Characterization of Potential Endocrine-Related Health Effects at Low-Dose Levels of Exposure to PCBs

Abraham Brouwer,[1] Matthew P. Longnecker,[2] Linda S. Birnbaum,[3] Jim Cogliano,[4] Paul Kostyniak,[5] Jack Moore,[6] Suzan Schantz,[7] and Gerhard Winneke[8]

[1]Division of Toxicology, Department of Food Technology and Nutritional Sciences, Wageningen Agricultural University and Research Center, The Netherlands; [2]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina USA; [3]Experimental Toxicology Division, National Health and Environmental Effects Research Laboratories, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina USA; [4]National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC USA; [5]Toxicology Research Center, School of Medicine and Biomedical Sciences, University of Buffalo, Buffalo, New York USA; [6]Institute for Evaluating Health Risks, Washington, DC USA; [7]College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois USA; [8]University of Duesseldorf, Duesseldorf, Germany

This article addresses issues related to the characterization of endocrine-related health effects resulting from low-level exposures to polychlorinated biphenyls (PCBs). It is not intended to be a comprehensive review of the literature but reflects workshop discussions. "The Characterizing the Effects of Endocrine Disruptors on Human Health at Environmental Exposure Levels," workshop provided a forum to discuss the methods and data needed to improve risk assessments of endocrine disruptors. This article contains an overview of endocrine-related (estrogen and thyroid system) interactions and other low-dose effects of PCBs. The data set on endocrine effects includes results obtained from mechanistic methods/ and models (receptor based, metabolism based, and transport protein based), as well as from *in vivo* models, including studies with experimental animals and wildlife species. Other low-dose effects induced by PCBs, such as neurodevelopmental and reproductive effects and endocrine-sensitive tumors, have been evaluated with respect to a possible causative linkage with PCB-induced alterations in endocrine systems. In addition, studies of low-dose exposure and effects in human populations are presented and critically evaluated. A list of conclusions and recommendations is included. *Key words:* endocrine, estrogen, health, human, low dose, PCBs, risk, thyroid. — *Environ Health Perspect* 107(suppl 4):639–649 (1999).
http://ehpnet1.niehs.nih.gov/docs/1999/suppl-4/639-649brouwer/abstract.html

A National Institute of Environmental Health Sciences' workshop, "Characterizing the Effects of Endocrine Disruptors on Human Health at Environmental Exposure Levels," was held in Raleigh, North Carolina, on 11–13 May 1998. This workshop was to provide a forum for the discussion on methods and data needed to improve risk assessments of endocrine disruptors. The discussions presented in this paper address the persistent and toxic environmental pollutants polychlorinated biphenyls (PCBs).

PCBs cause a wide range of effects in experimental animals, wildlife, and humans (*1,2*). This article addresses toxic effects from low-level exposures to PCBs that may be related to endocrine-associated influences on health. The assigned task was a challenge, in part because *a*) PCBs are a complex mixture that comprise theoretically 209 congeners; *b*) the toxicity of PCBs is highly dependent on the number and position of chlorine atoms on the biphenyl rings; *c*) both parent PCBs and their metabolites can cause toxic effects, with the metabolites being mainly associated with endocrine-associated effects; and *d*) there is a large species difference in toxic effects of PCBs. We also recognize that humans are exposed to high levels of natural compounds in foods that may alter the endocrine activity, if any, of PCBs in

humans. Nevertheless, much progress has been made through the study of specific PCB congeners and their metabolic products, which enables an evaluation of potential endocrine-associated adverse health effects of PCBs.

This article is not intended to be a comprehensive review of the literature but reflects workshop discussions. Only data available from peer-reviewed articles were considered for discussion. The following questions were addressed:

- What data and models are needed to estimate human risk?
- What is the spectrum of observed effects?
- What is the quality of the human exposure data?
- What has been the utility of specific animal and *in vitro* models?

The questions and discussions of these issues are addressed in the same order, with appropriate citations of relevant data. Conclusions and recommendations are given at the end of this article.

What Data and Models Are Needed to Estimate Human Risk?

In principle, the strategy to estimate human risk at low-dose exposure to endocrine disrupting chemicals does not differ from

that used for other categories of toxic compounds in the sense that one needs exposure–effect relationships and dose-response data in experimental animals; in the case of a suggested problem one may rely on wildlife data or human epidemiologic data to support or reject claims of exposure-related adverse health effects. The complexity arises, however, when considering the vast number of different cells, tissues, and organs that could possibly be affected when the supportive endocrine system(s) are disrupted. Consequently, many different exposure-effect relationships may have to be studied to evaluate the full spectrum of adverse effects that could arise following endocrine disruption.

The complexity increases further when considering that most essential physiologic functions such as reproduction and behavior, as well as the processes involved in fetal development, are supported by multiple endocrine systems that may communicate (cross-talk) with each other. Hence, even when a chemical affects a physiologic end point such as reproduction or behavior, it may be difficult to prove that this effect results from disruption of an endocrine system. Finally, endocrine systems can be affected in various ways, e.g., at the receptor level, the various metabolic steps, the organ, processes of synthesis and/or release, the transport systems, and various steps of feedback regulation. This complicates the development of suitable and unequivocal mechanism-based (pre)screening and testing methods to predict potential low-dose health risks from

exposure to putative endocrine-disrupting chemical compounds or mixtures.

Although it is difficult to answer the question, What data and models are needed to estimate human risk? based on data and experiences from studies on the effects of PCBs, the PCB database can provide valuable insight on how to evaluate human health risks at environmental exposure levels.

In this article, a concise overview is given of the available data with regard to endocrine-related effects of PCBs, including the full spectrum of low-dose effects observed in animals and effects in humans following accidental or occupational exposure to PCBs. An attempt is made to delineate which of those low-dose end points are to be considered as causally linked to endocrine disruption. The information is structured in subclasses of methods and models used to obtain the data. Although a lot of information on hormonal alterations caused by PCBs was known well before endocrine disruption became a prominent issue, it should be noted that many of the data sets on effects of PCBs were not obtained originally with a focus on putative endocrine-disruptive effects. Categorizing data on effects of PCBs in different classes of methods and models is therefore mainly done in retrospect. It is, however, possible and helpful to use this data set and the experiences obtained with PCBs to assess possible human health risks from exposure to low levels of putative endocrine disruptors.

Overview of Endocrine-Related and Other Low-Dose Effects of PCBs

Endocrine Systems Affected by PCBs

There is extensive data on endocrine-related effects of PCBs. A wide variety of endocrine systems are affected by PCBs, including the estrogen and androgen system (3), the thyroid hormone system (4), retinoid system, corticosteroid system and several other endocrine pathways. In addition, the dioxin-like non-*ortho* and mono-*ortho* PCBs that exert their effects mainly through the arylhydrocarbon receptor (AhR) pathway may also affect a number of other pathways through cross-talk of the AhR and AhR nuclear transport protein (ARNT) transcription factors with other members of the Per-Arnt-Sim family of helix-loop-helix proteins (5). The work group agreed to focus their attention on interactions of PCBs with the thyroid and estrogen hormonal systems. The effects observed by PCBs are subdivided into a) endocrine effects on components of the endocrine system such as hormones, metabolic enzymes, carrier proteins, receptors, endocrine glands, and feedback regulation

systems, and b) effects that may be associated with changes in endocrine systems: neurodevelopment, reproduction, immune effects, and induction of endocrine-sensitive tumors.

Mechanism-Based Models for Endocrine Effects

Mechanism-based models that are used to study endocrine effects focus on certain aspects of the endocrine system. The *in vitro* models and methods are particularly suitable to study certain aspects of endocrine systems in isolation. These mechanism-based models may be quite useful as (pre)screening tools to identify putative endocrine-disruptive chemicals and may allow the analysis of large numbers of compounds in short periods of time.

Receptor-based methods and models. These methods and models include receptor-binding assays, receptor-mediated reporter gene assays, and hormone-dependent cell proliferation assays.

ESTROGEN RECEPTOR-MEDIATED EFFECTS OF PCBs. Several hydroxylated (OH)-PCBs show binding affinity for the estrogen receptor in *in vitro* receptor-binding assays (6–8). The most potent congener among the limited set of hydroxy-PCBs tested was 2,4,6,2',6'-pentachloro-4-biphenylol, which was about 5 times less potent than 17β-estradiol (7), although most compounds show much less potency (6,8). A number of OH-PCBs identified in human serum exhibited antiestrogenic activity in the rat uterine estrogen-binding assay and in the MCF-7 cell proliferation assay (9). OH-PCBs bound to the estrogen receptor and transactivated the estrogen receptor to DNA binding and altered gene expression in *in vitro* reporter gene assays (9,10). However, these compounds were mainly antiestrogenic rather than estrogenic in these reporter gene assays. PCB mixtures (Aroclors 1221, 1232, 1242, 1248, 1254, and 1260) did not exhibit antiestrogenic activity, based on secretion of procathepsin D, an estrogen-regulated gene in MCF-7 cells (11).

THYROID RECEPTOR-MEDIATED EFFECTS OF PCBs. There is little information on the direct effects of PCBs or their metabolites on the thyroid hormone receptor(s). McKinney and co-workers (12) found some indications that dioxins and dioxinlike PCBs may bind to the nuclear thyroid hormone receptor, based on graphics-assisted computer modeling. However, to date no report has been published showing a direct competitive binding of PCBs to thyroid hormone receptors.

Metabolism-based methods and models. Many different enzyme systems are involved in the synthesis, intracellular bioactivation, and degradation of hormones including

cytochrome P450 isozymes, uridine-5-diphosphate-glucuronyltransferases (UGTs), deiodinases (IDs), and sulfotransferases (SULTs). It is the intimate interplay between these enzyme systems that determine the intracellular concentrations of active hormones in target cells. Certain chemicals may affect endocrine systems by interfering in metabolic steps and thereby altering the availability of the active natural hormone.

EFFECTS OF PCBs ON THYROID HORMONE METABOLISM. PCB congeners are capable of inducing UGT enzymes in livers of exposed rats. There are at least two or possibly three UGT isozymes involved in thyroid hormone glucuronidation (13). The so-called bulky phenol types of UGT are inducible by PCBs, in particular by planar non-*ortho*-PCBs, with the induction mechanism involving the Ah-receptor. For example, T_4 thyroxine glucuronidation is markedly induced in livers of rats exposed to 3,3',4,4'-tetrachlorobiphenyl CB (PCB 77) (13), 3,3',4,4',5,5'-hexaCB (PCB 169) (14), 3,3',4,4',5-pentaCB (PCB 126), and 2,3,3',4,4',5-hexaCB (PCB 156) (15,16). However, nonplanar di-*ortho* PCBs, such as 2,2',4,4',5,5'-hexaCB (PCB 153) are also capable of inducing T_4 throxine glucuronidation, albeit through another isozyme (17). PCB mixtures are also capable of inducing T_4 glucuronidation (18–21). A negative correlation between plasma T_4 levels and hepatic T_4 UGT activity has been observed (17).

PCBs also affect thyroid hormone ID enzymes as well as SULT isozymes. Exposure of rats to 3,3',4,4'-tetraCB (PCB 77) resulted in an inhibition of hepatic ID-1 that converts T_4 into T_3 (triiodothyronine) as well as reverse T_3 activity (13,22). OH-PCB metabolites competitively inhibited ID-1 activity in isolated rat microsomes (22,23). On the other hand, brain ID-2 (converts T_4 into T_3 exclusively) was enhanced in fetal and neonatal rats born from dams exposed to 3,3',4,4',5,5'-hexaCB (PCB 169) and 3,3',4,4'-tetraCB (PCB 77) (14). OH-PCBs were also potent inhibitors of thyroid hormone sulfation, competitively inhibiting the SULT enzyme involved (24,25). Inhibition of ID activity or SULT activities may compromise the availability of intracellular T_3.

EFFECTS OF PCBs ON ESTROGEN METABOLISM. There is only limited information available on the effects of PCBs on estrogen metabolism. The main enzyme system involved in estrogen metabolism that is also affected by dioxinlike PCBs is the cytochrome P450 system. Catechol estrogen formation was decreased by 50–75% in hepatic microsomes obtained from 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD)-treated rats (26). 2,3,7,8-TCDD and related

compounds affect aromatase, thus interfering in the production of estradiol from testosterone (27). Yoshihara et al. (28) reported that 3,3',4,4',5-pentaCB (PCB 126) increased the 7α-hydroxylation but suppressed the 2α-, 6β-, and 16α-hydroxylation and 5α-reduction of progesterone and testosterone in liver microsomes.

Transport protein methods and models. Interference with intracellular and/or plasma proteins that transport hormones may greatly enhance (at least momentarily) the availability of free hormone for uptake and biological activity. This may be particularly important during pregnancy, when serum protein binding of hormones plays a crucial role in maintaining a suitable level of free hormone despite large fluctuations in hormone physiology and demand. In addition, transport protein binding may provide special facilitated routes of transport for exogenous chemicals, which may have a considerable impact on their toxicokinetics.

INTERFERENCE OF PCBs WITH THYROID HORMONE-BINDING PROTEINS. OH-PCBs competitively displace T_4 from the plasma-binding protein transthyretin (TTR), formerly known as prealbumin (29–31). This occurs both *in vitro* and *in vivo* in fetal brain and plasma as well as in maternal plasma. The structure–activity relationship of T_4-TTR competition by OH-PCBs is well known and involves hydroxylation on *meta* or *para* positions with one, but preferably two, adjacent halogen substituents on the phenolic ring. It should be noted that no competitive interaction on thyroxine-binding globulin takes place with OH-PCBs, which may indicate a lower impact of OH-PCBs on plasma T_4 levels in those species, such as humans and nonhuman primates, possessing thyroxine-binding globulin. The binding of OH-PCBs to TTR may be involved in the rapid and facilitated transfer of OH-PCBs instead of the natural hormone T_4 across the placental and blood–brain barriers, leading to relatively high levels (up to micromoles) of OH-PCBs accumulating in the fetus, particularly in the fetal brain (32,33).

INTERFERENCE OF PCBs WITH ESTROGEN-BINDING PROTEINS. There is very little information on possible interference of PCBs with estrogen-binding proteins such as sex hormone-binding globulin (SHBG) or uterotropinlike proteins. There is, however, information on the binding of methylsulfone-PCB metabolites to a uterotropinlike protein in lungs of rats (34)

All of the mechanisms indicated above may have implications on the availability of thyroid hormones in the fetal brain and other tissues where thyroid hormones play an essential role in normal development. However, in recently performed rat perinatal

exposure studies, Aroclor 1254 resulted in little or no effect on fetal and neonatal brain T_3 levels, despite the fact that plasma and brain T_4 levels were reduced by 90% (32). In addition, no changes in T_4-sulfate concentrations were observed in dams or fetuses, despite the fact that considerable amounts of phenolic organohalogens were present in the fetal compartment (35).

In Vivo Models for Endocrine Effects of PCBs

Experimental animal models. THYROID HORMONE SYSTEM EFFECTS. Most information on thyroid hormone-related effects of PCBs stem from relatively short-term single-dose exposure studies of experimental animals (rats, mice, marmoset monkeys) exposed to either mixtures of PCBs or individual PCB congeners. Exposure to PCB mixtures (Aroclors 1254 and 1260, Clophen A50) or to single PCB congeners [e.g., 3,3',4,4'-tetraCB (PCB 77), 2,3,3',4,4'-pentaCB (PCB 105), 2,3',4,4',5-pentaCB (PCB 118), 3,3',4,4',5-pentaCB (PCB 126), 3,3',4,4',5,5'-hexaCB (PCB 169), or 2,2',4,4',5,5'-hexaCB (PCB 153)] resulted in marked reductions in plasma levels of T_4, whereas T_3 levels were only marginally affected (14–16,21,36–40). Reductions in plasma thyroid hormone levels were observed 24–48 hr after a single intraperitoneal injection and remained low for prolonged periods of time (several days to months), depending on the half-life of the PCB congener (36).

Effects at lower doses were observed in experiments involving perinatal exposure protocols. For example, severely depressed T_4 levels (by 60–90%) were observed in fetal and neonatal rats following *in utero* exposure to 5 or 25 mg of Aroclor 1254/kg from gestation day 10 to gestation day 16 (32), while much higher doses of Aroclor 1254 were needed in adult rats to cause similar changes in plasma T_4 levels (36). At postnatal day 21, there were no observable reductions in plasma T_4 levels, indicating that perinatal exposure to PCBs did not cause permanent alterations in thyroid hormone levels in the offspring. Reduced plasma T_4 levels were also observed in rat pups born to dams exposed to 4 or 16 mg of 2,3',4,4',5-pentaCB (PCB 118) and 16 or 64 mg of 2,2',4,4',5,5'-hexaCB (PCB 153)/kg/day from gestation day 10 to gestation day 16 (41).

Effects of PCB exposure on thyroid hormone feedback regulation, as measured by thyroid-stimulating hormone (TSH) levels, are equivocal. Some reports indicate an increased TSH concomitant with a low T_4 level in adult rats after exposure to PCBs (19,42,43); other reports, mainly from *in utero* exposure to PCBs, have not observed an effect on TSH despite severely depressed T_4 levels (32).

Reduced brain T_4 levels (by 90%) were also observed in fetal rats following *in utero* exposure to Aroclor 1254 (32). However, brain T_3 levels were marginally affected because of increased activity of the enzyme ID-2, which bioactivates T_4 to the active hormone T_3. This indicates that an effective compensatory mechanism is operative in late gestational fetuses that may effectively cope with severely reduced T_4 levels in critical target organs such as the brain.

Sustained alterations in peripheral thyroid hormone levels will result in a prolonged activation of the hypothalamic–pituitary–thyroid (HPT) axis. Evaluation of thyroid gland morphology may therefore be a good indicator for compounds that interfere in thyroid hormone metabolism. Thyroid gland abnormalities have been reported following adult and *in utero* exposure to Aroclor 1254 in rats and adult exposure in cynomolgus monkeys (44–49). The ultrastructural lesions observed in rat thyroid follicular cells after PCB exposure were suggestive of an effect on thyroid hormone synthesis and secretion (47,50). However, these thyroid ultrastructural changes were also interpreted as a sustained thyroid activation through the HPT axis (41,51).

ESTROGEN SYSTEM EFFECTS. Decreases in serum estradiol and progesterone were reported in rhesus monkeys exhibiting reproductive dysfunction, following exposure to 2,3,7,8-TCDD (500 ppt in the diet for 7 months) (52) and polybrominated biphenyls (53). Exposure to Aroclor 1248 at doses of 2.5 and 5.0 ppm in the diet caused prolonged menstrual cycles and decreased peak progesterone levels in female rhesus monkeys (54). Aroclor 1242 caused an increase in basal leutinizing hormone and follicle-stimulating hormone secretion by the pituitary in rats (55).

The uterotropic assay, e.g., an increase in uterine wet weight in prepubertal rats following a short-term exposure (e.g., 4 days) to an estrogenic compound, is frequently used as a functional assay for putative estrogenic compounds. Aroclor mixtures, particularly the lower chlorinated ones including Aroclors 1221, 1232, and 1242, were weakly estrogenic in the uterotropic assay (55–58). The potency of the Aroclor mixtures was 4–5 orders of magnitude lower than that of 17β-estradiol. The non-*ortho* 3,3',4,4'-TeCB[PCB 77] was not estrogenic and in fact antagonized the increases in uterine wet weight induced by 17β-estradiol and Aroclor 1242. *ortho* substitution may be related to the weak estrogenic activity of PCB congeners (7,58).

Studies in wildlife species. Possible effects of putative endocrine disruptors in real life are difficult to assess because of the generally very low background levels of exposure.

However, studies in wildlife species that are situated at the top of food chains may be quite useful to establish if there are effects at environmentally relevant concentrations.

THYROID HORMONE SYSTEM EFFECTS. There is a considerable data set for the effects of PCBs on wildlife species. Some studies also provide information on thyroid hormone system effects. Changes in plasma T_4 levels were observed in wildlife species exposed either experimentally or environmentally to low doses of PCBs. Harbor seals (*Phoca vitulina*) held in captivity were exposed for almost 3 years to fish diets that were obtained from a highly polluted area (Baltic Sea) or from a relatively clean area (northeast Atlantic). The diets differed by a factor of 10 in PCB concentrations, and feeding was adjusted for fish protein intake (*59*). Seal reproduction was reduced in the group fed the highly polluted fish diet. In plasma samples taken from the seals during the dietary exposure period, significantly reduced total and free thyroxine (TT_4, FT_4) and total triiodothyronine (TT_3) levels were found in the highly polluted fish diet group (*60*).

Several studies have been performed on eggs of fish-eating birds that were taken from their nests and artificially hatched in a laboratory incubator. Significant dose-dependent reductions in plasma thyroid hormone concentrations TT_4 and FT_4 were observed in cormorant hatchlings from high PCB-contaminated breeding colonies compared to those from low PCB-contaminated colonies (*61*). The number of hatchlings and fledglings in the high-contaminated breeding colony was significantly reduced compared to that in the low-polluted colony. In addition, 28-day-old eider ducks exposed to 3,3',4,4'-tetraCB (PCB-77) under semifield conditions showed a significant negative correlation between plasma thyroid hormone levels T_3 and T_4 and liver 3,3',4,4'-tetraCB concentrations (*62*)

Extensive reports have been published on thyroid gland lesions observed in Great Lakes fish species, where a possible linkage with polyhalogenated aromatic hydrocarbon (PHAH) exposure has been suggested (*63*). However, other factors such as iodine deficiency or non-PHAH-like waterborne goitrogens may have been involved. Feeding Great Lakes fish diets to rodents resulted in thyroid responses similar to those found in rats exposed to PCBs (*64*). Thyroid enlargement was found in several Great Lakes populations of herring gulls sampled between 1974 and 1982 (*65*).

ESTROGEN SYSTEM EFFECTS. There is little information on estrogen system effects of PCBs in wildlife species. Adult female mink who were fed diets containing various PCBs 1 month prior to breeding and through

Table 1. Dose- and endocrine system-linked adverse effects induced • PCBs in experimental animals and/or their offspring.

Effect	Animal	Dose level	Endocrine link
Thyroid system			
T_4 depletion	Rodents/monkeys	Low[a]	T
T_3 depletion		Moderate[b]	T
TSH increase	(Adults)	Low/moderate	T
Thyroid gland abnormalities	Rodents/monkeys	Moderate	T
Estrogen system			
E_2 levels	Rodents/monkeys	Moderate/high[c]	E
Uterotropic effects	Rodents	Low/moderate	E
Growth reduction	Rodents/monkeys	Low/moderate	T?
Neurodevelopment			
Neurotransmitters (dopamine)	Rodents/monkeys	Moderate/high	T/?
Neuroproteins (GFAP/synapt)	Rats	Low/moderate	T/?
Neuromotor effects	Rodents/monkeys	Moderate/high	T/?
Cognitive development	Rodents/monkeys	Low/moderate	T/?
Auditory function	Rodent	Low/moderate	T
Reprodevelopment			
Gonad development	Rodents/monkeys	Low	E/?
Sex differentiation	Rodents	Low	E/?
Sex behavior	Rodents/monkeys	Low	E/?
F_1-fertility	Rodents/monkeys	Low/moderate	E/?
Endometriosis	Rodents/monkeys	Low	E/?
Immunotoxicity	Rodents/monkeys	Low	?
Endocrine tumors			
Thyroid	Rats	High	T
Mammary	Rats	High (reduction)	Anti-E/?
Dermal lesions	Monkeys	Low/moderate	?
Porphyria	Rodents	Moderate	?
Biochemical enzyme induction, etc.	Rodents/monkeys	Low	?

Abbreviations: E, estrogen system; E_2, estradiol; F_1, first-generation offspring; GFAP, glial fibrillary acidic protein; PCBs, polychlorinated biphenyls; synapt, synaptophysin; T, thyroid system; TSH, thyroid-stimulating hormone; T_3, triiodothyronine; T_4, thyroxine; ?, endocrine linkage unknown. [a]Lower than 1 mg/kg. [b]Lower than 50 mg/kg. [c]Above 50 mg/kg PCBs.

parturition developed severe reproductive problems and some changes in sex hormone levels. Aroclor 1254 at 2.5 ppm in the diet caused a decrease in plasma progesterone (*66*); however, 3,3',4,4',5,5'-hexaCB (PCB 169) caused an increase in plasma progesterone. In harbor seals fed fish diets with relatively high levels of PCBs for 2 years, a lower reproductive success was observed; changes in plasma levels of progesterone were also found (*59*).

Other Low-Dose Effects of PCBs in Experimental Animals That May Be Linked to Endocrine Disruption

PCBs induce a wide spectrum of toxic and biochemical effects in experimental animals. In Table 1, a number of the most important effects are given. In addition, the effects are categorized as low-, moderate-, or high-dose effects and whether there is a suggested link with endocrine disruption.

Endocrine linkage is clear for hormone and endocrine gland-related effects. This is not necessarily the case for the other effects listed in this table. For most of the end points listed in Table 1, it may be reasonable to suggest a linkage with alterations in an endocrine system; however, this does not

indicate that the effect is mediated by an endocrine mechanism.

Neurodevelopmental effects. It is well known that thyroid hormones are very important for normal brain development and that hypothyroidism during fetal and early neonatal life may have profound adverse effects on the developing brain. This may result in reduced axonal and dendritic size and complexity (*67*) and alterations in the growth and development of a number of different neuronal cell types and glial fibrillary acidic protein (GFAP)-positive astrocytes (*68*). In fact, changes in GFAP levels have been observed in several brain regions in postnatal day 90 rat offspring that were exposed *in utero* and via lactation to Aroclor 1254 (*69*). Hypothyroidism also results in changes in the serotonergic and dopaminergic neurotransmitter systems (*70*). It is well known that perinatal exposure of rodents to PCB mixtures or to dioxinlike as well as nondioxinlike PCBs causes changes in dopaminergic and serotonergic neurotransmitter concentrations and metabolism (*71–73*). In a perinatal exposure study (*74*), similar influences were observed on the serotonergic system by 3,3',4,4'-tetraCB (PCB 77) and the well-known thyroid suppressive agent

6-n-propyl-2-thiouracil in rat offspring. Retarded behavioral and cognitive development and profound deafness are consequences of fetal hypothyroidism (75). Rodent and monkey offspring born to mothers exposed to PCBs perinatally also exhibited learning and behavioral deficits and reduced auditory-evoked potentials at low frequencies (40,76–80). Although quite similar changes in neurodevelopment can be induced by fetal hypothyroidism and by perinatal exposure to PHAHs, this does not necessarily mean that the changes observed by PHAHs are causally linked to alterations in the thyroid hormone system. However, the changes observed in auditory-evoked potentials are more likely linked directly to alterations in the thyroid hormone system (40).

Effects on reproductive development.
Gonad development, sex determination, and reproductive success of the offspring are highly dependent on the sex hormonal systems. Disruption of the sex steroid system during fetal stages of life results in profound adverse developmental reproductive effects, as is well known from the diethylstilbestrol experience (3). The changes include decreased sperm counts; sperm abnormalities; male gonadal changes such as hypospadia, cryptorchidism, microphallus, testicular and prostate tumors; female gonadal changes such as ovarian cysts, malformations of the cervical canal, ovarian tumors, and vaginal adenocarcinoma; and infertility in both sexes. Changes in estrogen and androgen hormonal balances during fetal life may also impact on gonadal as well as sexual behavioral development. Alterations in reproductive development have been most extensively studied with 2,3,7,8-TCDD. 2,3,7,8-TCDD exposure during development causes changes in both male and female gonadal development, reductions in sperm counts, abnormal sperm, and changes in sexual behavior such as demasculinization and feminization of male offspring (81,82). There is only limited information on reproductive developmental effects following exposure to PCBs. Postnatal lactational exposure of infant male rats to Aroclor 1254 at maternal doses of 8, 32, and 64 mg/kg resulted in adverse effects on subsequent mating behavior and reproductive success (83). There were also decreases in ventral prostate weight and testicular weight in adult male rats exposed to Aroclor 1254 via lactation. In female rats exposed to Aroclor 1254 via lactation, several adverse effects were observed such as delayed puberty, decreased uterine weight, impaired fertility, and irregular estrus cycles (84). Although the reproductive abnormalities induced by 2,3,7,8-TCDD (and to a more limited extent by PCBs) would not be in conflict with an underlying estrogenlike mechanism of action, this possible relationship needs to be studied further.

Endocrine-sensitive tumors. It is well known that tumor incidences of endocrine-sensitive organs such as mammary gland, testis, prostate, ovary, and thyroid gland can be highly influenced by changes in sex steroids or thyroid hormone levels. In fact, quite a number of antitumor therapies take advantage of this knowledge by using antihormone or hormone antagonist treatments.

PCBs are carcinogenic in rodents. The principle tumor response to 2,3,7,8-TCDD and related dioxinlike PCBs is seen in the female rat liver (85). The fact that no liver tumors are observed with 2,3,7,8-TCDD in male rat livers suggests a possible role of estrogens in female rat liver tumor formation by 2,3,7,8-TCDD. There is some information on PCB-induced tumors in endocrine tissues. Small increased incidences of thyroid gland follicular cell adenomas were observed for males receiving Aroclor 1242, 1254, or 1260 at 25, 50, or 100 ppm in the diet for 2 years, but the increases did not continue proportionately above the lowest dose. Follicular cell hyperplasia (generally minimal or mild) was increased in a nondose-related manner for males exposed to Aroclor 1242, 1254, or 1260. Thyroid effects were not observed in males fed 50, 100, or 200 ppm Aroclor 1016 or in female Sprague-Dawley rats fed Aroclor 1016, 1242, 1254, or 1260 (86). The morphology of the tumors was characteristic of those that develop as a secondary response to chronic overstimulation by TSH.

A significant decreased trend in the incidence of spontaneously occurring mammary gland tumors was measured in female Sprague-Dawley rats fed up to 100 ppm Aroclors 1242, 1254, and 1260 in the diet for 2 years. The effect was not observed in females fed up to 200 ppm Aroclor 1016 (86). This effect was most consistently observed in groups receiving Aroclors 1242 and 1260. The authors speculated that the results may be the consequence of an alteration in the 2-hydroxylation rate versus the 16-α-hydroxylation rate of estradiol, as reported by others and demonstrated in this study (87). We also note that 2,3,7,8-TCDD decreases the amount of hepatic estrogen receptors in rats (88). Reduced incidences of mammary tumors in rats have been seen with studies of Aroclor 1260 in Sherman rats (89) or Sprague-Dawley rats fed 2,3,7,8-TCDD (84), but these may have been associated with decreases in body weight.

These data suggest that at relatively high doses, PCBs affect tumor incidence. This may be related to persistent effects on the thyroid system (hypothyroidy resulting in prolonged overstimulation of the HPT axis) or to effects on the estrogen system following chronic exposure.

Exposure and Associations with Health Effects in Human Populations

Data on human exposure to PCBs. Over the past 20 years, measurement of human exposure to PCBs has improved because of developments in methods of extracting PCBs from the specimen source and in clean-up to remove materials that interfere with the assay; control of the proportion of PCBs in the source specimen that is extracted for analysis (because of availability of deuterated or surrogate standards); and instrumentation that has resulted in lower detection limits and lower coefficients of variation.

Using modern methods it is now apparent, that the PCBs in human specimens typically comprise three dozen or more specific PCB congeners, with the number detected depending on the analytical sensitivity of the laboratory method, amount of sample, and the population studied. From the 1970s through the mid-1980s, however, PCBs were usually quantified by either the peak pattern method, where PCB peaks in the sample were compared with the corresponding peaks in commercial PCB mixtures, or by the perchlorination method, where all individual PCB congeners were converted to decachlorobiphenyl, followed by back calculation to commercial PCB mixtures, e.g., Aroclor 1260 (1,90,91). Subsequently, analysis of several chromatographic peaks (often seven) reflecting the concentration of the major congeners was used to provide a more precise estimate of total PCBs [using the Webb-McCall method (92)]. Recently, the focus has been on quantification of specific congeners using solid phase (93) or solvent extraction (94). With the earliest measures of PCBs, comparison of results across laboratories is unreliable. Results based on the Webb-McCall method allow a qualitative comparison of concentrations measured in different laboratories. With congener-specific methods, quantitative comparisons across laboratories are feasible but still complicated by variation across laboratories in procedures for extraction and clean-up, methods of analysis including selection of congeners measured, and data handling and presentation.

Expressing organochlorine concentration in relation to the amount of lipid in the specimens is desirable because otherwise variation in lipid introduces variation in wet weight concentration that does not reflect body burden. Furthermore, expressing concentration per unit lipid probably better represents the concentration of free organochlorine that may be directly responsible for toxicity, much like levels of free hormones deemphasize variation in total concentration because of differences in amounts of binding proteins present. Expressing concentration of organochlorines

relative to lipids, however, introduces another source of variation in measurement across laboratories. Not only do the major approaches to lipid determination (gravimetric and enzymatic) introduce possible variation in results, but for a given approach results may vary substantially across laboratories.

Measurement of specific congeners has become popular, in part, because the toxicology of the individual PCB congeners varies. For example, PCB congener 153, routinely detectable in humans by modern methods, induces phenobarbital-metabolizing enzymes (95). Congener 28 and several others are suspected of being neurotoxic. Congener 118, also routinely detectable in humans, has weak dioxinlike activity.

The dioxinlike activity in a sample can be completely characterized by measurements of PCB congeners that include those with the most potent dioxinlike activity (congeners with no *ortho* position chlorines [coplanar PCBs]) as well as polychlorinated dibenzodioxins (PCDDs; including dioxin itself), and related compounds such as polychlorinated dibenzofurans (PCDFs). These three types of compounds are present at very low concentrations in background-exposed people. Measurement of these very low concentration compounds is expensive and requires relatively large sample volumes. Whether or not these are measured, the total dioxinlike activity of a given sample can be estimated by taking into account the dioxinlike activity of each individual compound and the amount of each compound that is present (95). The dioxinlike activity of a compound is expressed as a toxic equivalency factor, a multiplier that reflects the compound's dioxinlike potency relative to dioxin (range 0–1). The sum of the concentration of each compound multiplied by its toxic equivalency factor yields a toxic equivalency for the mixture.

Recent studies suggest that among background-exposed people in Europe (96) and North America (97), levels of specific PCB congeners, PCDDs, and PCDFs are highly

correlated, raising questions regarding the additional information gained by measuring a large number of specific PCB congeners or related compounds, and about whether effects of specific compounds can be distinguished statistically. Furthermore, methods of grouping congeners with similar toxicologic properties have been proposed for use as exposure measures in epidemiologic analyses, but their value in practice remains to be established.

Finally, we note that with PCB levels expressed relative to the amount of lipid present, the concentrations in various tissues have been considered equivalent. However, few data are available to substantiate this, and the possibility that different congeners equilibrate differently has not been adequately addressed (98).

A comment on the scope of epidemiologic data reviewed. We have focused our discussion of epidemiologic results primarily on studies of health outcomes associated with exposure to PCBs. We have not included a general review of the health effects of exposure to PCDDs or PCDFs, although such data may be informative if PCBs are toxic through an AhR-mediated mechanism. The reader is referred elsewhere for more information on this topic (99). We note that studies on fish intake in relation to health outcomes may be informative regarding PCB effects, but such studies were in general not included in this review because of the possibility that other contaminants in fish might

account for health effects associated with fish consumption. Two studies of fish intake in relation to neurodevelopment outcome, however, are included in this review. We have focused primarily on those relations where the data suggest an association. Thyroid data are reviewed in detail because of the direct tie-in with endocrine disruption. More complete recent reviews are available elsewhere (100).

Epidemiologic associations between early-life PCB exposure and health effects. THYROID HORMONES. The results of the two studies of *in utero* exposure to PCBs and thyroid function in neonates are summarized in Table 2. Both studies were done in Dutch populations with no special PCB exposure. The laboratory assay for TSH used in the study by Koopman-Esseboom et al. (101) had a lower limit of detection. In that study higher PCB exposure was related to increased TSH. In the small study by Pluim et al. (102) also done among a Dutch population, both thyroxin (T4) and TSH increased with higher exposure to dioxin and dioxinlike compounds. Koopman-Esseboom et al. (101) also found that exposure to dioxin and dioxinlike compounds was associated with increased TSH and lower total T4. In another study among subjects in Japan who had no special dioxin exposure (103), exposure to dioxin and dioxinlike compounds was associated with decreased T4 and increased TSH, measured at 1 year of age. Overall, the results of the four modest-size studies on

Table 2. Summary of epidemiologic data on organochlorine exposure in relation to neonatal or infant thyroid function.

Exposure measure	Location of population studied (reference)	n	T4	TSH
PCBs	Netherlands (101)	78	↓	↑
	Netherlands (118)	93	No Δ	NR
PCDFs/PCDDs, or total TEQs	Netherlands (102)	38	↑	↑
	Netherlands (101)	78	↓	↑
	Japan (103)	71	↓	↑
	Netherlands (118)	93	No Δ	NR

Abbreviations: no Δ, no association; NR, not reported; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzodioxins; PCDFs, polychlorinated dibenzofurans; TEQs, toxic equivalents; TSH, thyroid-stimulating hormone; T4, thyroxine; ↑, statistically significant direct association; ↓, statistically significant inverse association.

Table 3. Summary of epidemiologic results on perinatal PCB or dietary fish exposure in relation to neurodevelopmental outcomes.[a]

Outcome	Age outcome measured	Location of population studied (reference)	Effect of ↑ PCB intake	Exposure measure used Fish	Measured PCBs
Neonatal reflexes	60 hr	Michigan (119)	Hyporeflexia	✓	
	60 hr	Michigan (119)	None		✓
	72 hr	North Carolina (120)	Hyporeflexia		✓
	14 days	Netherlands (121)	None[b]		✓
	36 hr	New York (122)	Abnormal reflexes	✓	
Neonatal muscle tone	60 hr	Michigan (119)	None	✓	
	72 hr	North Carolina (120)	Hypotonia		✓
	14 days	Netherlands (121)	Hypotonia[c]		✓
	36 hr	New York (122)	None	✓	
Psychomotor development[d]	6 months	North Carolina (123)	Delayed		✓
	36 months	North Carolina (124)	None		✓
	3 months	Netherlands (125)	Delayed		✓
	42 months	Netherlands (126)	None		✓
Mental development	6 months	North Carolina (123)	None		✓
	3 months	Netherlands (125)	None		✓
IQ	11 years	Michigan (127)	Decreased		✓
Visual recognition memory	7 months	Michigan (128)	Decreased		✓
Short-term memory	4 years	Michigan (129)	Decreased		✓
	4 years	North Carolina (130)	None		✓

Abbreviations: IQ, intelligence quotient; PCBs, polychlorinated biphenyls. [a]Representative findings are shown from each study; for brevity all results from each study are not included. [b]A relation with "neonatal optimality score," comprising reflex and tone components, was found for breast-fed children with PCBs measured in breast milk. However, with PCBs in cord blood as the exposure measure in the entire study group, including children who were not breast fed, however, a relation was not present. [c]True only in breast-fed children, with PCBs measured in breast milk. [d]Dutch neurologic exam findings for children (not neonates) are classified with psychomotor development results in this table.

early-life organochlorine exposure suggest that associations with thyroid function exist and specifically that higher exposure increases TSH. The higher TSH could indicate mild hypothyroidism, which in turn could adversely affect neurodevelopment.

NEURODEVELOPMENT. The results of studies of perinatal PCB or dietary fish exposure in relation to neurodevelopmental outcomes are summarized in Table 3. The populations studied all had essentially background-level PCB exposure typical of the region, though the range of exposure in Michigan may have been increased somewhat due to frequent fish consumption (104). Use of fish intake as a surrogate measure of PCB exposure should attenuate associations due to measurement error, and potential confounding of associations with the fish measure by other neurotoxic contaminants of fish, such as methyl mercury, are a concern. "Measured PCBs" in the right-hand column of Table 3 refers to measurements in mother's milk, cord blood, maternal serum, etc., which are all highly correlated (96,105). The methods of assessing neurodevelopment have been somewhat similar across cohorts. Some parallel findings across cohorts are evident. For example, in the North Carolina and the breast-fed Netherlands subjects, higher PCB exposure was associated with hypotonia at birth and with delayed psychomotor development within 6 months of birth. Furthermore, in both studies the PCB-associated psychomotor delays were no longer evident later in childhood. Findings have not been consistently replicated across studies, however. For example, hypotonia at birth and subsequent delay in psychomotor aspect of the Bayley scales of infant development showed associations with PCB exposure in the Netherlands and North Carolina studies (106) but not in the Michigan study (not shown in Table 3). Detrimental effects of PCBs on cognition (IQ, visual recognition memory, and short-term memory) have been suggested in the Michigan data (Table 3), but confirmatory findings have not been reported in other studies to date. Despite discrepancies among studies, overall the data suggest subtle neurodevelopmental delays observable in human infant populations exposed *in utero* to background levels of PCBs.

OTHER OUTCOMES. A host of other outcomes have been examined in relation to early-life PCB exposure. Generally, the results are mixed or isolated positive findings remain uncorroborated (100). For example, data are mixed regarding an association of *in utero* background-level exposure to PCBs with lower birth weight and shorter gestational age (100,107); one occupational study supports such an association (108). The possibility of

detrimental immunologic effects has also been raised (100).

Epidemiologic associations between adult PCB exposure and health effects.
CANCER. In the study by Loomis et al. (109) on occupational PCB exposure in relation to cancer risk, a dose–response association was found for malignant melanoma. In that study, 88 deaths due to malignant melanoma occurred among exposed workers. Similarly, a meta-analysis including data from all earlier studies of cancer among those occupationally exposed to PCBs (100) also showed an increased risk of skin cancer. That study, in comparison, included only 15 deaths from skin cancer of all types. Previously, the cancers most suspected of being linked to occupational PCB exposure were liver, brain, and malignant melanoma. The results of Loomis et al. (109) and the meta-analysis are inconsistent with one another regarding whether risk is increased for cancer of the liver. At best, both provide weak support for increased risk of brain cancer.

Among nonoccupationally exposed women, the relation of PCB blood levels to risk of breast cancer has been examined in a number of large studies (110–113). Overall the data do not support an association. In a recent prospective study among subjects with background-level exposure to PCBs, a strong monotonic relation was observed between blood levels of PCBs and subsequent increased risk of non-Hodgkin lymphoma (114).

OTHER OUTCOMES. Occupational exposure to PCBs has consistently been associated with abnormal liver function tests and chloracne (99). In men occupationally exposed to PCBs, thyroid hormone level bore no clear relation to measured PCB levels (115). As with early-life exposures, a host of other outcomes have been examined in relation to PCB exposure. Generally, the results are mixed or isolated positive findings remain uncorroborated.

THE ASIAN (YUSHO/YU-CHENG) MASS POISONINGS. Mass poisonings with PCB-containing mixtures have occurred twice— once in Taiwan and once in Japan (116,117). In each instance contaminated rice oil was the source. The contaminating mixture contained PCBs, PCDFs, and other related compounds. The toxic effects of the mixture could be due to dioxinlike activity, with PCDFs the major contributor, or to effects mediated by other mechanisms of potential importance, as the mixture contained primarily PCBs. Studies of the health effects in these incidents are informative about high-dose adult and *in utero* exposures to the mixtures. Much of the available data on health effects are from case series and may reflect findings in the most severely affected. The standardized morality ratio for the studied, exposed adult populations in

Table 4. Effects observed in infants born to mothers with Yu-cheng.

Small for gestational age	More otitis media
Small head circumference	Increased porphyrin excretion
Hyperpigmentation of skin	Psychomotor delays
Skin desquamation	Hypotonia
Black color of nose	Hypo/hyperreflexia
Deformed nails	Cognitive deficits (IQ down 5 points)
Swelling of eyelid	Menstrual irregularities
Respiratory distress	Hair loss
More bronchitis	

IQ, intelligence quotient.

Taiwan (n-1900) and Japan (n-1800) 10 and 20 years after the incident, respectively, was 1.1 in both populations. Deaths from nonmalignant liver disease were increased in both populations. In Japan an excess of deaths from liver cancer was evident (99). In both populations, the exposed subjects had increased prevalence of chloracne, localized hyperpigmentation, abnormalities of the dermal tissues adjacent to the eye, slowed nerve conduction, abnormalities of a number of laboratory tests on blood, and other symptoms. A group of exposed subjects from Japan had elevated T_3 and T_4 levels 16 years later (116).

The Taiwanese children exposed to the mixture *in utero* were systematically followed and studied. Within 4 years of birth, 21% had died. The children had many of the same manifestations of toxicity as the adults, and in addition had delayed, inhibited growth, permanent intellectual impairment, disordered behavior, more frequent infections, and other problems (117). Table 4 is a list of the major findings in the children born to Yu-cheng mothers. Several of the symptoms in human infants resemble the developmental effects observed in experimental animals exposed *in utero* to PCBs. It is not known if the reproductive, cognitive, and psychomotor effects and the hearing difficulties have been linked with possible endocrine disruptive effects of PCBs in the Yu-cheng and Yusho infants.

Summary and Conclusions

After extensive discussions of the available peer-reviewed literature, the working group on PCBs arrived at the following conclusions:
- Numerous effects of PCBs have been demonstrated in experimental animals, some of which can be induced at tissue concentrations not far from body burdens that are present in background exposure populations in industrialized countries.
- The most sensitive time window of sensitivity for adverse effects by PCBs appears to be the prenatal and the early postnatal period.

- Developmental effects on neurobehavior, on the reproductive tract, and on the immune and thyroid systems are induced following exposure to some PCBs in the prenatal and early postnatal periods in experimental animals.
- In human infants from accidentally exposed populations (Yusho/Yu-cheng), developmental effects were caused by prenatal exposure to PCDFs or PCBs and included retarded growth, neurobehavioral effects, immune effects, chloracne, and teeth and nail deformities.
- In several studies involving human infants at background environmental exposure levels, subtle changes were observed in thyroid hormone levels and neurobehavioral parameters, associated mainly, but probably not exclusively, with prenatal exposure to PCBs.

However, it should be noted that there were some inconsistencies in the neurobehavioral and thyroid hormone findings in the human infant studies, which emphasizes the need for further investigations.

In the following paragraphs information on effects of PCBs on endocrine systems and in experimental animals, wildlife, and in human individuals is evaluated and used to answer the questions raised at the outset of the workshop.

What Data and Models Are Needed to Estimate Human Risk?

Extensive data from experimental animal studies and data from a modest number of human populations are available to estimate human risk from PCB exposure. However, the question is considerably more difficult to answer when we are asked to estimate human risk from endocrine-based effects of PCBs specifically. Several endocrine systems are affected by PCBs and their hydroxylated metabolites. Most information is available on thyroid hormone perturbations; however, the estrogen, androgen, and other systems may also be affected by PCBs. In experimental animal studies, reductions in thyroid hormone levels have been observed in addition to developmental effects on brain, behavior, and reproduction following developmental exposure to PCBs. Although it is tempting to propose an endocrine linkage for the adverse effects, there is evidence only for selected end points. Also, in human infants, associations have been demonstrated between serum or human milk PCB levels and thyroid hormone and neurobehavioral changes. However, these findings do not necessarily imply that neurobehavioral changes are also causally related to thyroid changes. Therefore, it is reasonable to conclude that we do not currently have the data and models that could answer the question

on estimates of human risks from low-dose endocrine-related effects.

One of the major problems is that multiple PCB congeners may impact upon multiple endocrine systems, and several of those endocrine systems may be involved in regulating and maintaining the same end point. Given the complexity of PCB mixtures in environmental exposures, it is unlikely that a single endocrine perturbation will exclusively drive all of the observed adverse effects. End points such as auditory thresholds, which seem to be more exclusively regulated by thyroid hormones, may be good candidates for further development into models for low-dose endocrine (thyroid hormone)-related effects. Another problem is the complexity and differences in the mixtures of PCBs that are available commercially, that are present in the environment, or are measured in human samples, e.g., human milk. Different compositions may result in different spectra of toxicity outcomes. Models using defined reconstituted mixtures, relevant to human exposure, may enhance the consistency of findings between different laboratories.

What Is the Quality of the Human Exposure Data (Dose, Timing)?

Although we have voluminous data on PCBs in several studies, the quality of the exposure data from the various human cohorts studied is highly variable. In some cases there is extensive data on congener-specific PCB, PCDD, and PCDF analysis in repetitive samples taken from the same individual over time; in others there may be only a sum PCB measure or just fish consumption data. The methodology used for analyzing PCBs by different laboratories varies greatly. This includes issues such as lipid-adjusted (gravimetrically or triglyceride/cholesterol basis) or wet-weight adjusted, differences in limits of detection, types and numbers of congeners analyzed, etc.

Although there is extensive research literature on the effects of PCBs, failure to adequately describe the test substance or verify the nominal test concentration compromises the utility of some results. Another limitation is the relatively small range of exposure levels within a given human study; this hampers the establishment of significant dose–response relationships. Intercalibration of laboratory analyses may allow comparisons between several cohorts with different background exposure levels.

What Is the Spectrum of Observed Effects?

The reader is referred to the tables and text presented in this paper.

What Has Been the Utility of Specific Animal and in Vitro Models?

In general, the animal data have been extremely useful in establishing biologic plausibility and suggesting directions for human studies. In vitro and in vivo models have been pivotal in the advancement of our understanding of mechanism(s) of action, of dose-response relationships, of susceptibility differences between strains or species, of critical time windows of exposure and effects, and on hazard identification in general. Studies using wildlife species are of value in assessing health effects when observed effects can be shown not to be due to concomitant exposures.

Recommendations

Confirmation and extension of previous findings in humans should be further investigated. Areas of focus should include neurodevelopmental, thyroid, immunologic, and reproductive effects.

Studies assessing the relation between early-life PCB exposure, thyroid hormone levels, and sensorineural hearing impairment in children are needed. If changes in sensorineural hearing impairment are observed, children should be followed to determine if early impairment results in later deficits in verbal ability.

End points that are more exclusively regulated by single hormonal influences, such as sensorineural hearing and thyroid hormone regulation, should be further studied and developed into validated models for in vivo tests for potential effects of endocrine disruptors.

Given the complex nature of mixtures of PCBs that human individuals are exposed to and the dependency of toxic outcome to the congener composition of mixtures, it is essential to design and distribute environmentally relevant reconstituted PCB mixtures to improve comparability of studies and consistency of results.

A research priority is for studies in appropriate animal models (including wildlife animal models) to determine if there is a causal relationship between PCB congeners, endocrine changes, and developing neurologic parameters. Characterization of dose–response relationships should be a central objective of such studies.

When interpreting study results that appear to be key to establishing an effect (or lack of effect), the quality and completeness of the characterization of the test materials should be part of the interpretation. Analytical chemical criteria should be developed to aid in the objective analysis of such data. The analytical criteria should promote consistency among studies in the PCB congeners measured, with detailed methodology

and quality assurance/quality control information presented for all studies. Details on methods of PCB determination for individual congeners, including recoveries and limits of detection, should be reported. The method of lipid determination and adjustment should be included. For truncated data, the number of samples included in any averaged data set should be stated and the methods used to deal with values below the limit of detection must be explained.

Because the distribution of PCB congeners varies with lipid content, this distribution must be determined in order to apply a lipid adjustment to a particular sample matrix to be used as an indication of body burden.

Ideally, a proficiency program would be established to certify all laboratories performing congener-specific analyses. Proficiency samples would be available for all relevant sample matrices, including serum, plasma, whole blood, milk, fat, and relevant tissues as needed. Proficiency would be demonstrated by participating laboratories on a regular basis. Each matrix would contain the analytes at concentrations normally encountered in population studies. Lipid analysis would be included in the proficiency program.

Vectors of exposure and the resulting levels of PCBs in human populations need to be characterized more fully. There is little data on foods that include meats, fish, fruits and vegetables, processed foods, beverages, etc. The contribution from inhalation exposures, both indoor and outdoor, need to be characterized.

Characterization of congener distributions in human tissues of interest is needed, especially for the OH- and sulfonated PCB metabolites.

Interlaboratory quality control of exposure data should stimulate the interactions and comparability of data from various study cohorts with different background exposure levels to PCBs. This will enhance the quality of estimates of dose–response relationships.

Issues of analytical quality and composition of mixtures for experimental studies should be coordinated by a working group under the auspices of an international organization.

REFERENCES AND NOTES

1. Kimbrough RD, Jensen AA. Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products. ISBN 0-444-41597-1. New York:Elsevier, 1989.
2. Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24:87–149 (1994).
3. Golden RJ, Noller KL, Titus-Ernstoff I, Kaufman RH, Mittendorf R, Stillman R, Reese EA. Environmental endocrine modulators and human health: an assessment of the biological evidence. Crit Rev Toxicol 28(2):109–227 (1998).
4. Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, Bergman A, Visser TJ. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicol Ind Health 14(1/2):59–84 (1998).

5. Poellinger L. Mechanism of signal transduction by the basic helix-loop-helix dioxin receptor. In: Inducible Gene Expression, Vol 1 (Baeuerle PA, ed). Boston:Birkhauser, 1995;177–205.
6. Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD. Estrogen receptor-binding activity of polychlorinated hydroxy-biphenyls conformationally restricted structural probes. Mol Pharmacol 33:120–126 (1987).
7. Fielden MR, Chen I, Chittim B, Safe SH, Zacharewski TR. Examination of the estrogenicity of 2,4,6,2′,6′-pentachlorobiphenyl (PCB 104), its hydroxylated metabolite 2,4,6,2′,6′-pentachloro-4-biphenylol (HO-PCB 104), and a further chlorinated derivative, 2,4,6,2′4′6′-hexachlorobiphenyl (PCB 155). Environ Health Perspect 105:1238–1248 (1997).
8. Connor K, Ramamoorthy K, Moore M, Mustain M, Chen I, Safe S, Zacharewski T, Gillesby B, Joyeux A, Balaguer P. Hydroxylated polychlorinated biphenyls (PCBs) as estrogens and antiestrogens: structure-activity relationships. Toxicol Appl Pharmacol 145:111–123 (1997).
9. Moore M, Mustain M, Daniel K, Chen I, Safe S, Zacharewski T, Gillesby B, Joyeuxz A, Balaguer P. Antiestrogenic activity of hydroxylated polychlorinated biphenyl congeners identified in human serum. Toxicol Appl Pharmacol 142:160–168 (1997).
10. Kramer VJ, Klasson-Wehler E, Bergman A, Giesy JP. Hydroxylated PCB metabolites are antiestrogenic in a stably transfected human breast adenocarcinoma (MCF7) cell line. Toxicol Appl Pharmacol 144:363–376 (1997).
11. Krishnan V, Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), and dibenzofurans (PCDFs) as antiestrogens in MCF-7 human breast cancer cells: quantitative structure-activity relationships. Toxicol Appl Pharmacol 120:55–61 (1993).
12. McKinney JD, Chae K, Oatley SJ, Blake CCF. Molecular interactions of toxic chlorinated dibenzo-p-dioxins and dibenzofurans with thyroxine binding prealbumin. J Med Chem 28:375–381 (1985).
13. Visser TJ, Kaptein E, Van Toor H, Van Raay JAGM, Van den Berg KJ, Tjong Tjin Joe C, Van Engelen JGM, Brouwer A. Glucuronidation of thyroid hormone in rat liver: effects of in vivo treatment with microsomal enzyme inducers and in vitro assay conditions. Endocrinology 133:2177–2186 (1993).
14. Morse DC, Groen D, Veerman M, Van Amerongen CJ, Koeter HBWM, Smits-Van Prooije AE, Visser TJ, Koeman JH, Brouwer A. Interference or polychlorinated biphenyls in hepatic and brain thyroid hormone metabolism in fetal and neonatal rats. Toxicol Appl Pharmacol 122:27–33 (1993).
15. Van Birgelen APJM, Van der Kolk J, Fase K, Bol I, Poiger H, Brouwer A, Van den Berg M. Toxic potency of 3,3′,4,4′,5-pentachlorobiphenyl relative to and in combination with 2,3,7,8-tetrachlorodibenzo-p-dioxin in a subchronic feeding study in the rat. Toxicol Appl Pharmacol 127:209–221 (1994). •
16. Van Birgelen APJM, Van der Kolk J, Fase K, Bol I, Poiger H, Van den Berg M, Brouwer A. Toxic potency of 2,3,3′,4,4′,5-hexachlorobiphenyl relative to and in combination with 2,3,7,8-tetrachlorodibenzo-p-dioxin in a subchronic feeding study in the rat. Toxicol Appl Pharmacol 126:202–213 (1994).
17. Van Birgelen APJM, Smit EA, Kampen IM, Groeneveld CN, Fase KM, Van der Kolk J, Poiger H, Van den Berg M, Koeman JH, Brouwer A. Subchronic effects of 2,3,7,8-TCDD or PCBs on thyroid hormone metabolism: use in risk assessment. Eur J Pharmacol [Environ Toxicol Pharmacol Section] 293:77–85 (1995).
18. Bastomsky CH. Effect of a polychlorinated biphenyl mixture (Aroclor 1254) and DDT on biliary thyroxine excretion in rats. Endocrinology 101:1150–1155 (1975).
19. Bastomsky CH, Murthy PVN. Enhanced in vitro hepatic glucuronidation of thyroxine in rats following cutaneous application or ingestion of polychlorinated biphenyls. Can J Physiol Pharmacol 54:23–26 (1976).
20. Saito K, Kaneko H, SK, Yoshitake A, Yamada H. Hepatic UDP-glucuronyl transferase(s) activity toward thyroid hormones in rats: induction and effects on serum thyroid hormone levels following treatment with various enzyme inducers. Toxicol Appl Pharmacol 111:99–106 (1991).
21. Barter RA, Klaassen CD. Reduction of thyroid hormone levels and alteration of thyroid function of four representative UDP-glucuronyl transferase inducers in rats. Toxicol Appl Pharmacol 128:9–17 (1994).
22. Adams C, Lans MC, Klasson-Wehler E, Van Engelen JGM, Visser TJ, Brouwer A. Hepatic thyroid hormone 5′-deiodinase another target protein for monohydroxy metabolites of 3,3′,4,4′-tetrachlorobiphenyl. Organohalogen Comp 1:51–54 (1990).
23. Rickenbacher U, Jordan S, McKinney JD. Structurally specific interaction of halogenated dioxin and biphenyl derivatives with iodothyronine-5′-deiodinase in rat liver. ACS Symp Series 413:354–365 (1989).
24. Schuur AG, Legger FF, Van Meeteren ME, Moonen MJH, Van

Leeuwen-Bol I, Bergman A, Visser TJ, Brouwer A. In vitro inhibition of thyroid hormone sulfation by hydroxylated metabolites of halogenated aromatic hydrocarbons. Chem Res Toxicol 11:1075–1081 (1998).
25. Schuur AG, Van Leeuwen-Bol I, Jong WMC, Bergman A, Coughtrie MWH, Brouwer A, Visser TJ. In vitro inhibition of thyroid hormone sulfation by polychlorobiphenylols: isozyme specificity and inhibition kinetics. Toxicol Sci 45: 188–194 (1998)
26. Shiverick KT, Muther TF. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) effects on hepatic microsomal steroid metabolism and serum estradiol of pregnant rats. Biochem Pharmacol 32:991–995 (1983).
27. Drenth HJ, Bouwman CA, Seinen W, Van den Berg M. Effects of some persistent halogenated environmental contaminants on aromatase (CYP 19) activity in the human choriocarcinoma cell line JEG-3. Toxicol Appl Pharmacol 148:50–55 (1998).
28. Yoshihara S, Nagata K, Wada I, Yoshimura H, Kuroki H, Masuda Y. A unique change of steroid metabolism in rat liver microsomes induced with highly toxic polychlorinated biphenyl (PCB) and polychlorinated dibenzofuran (PCDF). J Pharm Dyn 5:994–1004 (1982).
29. Brouwer A, Van den Berg KJ. Binding of a metabolite of 3,4,3′,4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol Appl Pharmacol 85:301–312 (1986).
30. Lans MC, Klasson-Wehler E, Willemsen M, Meussen E, Safe S, Brouwer A. Structure-dependent competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-p-dioxins and -dibenzofurans with human transthyretin. Chem-Biol Interact 88:7–21 (1993).
31. Lans MC, Spiertz C, Brouwer A, Koeman JH. Different competition of thyroxine binding to transthyretin and thyroxine-binding globulin by hydroxy-PCBs, PCDDs and PCDFs. Eur J Pharmacol [Environ Toxicol Pharmacol Section] 270:129–136 (1994).
32. Morse DC, Klasson-Wehler E, Wesseling W, Koeman JH, Brouwer A. Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). Toxicol Appl Pharmacol 136:269–279 (1996).
33. Darnerud PO, Morse DC, Klasson-Wehler E, Brouwer A. Binding of a 3,3′,4,4′-tetrachlorobiphenyl (CB-77) metabolite to fetal transthyretin and effects on fetal thyroid hormone levels in mice. Toxicology 106:105–114 (1996).
34. Lund J, Brandt I, Poellinger L, Bergman A, Klasson-Wehler E, Gustafsson J-A. Target cells for the polychlorinated biphenyl metabolite 4,4′-bis(methylsulfonyl)-2,2′,5,5′-tetrachlorobiphenyl: characterization of high affinity binding to rat and mouse lung cytosol. Mol Pharmacol 27:314–323 (1985).
35. Schuur AG. Interactions of Halogenated Aromatic Hydrocarbons with Thyroid Hormone Metabolism. PhD Thesis. ISBN 90-5485-940-7. Wageningen, The Netherlands:Agricultural University, 1998.
36. Brouwer A. Inhibition of thyroid hormone transport in plasma of rats by polychlorinated biphenyls. Arch Toxicol Suppl 13:440–445 (1989).
37. Van den Berg KJ, Zurcher C, Brouwer A. Effects of 3,4,3,4-tetrachlorobiphenyl on thyroid function and histology in marmoset monkeys. Toxicol Lett 41:77–86 (1988).
38. Byrne JJ, Carbonne JP, Hanson EA. Hypothyroidism and abnormalities in the kinetics of thyroid hormone metabolism in rats treated chronically with polychlorinated biphenyl and polybrominated biphenyl. Endocrinology 121:520–527 (1987).
39. Gray LJr, Ostby J, Marshall R, Andrews J. Reproductive and thyroid effects of low-level polychlorinated biphenyl (Aroclor 1254) exposure. Fundam Appl Toxicol 20:288–294 (1993).
40. Goldey ES, Kehn LS, Lau C, Rehnberg GL, Crofton KM. Developmental exposure to PCBs (Aroclor 1254) reduce circulating thyroid hormone concentrations and causes hearing deficits in rats. Toxicol Appl Pharmacol 135:77–88 (1995).
41. Ness DK, Schantz SL, Moshtaghian J, Hansen LG. Effects of perinatal exposure to specific PCB-congeners on thyroid hormone concentrations and thyroid histology in the rat. Toxicol Lett 68:311–323 (1993).
42. Bastomsky CH. Enhanced thyroxine metabolism and high uptake goiters after a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Endocrinology 101:292–296 (1977).
43. Brewster DW, Elwell MR, Birnbaum LS. Toxicity and disposition of 2,3,4,7,8-pentachlorodibenzofuran (peCDF) in the rhesus monkey (Macaca mulatta). Toxicol Appl Pharmacol 93:231–246 (1988).
44. Collins WT, Capen CC, Kasza L, Carter C, Dailey RE. Effect of polychlorinated biphenyl (PCB) on the thyroid gland of rats. Ultrastructural and biochemical investigations. Am J Pathol 89:119–130 (1977).
45. Collins WT, Capen CC. Biliary excretion of 125I-thyroxine and fine structural alterations in the thyroid glands of Gunn rats fed polychlorinated biphenyls (PCBs). Lab Invest 43:158–164 (1980).

46. Collins WT, Capen CC. Fine structural lesions and hormone alterations in thyroid glands of perinatal rats exposed *in utero* and by milk to polychlorinated biphenyls. Am J Pathol 99:125–142 (1980).

47. Collins WT, Capen CC. Ultrastructural and functional alterations of the rat thyroid gland produced by polychlorinated biphenyls compared with iodide excess and deficiency, and thyotropin and thyroxine administration. Virchows-Arch B Cell Path 33:213–231 (1980).

48. Sepkovic DW, Byrne JJ. Kinetic parameters of L-[125I]-triiodothyronine degradation in rats pretreated with polyhalogenated biphenyls. Food Chem Toxicol 22:743–747 (1984).

49. Tryponas L, Truelove J, Zawidzka Z, Wong J, Mes J, Charbonneau S, Grant DL, Campbell JS. Polychlorinated biphenyl (PCB) toxicity in adult cynomolgus monkeys (*Macaca fascicularis*): a pilot study. Toxicol Pathol 12:10–25 (1984).

50. Kazka L, Collins WT, Capen CC, Garthoff LH, Friedman L. Comparative toxicity of polychlorinated biphenyl and polybrominated biphenyl in the rat thyroid gland. Light and electron microscopic alterations after subacute dietary exposure. J Environ Pathol Toxicol 1:587–599 (1978).

51. Gupta BN, McConnell EE, Goldstein JA, Harris MW, Moore JA. Effects of polybrominated biphenyl mixture in the rat and mouse. I: Six month exposure. Toxicol Appl Pharmacol 68:1–18 (1983).

52. Barsotti DA, Abrahamson LJ, Allen JR. Hormonal alterations in female rhesus monkeys fed a diet containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxins. Bull Environ Contam Toxicol 21:463–469 (1979).

53. Lambrecht LK, Barsotti DA, Allen JR. Responses of nonhuman primates to a polybrominated biphenyl mixture. Environ Health Perspect 23:139–145 (1978).

54. Allen JR, Barsotti DA, Lambrecht LK, Van Miller JP. *Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates.* Ann N Y Acad Sci 330:419–425 (1979).

55. Jansen HT, Cooke PS, Porcelli J, Liu TC, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: *in vitro* and *in vivo* studies. Reprod Toxicol 7:237–248 (1993).

56. Gellert JR. Uterotrophic activity of polychlorinated biphenyls and induction of precocious reproductive ageing in neonatally treated female rats. Environ Res 16:123–130 (1978).

57. Cecil HC, Bitman J, Harris SJ. Estrogenicity of *o,p'*-DDT in rats. J Agr Food Chem 19:61–65(1971).

58. Ecobichon DJ, MacKenzie DO. The uterotropic activity of commercial and isomerically-pure chlorobiphenyls in the rat. Res Commun Chem Pathol Pharmacol 9:85–95 (1974).

59. Reijnders PJH. Reproductive failure in common seals feeding on *fish from polluted coastal waters.* Nature 324:456–457 (1986).

60. Brouwer A, Reijnders PJH, Koeman JH. Polychlorinated biphenyl (PCB)-contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (*Phoca vitulina*). Aquatic Toxicol 15:99–106 (1989).

61. Van den Berg M, Craane BLHJ, Sinnige T, Van Mourik S, Dirksen S, Boudewijn T, Van der Gaag M, Lutke-Schipholt IJ, Spenkelink A, Brouwer A. Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant (*Phalacrocorus carbo*) after *in ovo* exposure. Environ Toxicol Chem 13:803–816 (1994).

62. Murk AJ, Van den Berg JHJ, Fellinger M, Rozemeijer MJC, Swennen C, Duiven P, Boon JP, Brouwer A, Koeman JH. Toxic and biochemical effects of 3,3',4,4'-tetrachlorobiphenyl (CB-77) and Clophen A-50 on eider ducklings (*Somateria mollissima*) in a semi-field experiment. Environ Pollut 86:21–30 (1994).

63. Leatherland JF. Field observations on reproductive and developmental dysfunction in introduced and native salmonids from the Great Lakes. J Great Lakes Res 19:737–751 (1993).

64. Leatherland JF. Changes in thyroid hormone economy following consumption of environmentally contaminated Great Lakes fish. Toxicol Ind Health 14(1/2):41–57 (1998).

65. Moccia RD, Fox GA, Britton A. A quantitative assessment of thyroid histopathology of herring gulls (*Larus argentatus*) from the Great Lakes and a hypothesis on the causal role of environmental contaminants. J Wildl Dis 22:60–70 (1986).

66. Auerlich RJ, Bursian SJ, Breslin WJ, Olson BA, Ringer RK. Toxicological manifestations of 2,4,5,2',4',5'-, 2,3,6,2',3',6'-, and 3,4,5,3',4',5'-hexachlorobiphenyl and Aroclor 1254 in mink. J Toxicol Environ Health 15:63–79 (1985).

67. Stein SA, Kirkpatrick LL, Shanklin DR, Adams PM, Brady ST. Hypothyroidism reduces the rate of slow component A (Sca) axonal transport and the amount of transported tubulin in the hyt/hyt mouse optic nerve. J Neurosci Res 28:121–133 (1991).

68. Gould E, Frankfurt M, Westlind-Danielsson A, McEwen BS. Developing forebrain astrocytes are sensitive to thyroid hormone. Glia 3:283–292 (1990).

69. Morse DC, Plug A, Wesseling W, Van den Berg KJ, Brouwer A. Persistent alterations in regional brain glial fibrillary acidic protein and synaptophysin levels following pre- and postnatal polychlorinated biphenyl exposure. Toxicol Appl Pharmacol 139:252–261 (1996).

70. Baumgartner A, Campos-Barros A. Thyroid hormones and depressive disorders—clinical overview and perspectives. Part 2: Thyroid hormones and the central nervous system-basic research. Nervenartz 64:11–20 (1993).

71. Seegal RF, Shain W. Neurotoxicity of polychlorinated biphenyls. In: The Vulnerable Brain and Environmental Risks. Vol 3 (Isaacson RL, Jensen KF, eds). New York:Plenum Press, 1992;169–195.

72. Seegal. RF. The neurochemical effects of PCB exposure are age-dependent. Arch Toxicol Suppl 16:128–137 (1994).

73. Morse DC, Seegal RF, Brosch KO, Brouwer A. Long-term alterations in regional brain serotonin metabolism following maternal polychlorinated biphenyl exposure in the rat. Neurotoxicology 17:631–638 (1996).

74. Lilienthal H, Weinand-Harer A, Winterhoff H, Winneke G. Effects of maternal exposure to 3,3',4,4'-tetrachlorobiphenyl or propylthiouracil in rats trained to discriminate apomorphine from saline. Toxicol Appl Pharmacol 146:162–169 (1997).

75. Freeman S, Sohmer H. Effect of thyroxine on the development of somatosensory and visual evoked potentials in the rat. J Neurol Sci 128:143–150 (1995).

76. Schantz SL, Bowman RE. Learning in monkeys exposed perinatally to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Neurotoxicol Teratol 11:13–19 (1989).

77. Schantz SL, Levin ED, Bowman RE, Heironimus MP, Laughlin NK. Effects of perinatal PCB exposure on discrimination-reversal learning in monkeys. Neurotoxicol Teratol 11:243–250 (1989).

78. SL, Ferguson SA, Bowman RE. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on behavior of monkeys in peer groups. Neurotoxicol Teratol 14:433–446 (1992).

79. Sauer PJJ, Huisman M, Koopman-Esseboom C, Morse DC, Smits-Van Prooije AE, Van den Berg KJ, Tuinstra LGMTh, Van der Paauw CG, Boersma ER, Weisglas-Kuperus N, et al. Effects of polychlorinated biphenyls (PCBs) and dioxins on growth and development. Hum Exp Toxicol 13:900–906 (1994).

80. Lilienthal H, Winneke G. Sensitive periods for behavioral toxicity of polychlorinated biphenyls: determination by cross-fostering in rats. Fundam Appl Toxicol 17:368–375 (1991).

81. Peterson RE, Theobald HM, Kimmel GL. Developmental and reproductive toxicity of dioxin and related compounds: crossspecies comparison. Crit Rev Toxicol 23:283–335 (1993).

82. Gray LE, Kelce WR, Monosson E, Ostby JS, Birnbaum LS. Exposure to TCDD during development permanently alters reproductive function in male LE rats and hamsters: reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. Toxicol Appl Pharmacol 131:108–118 (1995).

83. Sager DB. Effects of postnatal exposure to polychlorinated biphenyls on adult male reproductive function. Environ Res 31:76–94 (1983).

84. Sager DB, Giraud DM. Long-term effects on reproductive parameters in female rats after translactational exposure to PCBs. Environ Res 66:52–76 (1994).

85. Kociba RJ, Keyes DC, Beyer JE, Carreon RM, Wade EE, Dittenber DA, Kalnins RP, Franson LF, Park DN, Barnard SD, Hummel RA, Humiston CG. Results of a two-year chronic toxicity and carcinogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats. Toxicol Appl Pharmacol 46:279–303 (1978).

86. Mayes BA, McConnell EE, Neal BH, Brummer MJ, Hamilton SB, Sullivan TM, Peters AC, Ryan MJ, Toft JD, Singer AW, Brown JF, Menton RG, Moore JA. Comparative carcinogenicity in SD rats of the polychlorinated biphenyl mixtures Aroclors 1016, 1242, 1254 and 1260.Toxicol Sci 41:62–76 (1998).

87. Amaratunga M, Fish KM, Brown JF, Mayes BA, Malome J, May RJ, Silkworth JB. Estrogen metabolism by hepatic microsomes from rats chronically dosed with Aroclors 1016,1242,1254 and 1260. Toxicologist 36: 88 (1997).

88. Romkes M, Piskorska-Pliezczyneka J, Safe S. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic and uterine estrogen receptor levels in rats. Toxicol Appl Pharmacol 87:306–314 (1987).

89. Kimbrough RD, Squire RA, Linder RE, Strandberg JD, Montali RJ, Burse VW. Induction of liver tumor in Sherman strain

female rats by polychlorinated biphenyl Aroclor 1260. J Natl Cancer Inst 55:1453–1459 (1975).

90. McKinney JD, Moore L, Prokopetz A, Walters DB. Validated extraction and cleanup procedures for polychlorinated biphenyls and DDE in human body fluids and infant formula. J Assoc Off Anal Chem 67:122–129 (1984).

91. Sawyer LD. Quantitation of polychlorinated biphenyl residues by electron capture gas-liquid chromatography. J Assoc Off Anal Chem 61:272–291 (1978).

92. Webb RG, McCall AC. Quantitative PCB standards for electron capture gas chromatography. J Chromatogr Sci 11:366–373 (1973).

93. Brock JW, Burse VW, Ashley DL, Najam AR, Green E, Korver MP, Powell MK, Hodge CC, Needham LL. An improved analysis for chlorinated pesticides and polychlorinated biphenyls (PCBs) in human and bovine sera using solid-phase extraction. J Anal Toxicol 20:528–536 (1996).

94. Greizertein HB, Gigliotti P, Vena J, Freudenheim J, Kostyniak PJ. Standardization of a method for the routine analysis of polychlorinated biphenyl congeners and selected pesticides in human serum and milk. J Anal Toxicol 21:558–566 (1997).

95. Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24:87–149 (1994).

96. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus, Van der Paauw CG, Tuinstra LGMT, Boersma ER, Sauer PJJ. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 28:1721–32 (1994).

97. Gladen BC, Longnecker MP, Schecter AJ. Correlations among polychlorinated biphenyls, dioxins, and furans in humans. Am J Ind Med 35:15–20 (1999).

98. Parham FM, Kohn MC, Matthews HB, DeRosa C, Portier CJ. Using structural information to create physiologically based pharmacokinetic models for all polychlorinated biphenyls. Toxicol Appl Pharmacol 144:340–347 (1997).

99. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Polychlorinated dibenzo-*para*-dioxins and polychlorinated dibenzofurans. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol 69. Lyon:International Agency for Research on Cancer, 1997.

100. Longnecker MP, Rogan WJ, Lucier G. The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBs (polychlorinated biphenyls) and an overview of organochlorines in public health. Annu Rev Public Health 18:211–244 (1997).

101. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LGMT, Brouwer A, Sauer PJJ. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 36:468–473 (1994).

102. Pluim HJ, de Vijlder JJM, Olie K, Kok JH, Vulsma T, van Tijn DA, van der Slikke JW, Koppe JG. Effects of pre- and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. Environ Health Perspect 101:504–508 (1993).

103. Nagayama J, Iida T, Hirakawa H, Matsueda T, Okamura K, Hasegawa M, Sato K, Ma HY, Yanagawa T, Igarashi H, et al. Effects of lactational exposure to chlorinated dioxins and related chemicals on thyroid functions in Japanese babies. Organohalogen Comp 33:446–450 (1997).

104. Jacobson JL, Jacobson SW. Teratogen update: polychlorinated biphenyls. Teratology 55:338–347 (1997).

105. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, Tinglestad J, Tully M. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: effects of maternal factors and previous lactation. Am J Public Health 76:172–177 (1986).

106. Tilson HA, Jacobson JL, Rogan WJ. Polychlorinated biphenyls and the developing nervous system: cross-species comparison. Neurotoxicol Teratol 12:239–248 (1990).

107. Rylander L, Stromberg U, Dyremark E, Ostman C, Nilsson-Ehle P, Hagmar L. Polychlorinated biphenyls in blood plasma among Swedish female fish consumers in relation to low birth weight. Am J Epidemiol 147:493–502 (1998).

108. Taylor PR, Stelma JM, Lawrence CE. The relation of polychlorinated biphenyls to birth weight and gestational age in the offspring of occupationally exposed mothers. Am J Epidemiol 129:395–406 (1989).

109. Loomis D, Browning SR, Schenck AP, Gregory E, Savitz DA. Cancer mortality among electric utility workers exposed to polychlorinated biphenyls. Occup Environ Med 54:720–728 (1997).

110. Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N. Blood levels of organochlorine residues and risk of breast cancer. J Natl Cancer Inst 85:648–652 (1993).

111. Krieger N, Wolff MS, Hiatt RA, Rivera M, Vogelman J, Orentreich N. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. J Natl Cancer Inst 86:589–599 (1994).

112. Hunter DJ, Hankinson SE, Laden F, Colditz GA, Manson JE, Willett WC, Speizer FE, Wolff MS. Plasma organochlorine levels and the risk of breast cancer. N Engl J Med 337:1253–1258 (1997).

113. Moysich KB, Ambrosone CB, Vena JE, Shields PG, Mendola P, Kostyniak P, Greizerstein H, Graham S, Marshall JR, Schisterman EF, et al. Environmental organochlorine exposure and postmenopausal breast cancer risk. Cancer Epidemiol Biomarkers Prev 7:181–188 (1998).

114. Rothman N, Cantor KP, Blair A, Bush D, Brock JW, Helzlsouer K, Zahm SH, Needham LL, Pearson GR, Hoover RN, et al. A nested case-control study of non-Hodgkin lymphoma and serum organochlorine residues. Lancet 350:240–244 (1997).

115. Emmett EA, Maroni M, Jefferys J, Schmith J, Levin BK, Alvares A. Studies of transformer repair workers exposed to PCBs: II. Results of clinical laboratory investigations. Am J Ind Med 14:47–62 (1988).

116. Murai K, Okamura K, Tsuji H, Kajiwara E, Watanabe H, Akagi K, Fujishima M. Thyroid function in "Yusho" patients exposed to polychlorinated biphenyls (PCB). Environ Res 44:179–187 (1987).

117. Hsu C-C, Yu M-LM, Chen Y-CJ, Guo Y-LL, Rogan WJ. The Yu-cheng rice oil poisoning incident. In: Dioxins and Health (Schecter A, ed). New York:Plenum Press, 1994;661–680.

118. Fiolet DCM, Cijpers CEJ, Lebret E. Exposure to polychlorinated organic compounds and thyroid hormone plasma levels of human newborns. Organohal Comp 34:459–465 (1997).

119. Jacobson JL, Jacobson SW, Fein GG, Schwartz PM, Dowler JK. Prenatal exposure to an environmental toxin: a test of the multiple effects model. Dev Psychol 20:523–532 (1984).

120. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, Tinglestad J, Tully M. Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr 109:335–341 (1986).

121. Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, van der Paauw CG, Tuinstra LGMTh, Weisglas-Kuperus N, Sauer PJJ, Touwen BCL, Boersma ER. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 41:111–27 (1995).

122. Lonky E, Reihman J, Darvill T, Mather J Sr, Daly H. Neonatal behavioral assessment scale performance in humans influenced by maternal consumption of environmentally contaminated Lake Ontario fish. J Great Lakes Res 22:198–212 (1996).

123. Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad J, Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. J Pediatr 113:991–995 (1988).

124. Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr 119:58–63 (1991).

125. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer-PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 97:700–706 (1996).

126. Lanting CI, Patandin S, Fidler V, Weisglas-Kuperus N, Sauer PJJ, Boersma ER, Touwen BCL. Neurological condition in 42-month-old children in relation to pre- and postnatal exposure to polychlorinated biphenyls and dioxins. Early Hum Dev 50:283–292 (1988).

127. Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. N Engl J Med 335:783–789.

128. Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, Dowler JK. The effect of intrauterine PCB exposure on visual recognition memory. Child Dev 56:853–860 (1985).

129. Jacobson JL, Jacobson SW, Humphrey HEB. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 116:38–45 (1990).

130. Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr 119:58–63 (1991).

Editorial Policy

Environmental Health Perspectives (EHP) is a forum for the discussion of issues in environmental health, and several formats have been devised for that purpose. All scientific articles are subject to rigorous peer review. The primary criteria for publication are environmental significance and scientific quality. Articles are generally published online within eight weeks after acceptance. Publication in paper form will then occur within about three to four months after Internet publication.

Environmental science and medicine is made up of many fields; therefore, we are prepared to consider scientific progress in all of them. Cross-fertilization and serendipity have proven to be extremely important processes in the advancement of science in general, and this must hold true for the science of environmental health. We will consider for publication articles ranging from the most basic molecular biology to environmental engineering. We particularly encourage those researchers concerned with mechanisms of toxicity and new approaches for detecting and/or remedying environmental damage. We also encourage the submission of articles related to the identification and characterization of genes involved in the manifestation of environment-related diseases.

Opinions and ideas based on scientific observation and argument are welcome. Although the expression of opinions may lead to debate and disagreement, such reactions are healthy and can lead to new research and discoveries. Presentation of ideas and opinions will be promoted, but our policy is to strive for objectivity and balance.

While the condition of all forms of life is of concern to our readership, the ultimate effect of environmental degradation is on human health and, in this regard, our intention is to serve both the scientific and medical communities. Environment-related diseases are a reality of everyday life for practicing physicians and, in order that they might be rendered more aware of current problems, we feature a section dedicated to the examination and evaluation of specific case records. Cases presented must be scholarly, scientifically rigorous, and devoid of unsubstantiated anecdotal opinion.

In addition to scientific articles and discussion, we publish news of issues that affect the environment and human health. The Environews section disseminates technical scientific and related information in a manner that is comprehensible and usable by an informed lay, medical, or scientific audience.

Research articles accepted for publication in *EHP* will be published initially on the Internet at http://ehis.niehs.nih.gov and subsequently in printed form. This process substantially reduces time to publication, thus enabling the establishment of priority. Research articles accepted for publication in *Environmental Health Perspectives Supplements* are not published in advance of the printed version.

Page charges are $55.00/page for *EHP* and $40.00/page for *EHP Supplements.* The corresponding author will receive one copy of the journal free of charge for each author. Reprints may be purchased by returning an order form with the page proofs. Late reprint orders will incur an administrative fee.

PERSPECTIVES

The journal is a forum for the expression of ideas and opinions. Opinions and ideas should be carefully considered and based on scientific principles. All articles are subject to peer review. Four formats are offered:

Editorial statements are published by our editors, members of our editorial board, and occasional guest editors. These statements focus attention on important or neglected areas of environmental health, offer opinions and ideas, and stimulate discussion.

Commentaries are up-to-date articles that present ideas and opinions offering perspective and insight on a particular topic. Data may be included to substantiate arguments. Abstracts are required and articles must be appropriately referenced.

Reviews are narrowly focused articles that emphasize recent developments in a particular field of research. Lengthy historical perspectives are not appropriate.

Correspondence is encouraged. Opinions, perspectives, and insight are welcome. Comments on articles published in *EHP* are also welcome, but criticism will always be balanced by the opportunity for defense and clarification.

MEDICINE

This section is targeted to medical workers and others engaged in the environmental and occupational health arenas. Articles are subject to peer review.

Grand Rounds in Environmental Medicine are case presentations of actual patients that elegantly demonstrate a scenario that arises not uncommonly in environmental medicine. The discussant should be a recognized expert who provides a state-of-the-art approach to evaluating and managing the patient as well as a well-referenced but succinct discussion of the scientific issues involved. Emphasis should be placed on selecting cases based on environmental significance, the rationale and justification provided for the clinical approach, an insightful and balanced discussion of scientific issues, and the conciseness and clarity of the writing. The discussant may also touch on broader (e.g., public health, legal, ethical) issues raised by the case.

RESEARCH

To ensure fairness in the review process, we routinely seek opinions from three reviewers. Suggestions for reviewers of manuscripts will be considered. The research portion of the journal consists of three formats:

Research Articles are original manuscripts reporting scientific research and discovery in the broad field of environmental health. Research articles may come from any field of scientific research, from the most basic molecular biology and biochemistry to atmospheric physics, ecology, and engineering. The criteria for publication are weighted toward scientific quality and environmental significance. The work will be assessed according to its originality, scientific merit, and experimental design; the manuscript will be evaluated on the basis of its conciseness, clarity, and presentation. We also attempt to address certain ethical problems during the review process. We require assurances that all human and animal subjects have been treated humanely and with due regard for the alleviation of suffering. Manuscript review also considers scientific integrity as part of the process.

Meeting Reports are short summaries of conferences, symposia, or workshops in which the scientific objectives and achievements of a meeting are described.

Workshop Summaries are reports by expert scientific committees that include reviews of existing information and that summarize research findings on specific topics, present new information, and recommend methods, courses of action, or further research needs for the scientific community. Abstracts are required for Workshop Summaries.

ENVIRONEWS

The Environews section provides up-to-date information on important issues in environmental health, covering a variety of areas including but not limited to policy, legislative, and regulatory actions; innovative technological and conceptual research advances; conference and meeting summaries; and emerging environmental problems. The Environews section consists of several components:

Forum articles are brief reports on topics of environmental health significance such as recent research advances, national and international meetings, contamination