testosterone (Ts) must be secreted by the fetal testes at the appropriate time. The Ts profile permits sex organs to mature normally and for patterns of male sexual behavior to be imprinted. If the Ts profile for male development is altered, the default phenotype, female, is expressed.

In previous studies (1-3), TCDD when administered to pregnant female rats (single oral dose of 0.064-1.00 µg/kg on gestational day 15) affected a variety of androgenic status indices [spermatogenesis, adult sexual behavior, luteinizing hormone (LH) secretion pattern] in the male offspring in a dose-dependent manner. This maternal TCDD exposure also reduced in male offspring the prebirth plasma Ts levels and the postparturition surge of Ts.

In follow-up experiments, male offspring of TCDD-dosed females (single 0.7 µg/kg oral dose on gestational day 15) were castrated on day 63 (postpuberty) and implanted with Ts-containing silastic capsules to maintain physiological levels of serum Ts (2 ng/ml). These male offspring of TCDD-treated dams had reductions in what would normally be Ts-induced weight gains in the ventral prostate. The reductions were not due to decreases in either cell numbers or levels of Ts or dihydro-Ts in the ventral prostate, or to changes in Ks or Bmax values for androgen receptors. Weights of seminal vesicles were also decreased in a dose-dependent manner in these male offspring of TCDD-treated dams. In contrast to the ventral prostate, no TCDD effect was seen in castrated or implanted males in wet weight gain, protein and DNA content or amount of Ts or dihydro-Ts in seminal vesicles.

Male offspring from TCDD-treated dams also had dose-dependent alterations in sexual behaviour (demasculinization, feminization, feminized pattern of LH secretion). Sexual differentiation in the central nervous system of male rats is dependent upon levels of 17β-estradiol (related to perinatal Ts). TCDD has been shown to alter Ts levels in male offspring. To investigate whether it altered estradiol as well, experiments were conducted to discern effects of TCDD on sexual dimorphism. Perinatal exposures to TCDD did not alter the volumes of medial preoptic nuclei or the distribution patterns of estrogen receptors in the brain, two features of sexual dimorphisms in rats.

Spermatogenesis in rats is sensitive to prenatal exposures to TCDD: reductions in daily sperm production and sperm counts in the caudal epididymis continued to occur in the sexually mature (day 120) male offspring of dams treated with TCDD at dose levels as low as 64 ng/kg (gavage on gestational day 15). Flow cytometric methods were used to investigate Sertoli cell division but revealed no effects.
of TCDD on Sertoli cell labeling index (LI) at parturition or on ploidy levels in the testes. Future experiments are planned to examine the Sertoli cell LI at preparturition and in the adult, to undertake quantitative testes morphology.

Dr. Schantz reviewed her published studies on the effects of perinatal exposures on thyroid function. Exposures of adult animals to PCB mixtures (Aroclor 1254) increased the metabolism of thyroxine (T4); decreased the serum levels of thyroid hormones [TH, T3 (triiodothyronine) and T4]; diminished the response of serum TH levels to exogenous thyroid stimulating hormone (TSH) as well as the binding of congeners and/or metabolites to the main rat TH-transport protein (transhre- tin), and had indirect/direct toxic effects on the thyroid gland.

In a study initially designed to investigate the behavioral effects associated with perinatal exposure to individual PCB congeners in rats, offspring TH status and thyroid gland histology were investigated (4). Three PCB congeners were selected: IUPAC Nos. 28, 118 and 153 (2,4,4'; 2,3',4,4',5; 2,2',4,4',5,5', respectively) on the basis of their environmental and human occurrence and suspected effect on brain catecholamine function. These three congeners had been previously shown to comprise up to 25% of the total PCB content of human breast milk samples (5).

Doses of the congeners, preselected to minimize developmental toxicity, were administered to SD rats on gestational days 10 to 16 by gavage. At weaning (day 21 postparturition), samples were collected for serum TH analysis and thyroid histology.

None of the reproductive parameters measured (litter size, percent live births, pup survival to weaning, dam weight gain, or liver weights) were affected by the PCBs. Pups in the high-dose congener 118 group (16 mg/kg/day), however, exhibited significantly reduced body weights (25%) in both sexes up to weaning. In the case of the male pups, this effect was still evident by day 90. Pup serum T4 levels in both sexes were unaffected by congener 28, reduced approximately 50% in the high dose group of congener 153 (64 mg/kg/day), and drastically decreased in the high dose group of congener 118 (approximately 90%). Pup T3 levels, as well as dam TH levels, were not affected.

Thyroid gland histology in the high dose congener 118 pups revealed an increase in the height of epithelial cells, a reduction in the colloid area and an increase in follicular cell cytoplasm vacuolation and amount of nuclear vesiculation. These effects are consistent with sustained TSH stimulation of the thyroid in response to low circulating levels of T4.

In a similar study involving non-ortho-substituted PCB congeners 77 and 169 (6), both maternal TT4 (total T4) and fetal FT4 (free T4) in Wistar rats were reduced by a single dose of PCB congener 169 (3,3',4,4',5,5') (1.8 mg/kg) on day 1 of gestation. In addition, neonatal TT4 levels were decreased in a group in which dams were exposed to PCB congener 169 (0.6 mg/kg) on gestational day 1 and PCB congener 77 (3,3',4,4') (1 mg/kg) on days 2 to 19. The observed consequences of the decreased fetal plasma T4 included increased brain type II 5'-deiodinase activity. One possible mechanism for the reduction of plasma T4 is the displacement of T4 from transshreitin by hydroxylated PCB metabolites, enhancing T4 metabolism. In a subsequent study, the induction of hepatic T4-UDPGT (uridine 5'-diphosphate (UDP)-glucuronotransferase) activity by PCB congener 169 is described as the probable cause of decreased neonatal and maternal, but not fetal, plasma T4 levels (7).

Thyroid hormone levels are critical for brain development in the rat, and the majority of fertil and neonatal thyroid T3 in the rat is derived from serum T4. Future experiments will examine whether neonatal hypothyroidism affects neurobehavioral development. In addition, T4 replacement studies are planned, since neonatal hypothyroid states decrease the dendritic branching pattern among hippocampal neurons.

**Physiology of the Thyroid**

Dr. Rhains reviewed the role of thyroid hormones in human fetal development, describing physiology of the thyroid, changes in thyroid hormone levels during pre- and postnatal development, and clinical thyroid function tests.

Figure 1 summarizes the synthesis, release, binding, and transport in serum, metabolism, and regulation by the hypothalamic–pituitary–thyroid axis (HPT) of thyroid hormones.

Proteolytic degradation of thyroglobulin in the thyroid results in the release of TH. In the blood, the majority of TH is bound to TBG (80%), with lesser quantities bound to TBP and albumin. TH are metabolized by three isozymes of 5'-monodeiodinases (classified on the basis of propylthiouracil sensitivity). Preferential conversion of T4, considered a prehormone, to the active T3 in peripheral tissues occurs primarily through type II 5'-monodeiodi- nase activity. Type I 5'-monodeiodinase activity functions in rodents, and possibly humans, to provide T3 to the plasma (8). Thyroid hormone-responsive tissues contain nuclear receptors with high binding affinity for T3 (90% of receptors are bound with T3). Regulation of gene expression by thyroid hormones requires their interaction with thyroid hormone nuclear receptors, and T3 levels are, therefore, important for maintaining the normal euthyroid state (9).

The relationship between maternal and fetal levels of TSH, TBG, free and total T4 (FT4, TT4), and T3 has not been established (10). Maternal transfer of TH to the neonate does not occur in physiologically significant amounts, due in part to 5'-deiodinase activity in the placenta. In addition, the placenta is impermeable to maternal TSH.

It is thought that production of T4 by the fetal thyroid gland is delayed until 10 to 12 weeks of gestation, and is followed at 18 to 22 weeks by an abrupt increase in the level of fetal serum TSH. At week 25, levels of TSH plateau until birth. At birth, they can surge upwards due to sevoring of the umbilical cord and cooling of the neonate. The increase in the neonatal free T4/TSH ratio after birth is due primarily to the decrease in serum TSH levels.

**Figure 1.** TSH synthesis and secretion by the anterior pituitary is under feedback control by both TRH produced in the hypothalamus and the level of circulating thyroid hormones (T3, T4). TSH activates both increased iodide uptake and thyroglobulin hydrolysis in the thyroid, resulting in the release of thyroid hormones. Thyroid hormones in the circulation are bound primarily to TBG for transport to peripheral tissues. The liver is the major site of thyroid hormone degradation via Phase II conjugation reactions and excretion into the bile. TSH, thyroid stimulating hormone (thyrotrphin); TRH, thyrotropin releasing hormone; T3, 3,5,3'-triiodothyronine; T4, 3,5,3',5'-tetraiodothyronine (thyroxine); TBG, thyroxine binding globulin.
Fetal serum total T4, free T4, and TBG values reach adult levels by week 36 of gestation, and reflect the continuing maturation process of the HPT axis and the liver. Infant TBG levels at birth are usually 1.5-fold higher than maternal values and remain elevated for up to the first 3 years.

Free and total T3 values in fetal serum are low by 30 to 32 weeks of gestation but then gradually increase until parturition. At birth, T3 levels remain lower than the adult, due to incomplete development of T4 conversion processes in peripheral tissue. The maturation of the neonatal hypothalamic-pituitary-thyroid axis is generally not complete until 1 to 2 months postpartum.

The determination of thyroid status can be made by quantification of blood levels of TSH, TBG, and free and total TH by radioimmunoassay (RIA) techniques. Free TH levels can also be measured by dialysis and by mathematical derivation using data from total T4 and T3 estimations. Thyroid functionality can be assessed by 131I uptake, TRH stimulation of the pituitary gland, and subsequent TSH secretion. The degree of saturation of TBG can be estimated by the resin T3 uptake test. The total concentration of serum TBG can be estimated by combining the results with total T4 levels. Various factors that can influence levels of TH, TSH, and TBG include: a) serum TBG concentrations and available binding sites, which will influence T4 values; b) brain androgen deprivation, which has a suppressive effect on TSH secretion; c) levels of estrogen and androgen, which increase and decrease, respectively, serum TBG values as well as basal and TRH-stimulated TSH levels; d) dopamine levels, which decrease the basal and TRH-stimulated TSH levels; and e) induction of hepatic enzymes (UDPGT), which increase the excretion of conjugated T4 into the bile.

**Human Studies**

Dr. Koopman-Esseboom reviewed an ongoing study comparing endocrine effects from perinatal exposures to low levels of DLCs in 200 Dutch children (100 breast fed, 100 formula fed). Infant (10 days and 3 months postpartum), maternal, and umbilical cord blood samples were obtained and analyzed for T3, T4, TSH, and FT4. Results were compared to breast milk levels of dioxins, coplanar PCBs and other PCB congeners (milk samples collected 10 days after birth, 24-hr collection).

The neonatal TSH levels were elevated at 10 days and 3 months. There was no effect on T4. The increased TSH levels were related to increased breast milk dioxin and coplanar PCB values. There was no correlation of levels of thyroid hormones with breast milk concentrations of PCB congeners 118, 138, 153, and 180. Previous results (11) had revealed no significant association between infant growth parameters (height, weight, cranial circumference) and breast milk PCB levels. A battery of infant neurobehavioral tests are also planned.

In a similar study (12), breast milk TCDD TEQ (PCDDs/PCDFs) values of 37.5 mg/kg milk fat (high exposure group) were associated with elevated levels of neonatal TT4 and TT4/TT3 at 1 and 11 weeks post partum. TSH levels were also increased in the neonates but only at 11 weeks of age.

All comparisons were made with the "low" exposure group, exposed to breast milk with TCDD TEQ levels of 18.6 ng/kg milk fat.

Dr. Rogan reported on the status of follow-up studies on the infant cohort from the North Carolina Breast Milk and Formula Project, which was initiated in 1978. The children are now 11 to 15 years old. They have been seen (11/93) pictorial representations of sexual development indices (Tanner Scale) and asked to perform a self-assessment, including pre-pubertal height and weight. All girls involved in the project are at Tanner Scale 2 and will continue to be assessed post-puberty.

One of the findings reported in the original study (13) was that breast milk DDE (dichlorodiphenyl dichloroethene) concentrations and duration of lactation seemed to be inversely correlated: women with the lowest 5% of breast milk DDE levels (<1.99 mg/kg milk fat) lactated on average for 26 weeks, while women with the upper 5% of DDE breast milk levels (>6.00 mg/kg milk fat) lactated for only 10 weeks. This relationship suggests that o,p'-DDE may act as an "environmental estrogen" and antagonize prolactin effects.

In a preliminary investigation to further explore this hypothesis (14), Dr. Rogan compared the breast milk DDE levels with the duration of lactation among 229 women from a region of Mexico where agricultural use of DDT is heavy. Mothers with breast milk DDE concentrations of 0 to 2.5 mg/kg milk fat breast fed an average of 7.5 months, while women with breast milk DDE concentrations of greater than 7.5 mg/kg milk fat breast fed for approximately 3.4 months.

Dr. Dewailly reported on several epidemiologic studies his group is undertaking. A cord blood study compared serum TSH levels (routine screening for hypothyroidism) among Inuit children with their exposures to contaminants in breast milk. No association was found between TSH and contaminant levels. Birth length of males, however, did show a statistically significant negative correlation with breast milk PCB congeners (IUPAC Nos. 118, 138, 153, 170, 180, 183, 187). In contrast, The birth length of females was positively associated with breast milk TCDD TEQs (coplanar PCBs, PCDDs/PCDFs) (15).

As part of the Arctic Monitoring and Assessment Program (AMAP), cord blood samples in future Inuit health surveys will be collected from birthing centers in the Northwest Territories, northern Labrador, and Quebec and analyzed for essential trace elements (Se, Cu, Zn), omega-3 fatty acids, and food chain contaminants (OC pesticides, PCB congeners, PCDDs/PCDFs, and toxic metals (Cd, Pb, Hg)). Dr. Ayotte described a number of biomarkers for health/susceptibility that will be investigated in this study. The control population will be obtained from southern Quebec. An additional cord blood study, which is similar to the AMAP study, will be undertaken using births to families of commercial fishermen of the Lower North Shore St. Lawrence River.

**Conclusions**

In experimental animals, perinatal exposure to dioxin- and non-dioxin-like compounds is capable of causing endocrine-related effects in offspring in the absence of overt signs of maternal toxicity. Consequences of this "maternal" exposure include: deficits in androgen-dependent differentiation (imprinting) and neurologic development in rodents; and alterations in thyroid homeostasis in rodents. These effects may be interrelated. Epidemiologic investigations indicate that exposure to contaminants in utero or via lactation may be associated with impairment of physical and neurologic development in the fetus/neonate. Future epidemiologic studies that use biomarkers in cooperation with data from animal studies can provide useful information for developing strategies for prevention and regulation.
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