Polychlorinated Biphenyls as Disruptors of Thyroid Hormone Action

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are well known to reduce the concentrations of thyroid hormones in the circulation of experimental animals (Bastomsky *et al.*, 1976; Brouwer *et al.*, 1998). Moreover, circulating levels of PCBs have been reported to co-vary with various measures of thyroid status in humans (Koopman-Esseboom *et al.*, 1997; Osius *et al.*, 1999). These observations form the basis for the hypothesis that PCBs disrupt thyroid hormone action by reducing circulating levels of thyroid hormone. This hypothesis is particularly important to explore because thyroid hormone is known to be essential in brain development and because PCB contamination is enormously widespread. Therefore, it is possible that PCBs may lead to neurological abnormalities in humans and animals by interfering with thyroid hormone action. Linking the known effects of PCB contamination on neurological development to disruption of thyroid hormone action is difficult for two basic reasons. First, there are many gaps in our understanding of the interaction of PCBs with the thyroid signaling system. Therefore, it is difficult to predict how PCBs may interfere with thyroid hormone action. Second, there are many gaps in our understanding of the mechanisms by which thyroid hormone acts during development. Therefore, it is difficult to determine whether the neurological effects of PCB exposure are similar to those effects predicted by the hypothesis of thyroid disruption. The goal of this review is to frame what is known about the interaction of PCBs with the thyroid system within the context of what is known about the mechanism of thyroid hormone action on brain development. The emphasis will be on PCB disruption of thyroid hormone *action* as opposed to thyroid *function*.

PCBS AND CIRCULATING THYROID HORMONE

Experimental Animals

Bastomsky was among the first to show that an industrial mixture of PCBs (Aroclor 1254) could reduce circulating levels of thyroid hormone in the rat (Bastomsky, 1974; Bastomsky *et al.*, 1976). This work was motivated in part by previous reports that PCBs increased thyroid gland size in seagulls (Jefferies and Parslow, 1972), and that hepatic accumulation of ^{125}I-thyroxine (T_4) was caused by other chlorinated hydrocarbons, such as chlordane (Bernstein *et al.*, 1968). Bastomsky found that dermal application of Aroclor 1254 reduced circulating levels of total T_4 by approximately 70%. However, total T_3 was not altered and TSH was not measured. The profound hypothyroxinemia following Aroclor 1254 administration has been amply confirmed (see the excellent review by Brouwer *et al.*, 1998). Most of these studies have administered the PCB mixture in the food (e.g., Byrne *et al.*, 1987) or by gavage (e.g., Goldey *et al.*, 1995a). In general, all reports document that mixtures of PCBs such as Aroclor 1254 profoundly decrease circulating total T_4, but that there is little or no effect on circulating total T_3 or free T_3.

Many studies have also investigated the ability of specific PCB congeners to reduce circulating levels of thyroid hormone. There are over 200 individual PCB congeners based on the pattern of chlorine substitutions. In general, PCB congeners can be broadly categorized according to their dioxin-like activity. PCBs with zero or one ortho chlorine, two para chlorines and at least two meta chlorines, can adopt a planar structure similar to that of TCDD and can bind to and activate the aryl hydrocarbon receptor (AhR) (Tilson and Kodavanti, 1997). In contrast, *ortho*-substituted PCBs may adopt a non-coplanar conformation that does not act through the AhR, but nevertheless produce neurotoxic effects (Fischer *et al.*, 1998; Seegal and Shain, 1992). Several studies have compared the hypothyroxinemic effects of specific PCB congeners that represent these different classes. Ness *et al.* (1993) found that the non coplanar PCB 153 and the mono-ortho coplanar PCB 118 reduced serum total T_4 in a dose-dependent manner. In contrast, PCB 28 did not reduce total T_4. Seo *et al.* (1995) reported a gender difference in the effects of PCB 77 and TCDD; low doses of PCB 77 and TCDD reduced total T_4 in females but not in males; whereas higher doses reduced total T_4 in both genders.

In general, congener-specific studies demonstrate that both *ortho*- and non *ortho*-substituted PCB congeners can reduce circulating levels of T_4. There are many differences in the design of these studies that make drawing general conclusions difficult. For example, the pattern and duration of PCB exposure and the timing of T_4 measurements, both relative to PCB exposure and timing during development, are quite different among the many studies. However, several studies indicate that T_4 levels in fetal or neonatal serum appear to be more sensitive to PCB exposure than in the adult (Morse *et al.*, 1993; Seo *et al.*, 1995).

At least three mechanisms may account for the ability of PCBs to reduce circulating levels of thyroid hormone (also reviewed by Brouwer *et al.*, 1998). First, PCBs have been reported to alter the structure of the thyroid gland, perhaps directly affecting thyroid function (Collins and Capen, 1980a; Collins and Capen, 1980b; Kasza *et al.*, 1978). These observations, though not extensively pursued since their publication, are consistent with the report of Byrne *et al.* that PCB exposure reduces the ability of TSH to increase serum T_4 *in vivo* (Byrne *et al.*, 1987). Thus, PCBs may directly interfere with the ability of the thyroid gland to respond to TSH. Second, PCBs can alter thyroid hormone metabolism. Early work demonstrated that

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PCB exposure increased the rate of bile flow and increased the biliary excretion of ^{125}I-T_4 (Bastomsky *et al.*, 1976). Moreover, PCB exposure induces the expression and activity of UDP-glucuronosyltransferase (UDP-GT) (Kolaja and Klaassen, 1998) and increases T_4-glucuronidation (Visser *et al.*, 1993). In addition, PCB exposure selectively activates the glucuronidation of T_4 not T_3 (Hood and Klaassen, 2000), suggesting that this mechanism may account for the failure of PCBs to alter circulating T_3. Thus, PCB exposure may facilitate T_4 clearance from serum through liver metabolism, reducing the half-life of T_4 in the blood. Finally, specific PCB congeners can bind to thyroid hormone binding proteins in the blood, and potentially can displace T_4 from the protein *in vivo* (Brouwer *et al.*, 1998; Chauhan *et al.*, 1999). These three mechanisms may combine to reduce the carrying capacity of the blood for T_4, reduce the serum half-life of T_4, and reduce the ability of the thyroid gland to respond to TSH. Though it is not clear which among these potential mechanisms are most important for mediating the effects of PCBs on circulating levels of thyroid hormone, it is likely that all are important in experimental systems.

Humans

The reports described above clearly indicate that exposure to PCBs in experimental animals can reduce circulating levels of T_4. Based in part on these experiments, several recent studies have reported the relationship between circulating PCBs and circulating thyroid hormones in humans. However, because nearly everyone is contaminated with some level of PCBs, these studies are structured so that associations can be made between circulating PCBs and circulating thyroid hormones, rather than comparing exposed and unexposed populations. For example, Osius *et al.* (1999) recently studied 7- to 10-year-old school children in three German municipalities and found that serum concentrations of individual PCB congeners were associated with circulating TSH. In particular, they found a significant positive correlation between the concentration of the mono-*ortho* congener PCB 118 and TSH. Moreover, they found a significant negative correlation between several PCB congeners and free T_3. There was no correlation between circulating levels of PCBs and T_4. In contrast, Koopman-Esseboom *et al.* (1994) measured dioxins and PCBs in human cord blood and breast milk and found that PCB exposure, estimated by toxic equivalents (TEQ), were negatively correlated with circulating T_4 in infants. It is important to recognize that the differences in circulating levels of thyroid hormones associated with PCBs are still within the normal range. Therefore, there is no evidence for background exposure to PCBs causing overt hypothyroidism as it does in experimental animals. However, it is important to recognize that small changes in serum T_4 and T_3 concentrations, within the normal range, alter serum TSH concentrations in individual subjects (Snyder and Utiger, 1972; Vagenakis *et al.*, 1974) because TSH and thyroid hormones are inversely related across the normal ranges as well as in disease states. Therefore, some individual measures fall outside the normal reference area for serum T_4-TSH relationship, without the values being clearly abnormal for either (Stockigt, 2000).

INTERACTION OF PCBS WITH THE THYROID HORMONE SIGNALING SYSTEM

Many reports support the notion that PCBs can produce deleterious effects on human brain development and that these effects may be mediated by PCB disruption of thyroid hormone action. Incidental exposure to PCBs is associated with deficits in gross motor performance and visual recognition memory (Longnecker *et al.*, 1997; Rogan and Gladen, 1992). The level of PCBs in serum collected from the umbilical cord at birth exhibits a significant correlation with shorter gestation, lower birth weight, and smaller head circumference (Fein *et al.*, 1984), and deficits in visual recognition memory at 7 months (Jacobson *et al.*, 1985). Data from four independent cohorts of newborns also shows a correlation between serum PCBs and/or dioxin and neurocognitive development (Gladen and Rogan, 1991; Jacobson and Jacobson, 1996; Koopman-Esseboom *et al.*, 1996; Stewart *et al.*, 2000). Reports of accidental PCB exposure of humans also support the concept that these chemicals disrupt thyroid hormone action. The first massive human exposure occurred in Japan in 1968, resulting in "Yusho" (oil disease) in which rice bran cooking oil became contaminated with PCBs and their thermal degradation products. Adults exposed to these high levels exhibited epidermal abnormalities, behavioral deficits and hypothyroxinemia (Kashimoto *et al.*, 1981). Children born to mothers who consumed this oil were exposed to organochlorines through the placenta and by breastfeeding (Masuda *et al.*, 1978; Nishimura *et al.*, 1977), and exhibited a number of physical and behavioral deficits including apathy, inactivity, hypothyroidism, and generally lower IQ scores. A similar incident occurred in Taiwan 10 years later, resulting in "Yu-cheng" (oil disease). Follow-up studies showed that children born as late as 12 years after their mothers' exposure exhibited delays in several neurological measures of development (Guo *et al.*, 1994; Rogan *et al.*, 1988; Yu *et al.*, 1991).

Developmental exposure to PCBs also produces neurological deficits in laboratory animals. For example, perinatal exposure to PCBs diminishes muscarinic receptor binding in the brain (Eriksson, 1988). It was later shown that pre- and post-natal treatment with Aroclor 1254 significantly reduced choline acetyltransferase activity in the cerebral cortex (Ku *et al.*, 1994). In addition, developmental exposure to A1254 produced hearing deficits (Goldey *et al.*, 1995a) that were similar to those observed in hypothyroid animals (Goldey *et al.*, 1995b). Several studies have evaluated the effects of PCB exposure on various behaviors in rats (Ku *et al.*, 1994; Schantz *et al.*, 1990; Seo *et al.*, 1995; Weinand-Harer *et al.*, 1997). Although many of these behavior disturbances are similar to those produced by perinatal hypothyroidism, most of these reports were not designed to provide information about the mechanism by which the behavioral deficits were produced.

The kinds of neurological deficits observed in relation to PCB exposure are not always consistent between studies, whether they be human or animal studies (see Hauser *et al.*, 1998). This may be attributable to the type of congener used in the experiment or measured in a clinical setting, to the dose or pattern of administration, to the specific test or measure used to evaluate neurological effects, or to species differences.

Evidence that PCBs exert adverse effects on neurodevelopment by interfering with thyroid hormone action

The association between background exposure to PCBs and clinical symptoms that are normally associated with congenital hypothyroid-

ism supports the concept that some of the developmental effects of PCB exposure are mediated by thyroid disruption. For example, deficits in motor coordination, cognitive development, and muscular hypotonia are some of the symptoms of congenital hypothyroidism that are correlated with background exposure to PCBs (Dussault and Walker, 1983; Porterfield, 1994). If PCBs affect brain development by interfering with thyroid hormone action, then PCBs should exert effects on developmental processes known to be responsive to thyroid hormone in experimental animals and these effects should be ameliorated by thyroid hormone replacement. Some of these predictions appear to be met by experimental studies. For example, perinatal exposure to PCB diminished choline acetyltransferase activity in the cerebral cortex, which was either partially or completely reversed by thyroxine replacement depending on brain area (Ku *et al.*, 1994). In addition, Goldey *et al.* demonstrated that developmental exposure to PCBs produced deficits in hearing that were similar to that produced by the goitrogen propylthiouracil (PTU) (Goldey *et al.*, 1995a), and these deficits were partially ameliorated by exogenous thyroxine (Herr *et al*, 1996). Finally, Cooke has shown that neonatal exposure to PCBs can produce the same effects on testis growth as that of PTU (Cooke *et al.*, 1993; Cooke *et al.*, 1996).

Taken together, these studies demonstrate that developmental exposure to PCBs in humans and animals can produce neurological deficits that are associated with reductions in circulating levels of thyroid hormone, and that in animals, PCB exposure affects neural development that can be partially ameliorated by thyroid hormone. However, not all of the effects of PCB exposure in animals are consistent. For example, it is unclear how PCBs can reduce circulating levels of T_4 without affect circulating TSH (Barter and Klaassen, 1992; Kolaja and Klaassen, 1998) because lower circulating levels of T_4 should cause a compensatory increase in circulating TSH (Taylor *et al.*, 1986). In addition, PCB exposure accelerates eye opening in rats (Goldey *et al.*, 1995), much like high levels of thyroid hormone (Wallace *et al.*, 1995). Finally, rat pups exposed to PCB concentrations that reduce circulating level of T_4 to undetectable levels do not exhibit reduced weight (Zoeller *et al.*, 2000). Considering that some PCB congeners may structurally resemble T_3 enough to interact with the thyroid hormone receptor (TR) (Chauhan *et al.*, 1999), it is possible that some PCB congeners, or their metabolites, may act as agonists, antagonists, or mixed agonists (McKinney and Waller, 1998) at the TR.

Thyroid Hormone Receptors. Thyroid hormone receptors (TRs) are members of the steroid/thyroid superfamily of ligand-dependent transcription factors (Lazar, 1993; Lazar, 1994; Mangelsdorf and Evans, 1995), indicating that effects on gene expression mediate the majority of biological actions of thyroid hormone. TRs are encoded by two genes, designated α and β c-*erb*A (Sap *et al.*, 1986; Weinberger *et al.*, 1986). These two genes produce three functional TRs: TRα1, TRβ1, and TRβ2 (Hodin *et al.*, 1989; Izumo and Mahdavi, 1988; Koenig *et al.*, 1988; Murray *et al.*, 1988; Thompson *et al.*, 1987). Although there are several TRs expressed, the binding affinity for T_3 or for T_4 are not different among the various forms (Oppenheimer, 1983; Oppenheimer *et al.*, 1994; Schwartz *et al.*, 1992). However, the TRs exhibit a 10-fold greater affinity for T_3 than for T_4, and T_3 is generally recognized as the physiologically important regulator of TR action (Oppenheimer, 1983). Despite this, TRα1 and TRβ1 exhibit different binding kinetics to the thyroid hormone analogue desethylamioderone (Bakker *et al.*, 1994; Beeren *et al.*, 1995). Therefore, it is possible that other exogenous compounds, specifically environmental chemicals, may bind differentially to these two TRs.

Studies focused on the molecular events transducing thyroid hormone action on gene expression have begun to provide some insight into the potential mechanisms that may account for the pleiotropic effects of thyroid hormone. An example of the pleiotropic action of thyroid hormone is provided by the observation that the gene encoding RC3/Neurogranin is regulated by thyroid hormone only in a subset of neurons that have TRs (Guadano-Ferraz *et al.*, 1997). Thus, the presence of thyroid hormone receptor in cells is necessary for thyroid hormone to regulate the expression of genes in that cell, but it is not sufficient. Two additional classes of proteins are known to impact on TRs. First, TRs can form heterodimers with other members of the steroid/thyroid hormone receptor superfamily, such as the retinoid receptors RXRs (Kliewer *et al.*, 1992; Mangelsdorf and Evans, 1995). Moreover, the type of heterodimer formed will direct the complex to a different structural motif in the hormone response element on the target gene. A second class of protein essential for TR function is the receptor cofactors (Koenig, 1998; Fondell, 1999; Ko, 2000; Arrieta, 2000). The interactions of TR with these classes of proteins are mediated by structural changes induced by ligand binding (Koenig, 1998). Therefore, if specific PCB congeners can bind to the thyroid hormone receptor, and there is presently no evidence that they do, they could have a different affinity for TRα compared to TRβ, and they may alter TR structure in a way that produces effects dissimilar from those of T_3.

If the effects of thyroid hormone are mediated through its receptors, then identifying when the TRs are expressed and in what neurons will help identify where and when thyroid hormone exerts effects on brain development. Thyroid hormone receptors are expressed in the fetal brain of humans (Bernal and Pekonen, 1984), and are differentially expressed in animals (Bradley *et al.*, 1992; Bradley *et al.*, 1989; Falcone *et al.*, 1994; Perez-Castillo *et al.*, 1985; Strait *et al.*, 1990). These findings suggest that thyroid hormone may influence gene expression in the fetal brain. It is also interesting to note that the TRα1 and TRβ1 exhibit different patterns of expression. For example, the β TRs are selectively expressed in the developing cochlea (Bradley *et al.*, 1994). In addition, the β1 TR is expressed in the proliferative zone of the developing cortex where cortical neurons proliferate, but the α1 TR is expressed in differentiating neurons (Bradley *et al.*, 1992). Thus, different TR isoforms may mediate different effects on the developing brain.

Thyroid Hormone Action in the Fetal Brain. The concept that thyroid hormone of maternal origin can affect brain development is also supported by the observation that T_4 from the maternal circulation can cross the placenta and be converted to T_3 (Calvo *et al.*, 1990; Contempre *et al.*, 1993; Escobar *et al.*, 1990; Escobar *et al.*, 1997; Vulsma *et al.*, 1989). However, few studies have examined thyroid hormone responsiveness of the fetal brain (Bonet and Herrera, 1988; Escobar *et al.*, 1997; Escobar *et al.*, 1988; Geel and Timiras, 1967; Hadjzadeh *et al.*, 1989; Porterfield, 1994; Porterfield and Hendrich, 1993). This lack of information about the molecular mechanism(s) of thyroid hormone action on fetal brain development has two important consequences. First, we have little appreciation for the molecular events or developmental processes by which thyroid hormone produces the effects observed in humans and animals

briefly discussed above. Second, we have no direct measures of thyroid hormone action in fetal brain. Therefore, we cannot directly test the hypothesis that specific chemicals can interfere with thyroid hormone action because the only measures available are indirect such as thyroid hormone concentration in serum or in specific tissues.

Therefore, our laboratory has recently begun to identify thyroid hormone-responsive genes expressed in the fetal brain before the onset of fetal thyroid function (Dowling *et al.*, 2000b). We have identified two genes expressed in the fetal cortex that are regulated by maternal thyroid hormone. One of these genes is a transcription factor. Oct-1 is a homeodomain-containing transcription factor that is differentially expressed in the developing brain (He *et al.*, 1989; Kambe *et al.*, 1993; Suzuki *et al.*, 1993). We have found that its expression is regulated only in the proliferative zone of the fetal cortex (Dowling *et al.*, 2000b). Likewise, Neuroendocrine-Specific Protein (NSP) is a gene encoding a protein that is inserted into the endoplasmic reticulum of neurons (Ninkina *et al.*, 1997; Senden *et al.*, 1996). This gene also is expressed selectively in the proliferative zone of the fetal cortex (Dowling *et al.*, 2000a). Although these studies have not resolved whether thyroid hormone exerts a direct receptor-mediated effect on the expression of these genes, we have also found that thyroid hormone of maternal origin can regulate the expression of the gene encoding RC3/Neurogranin in the fetal brain (Dowling and Zoeller, 2000). This gene is known to be directly regulated by thyroid hormone (Arrieta *et al.*, 1999; Iniguez *et al.*, 1993). These observations suggest that thyroid hormone from the maternal circulation can directly regulate the expression of genes selectively expressed in the part of the fetal cortex where neurons are born. These studies are important because they provide a tentative developmental process regulated by thyroid hormone in the fetal brain (cortical neurogenesis) and provide specific genetic markers of thyroid hormone action. These end-points can potentially be employed as markers of disruption of thyroid hormone action by PCBs.

Thyroid Hormone Action in the Postnatal Brain. In contrast, considerably more experimental work has focused on the period of the so-called brain growth spurt in the neonatal rat. During the first 3 weeks of life in the rat and mouse, cerebellar development is nearly completed and it is quite sensitive to thyroid hormone (Figueiredo *et al.*, 1993; Koibuchi and Chin, 1998; Thompson, 1996; Xiao and Nikodem, 1998). During this period, 3 genes have been most extensively studied for their responsiveness to thyroid hormone: RC3/Neurogranin, Myelin Basic Protein (MBP), and Purkinje Cell-Specific Protein-2 (PCP-2) (Iniguez *et al.*, 1993; Iniguez *et al.*, 1996; Marta *et al.*, 1998; Morte *et al.*, 1997; Zou *et al.*, 1994). The roles of RC3/Neurogranin and PCP-2 in brain development are not well-understood; however, the observation that thyroid hormone regulates the expression of MBP expression provides at least a partial mechanism for the important role thyroid hormone plays in the process of myelination (Figueiredo *et al.*, 1993; Gupta *et al.*, 1995; Ibarrola and Rodriguez-Pena, 1997; Jagannathan *et al.*, 1998; Rodriguez-Pena *et al.*, 1993).

Because there is more information about the role of thyroid hormone in postnatal brain development, it is possible to directly test the effects of PCB exposure on thyroid hormone action in the developing brain. Therefore, we recently examined the effect of A1254 exposure on RC3/Neurogranin and MBP expression in the postnatal cerebellum (Zoeller *et al.*, 2000). The PCB exposure paradigm we used was that of Goldey *et al.* (1995). This paradigm reduces circulating levels of total T_4, in a dose-dependent manner, below detection limits for the radioimmunoassay. The lowest dose of A1254 exposure (1 mg/kg) significantly reduced MBP mRNA levels in the developing brain. However, higher doses (4 and 8 mg/kg) restored MBP expression to normal. In addition, we found that the higher doses of A1254 increased the cellular level of RC3/Neurogranin expression in cells of the retrosplenial granular nucleus (RSG). Considering that thyroid hormone increases the cellular expression of RC3/Neurogranin in the RSG by a transcriptional mechanism (Guadano-Ferraz *et al.*, 1997), it is possible that A1254 also is affecting both RC3/Neurogranin and MBP mRNA levels by a transcriptional mechanism.

Several aspects of our findings supported the interpretation that A1254 altered MBP and RC3/Neurogranin expression through the thyroid hormone signaling pathway. For example, thyroid hormone does not alter MBP expression in the cerebellum on postnatal day 5 (P5) or P30, as shown by treatment with the goitrogen methimazole (MMI) (Ibarrola and Rodriguez-Pena, 1997). However, on P15, MMI treatment significantly reduces the expression of MBP in cerebellum. Thus, MBP expression exhibits the same temporal pattern of sensitivity to thyroid hormone and A1254. RC3/Neurogranin expression also is unaffected by MMI (Iniguez *et al.*, 1993) or A1254 on P5. However, on P15, thyroid hormone regulates the expression of RC3/Neurogranin in the RSG and dentate gyrus, but not in CA1, CA2, CA3, or layers IV-II in the occipital cortex (Guadano-Ferraz *et al.*, 1997). Considering that A1254 affected RC3/Neurogranin expression only in brain regions known to be sensitive to thyroid hormone, the effects of A1254 also appear to be exerted through the thyroid hormone signaling pathway.

The most parsimonious explanation for our findings is to propose that individual PCB congeners, or classes of congeners, can directly activate the thyroid hormone receptor either as parent congeners or following hydroxylation or methylation. If this is true, then individual PCB congeners should be able to bind to the TR (or TRs) with high affinity. Presently, only one report has tested this hypothesis (Cheek *et al.*, 1999), and while they found that individual hydroxylated PCB congeners can bind to the TRβ1 TR with low affinity (K_1~32μM), it is questionable that this level of binding is physiologically meaningful. Thus, this prediction remains to be stringently tested.

CONCLUSION

Many studies clearly support the conclusion that PCBs can reduce circulating levels of thyroid hormone in experimental animals. However, the lowest dose of PCB required to produce this effect is not well established. In addition, it is unclear whether animals of different genders or age (developmental stage) are differentially sensitive to the hypothyroxinemic effect of PCB exposure. Finally, the most potent congener or combination of congeners that reduce circulating T_4 have not been unequivocally determined, nor has the mechanism(s) accounting for their effects. Likewise, it is not clear why PCB exposure appears to produce both antithyroid and thyroid hormone like effects on different developmental events. Do individual PCB congeners or congener metabolites bind to the thyroid hormone receptor or otherwise affect thyroid hormone receptor activation? In addition to these questions about the interaction of PCBs

with the thyroid signaling system, it will be important to delineate the developmental processes affected by thyroid hormone during brain development and the mechanisms by which thyroid hormone exerts these effects. Despite these broad areas of ambiguity, viewing thyroid hormone action on brain development from the perspective of environmental endocrine disruption can provide a powerful and effective way of prioritizing questions that require research focus. Without answering these fundamental questions in experimental systems, interpreting the epidemiological evidence on PCBs, neurological function, and thyroid hormone will be severely compromised.

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