Polychlorinated Biphenyls as Hormonally Active Structural Analogues

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Among the environmental chemicals that may be able to disrupt the endocrine systems of animals and humans, the polychlorinated biphenyls (PCBs) are a chemical class of considerable concern. One possible mechanism by which PCBs may interfere with endocrine function is their ability to mimic natural hormones. These actions reflect a close relationship between the physicochemical properties encoded in the PCB molecular structure and the responses they evoke in biological systems. These physicochemical properties determine the molecular reactivities of PCBs and are responsible for their recognition at biological acceptors and receptors, as well as for triggering molecular mechanisms that lead to tissue response. “Coplanarity” of PCB phenyl rings and “lat-erality” of chlorine atoms are important structural features determining specific binding behavior with proteins and certain toxic responses in biological systems. We compare qualitative structure–activity relationships for PCBs with the limited information on the related non-coplanar chlorinated diphenoxyethers, providing further insights into the nature of the molecular recognition processes and support for the structural relationship of PCBs to thyroid hormones. Steroidlike activity requires conformational restriction and possibly hydroxylation. We offer some simple molecular recognition models to account for the importance of these different structural features in the structure–activity relationships that permit one to express PCB reactivities in terms of dioxin, thyroxine, and estradiol equivalents. The available data support the involvement of PCBs as mimics of thyroid and other steroid hormones. The potential for reproductive and developmental toxicity associated with human exposure to PCBs is of particular concern. Key words: dioxin/thyroxine/estradiol equivalents, PCBs, structure–activity relationships. Environ Health Perspect 102:290–297 (1994)

There is growing concern (1) that a large number of man-made chemicals that have been released into the environment can disrupt the endocrine systems of animals and humans. Among the chemicals of concern are the persistent, bioaccumulative organohalogen compounds that include some pesticides and industrial chemicals such as polychlorinated aromatics, other synthetic products, and some metals. Possible mechanisms by which these chemicals may interfere with endocrine function include their ability to mimic natural hormones (both as agonists and antagonists), including recognition of their specific binding sites, ability to react directly or indirectly with hormone structure to alter it, and ability to alter the pattern of hormone synthesis or modulate hormone receptor numbers and affinities. The polychlorinated biphenyls (PCBs) are known or suspected to express biological activities through most, if not all, of these mechanisms.

PCBs are mixtures of chlorinated aromatic chemicals manufactured by chlorination of biphenyl under suitable conditions for varying the percentage by weight of chlorine incorporated. The chemical structure (Fig. 1) of PCBs can be represented in a general way, where n,n′ is the number of chlorine atoms replacing hydrogen atoms in each ring. A total of 209 different congeners are theoretically possible, but less than half that number appear to account for nearly all of the environmental contamination attributable to PCBs. If potential toxicity, environmental prevalence, and relative abundance in animal tissues are considered as criteria, the number of environmentally threatening congeners decreases to fewer than 50. Furthermore, fewer than 25 of these may account for most of the total PCB burden in tissues of invertebrates, fish, birds, and mammals (2).

PCBs were discovered before the turn of the century, and their usefulness in industry as, for example, diellectric and heat-exchange fluids with desirable physical properties, was recognized early. Their chemical resistance and neutral organic chemical nature have led to widespread distribution in the environment, including food webs. Now PCBs are almost entirely restricted to use in closed systems. Human exposure to PCBs has resulted largely from the consumption of food, but occupational exposure also results from inhalation and skin absorption. PCBs accumulate in the fatty tissues of humans and other animals. In turn, PCBs have caused toxic effects in humans and animals. The skin and liver are major sites of pathologic change, and other targets include the gastrointestinal tract, the immune system, and the nervous system. Studies in animals suggest that some PCB congeners may be carcinogenic and that they can promote the carcinogenicity of other chemicals. Such toxicological properties, both qualitatively and quantitatively, often are congener specific with remarkable dependence on number and position of chlorine substitutions. Structural patterns can thus be used to discriminate among PCB congeners on the basis of toxic potential, if not entirely on toxicity per se (3,4).

The toxic effects of PCBs appear to involve at least three types of basic mechanisms of action: 1) reversible interaction (binding) of the PCB with specific molecular sites of action such as receptors, enzymes, etc., 2) irreversible covalent interaction (binding) between the PCB (probably a reactive metabolite) and target molecules (particularly macromolecules such as DNA and proteins), and 3) accumulation of highly lipid-soluble, metabolically stable PCBs in lipid-rich tissues or tissue compartments. The same or different structural properties may be involved in the expression of these toxic effects. Of particular interest to this discussion is the use of

Figure 1. General structure for PCBs. o, m, p denote ortho, meta, and para positions, respectively.
structure–activity relationship (SAR) approaches to study the reversible interaction of PCB congeners with specific molecular sites in biological systems that normally control or mediate hormonal activities in the body. In the study of SARs, the basic philosophy is to draw conclusions by analogy, assuming that similarity of PCBs with respect to certain chemical properties/reactivities will result in similar biological responses (5). Thus, the problem is one of determining these properties and how they are linked to specific biological activities of interest.

**PCB Chemical Reactivities Underlying Toxicity**

PCB chemical reactivities are encoded within the chemical structure of the PCB molecule. Because chemical reactivities underlie biological activities (6), it is important to understand how structure determines the important reactivities and, in turn, how these reactivities are linked to toxicity. One of the more important applications of SAR approaches is to help guide mechanistic research and permit testing of mechanistic hypotheses (7). Simplifying and classifying relevant chemical reactivities can be an important first approach to studying molecular mechanisms of toxicity.

**Stacking Interactions and Coplanarity of Structure**

The importance of coplanarity of PCB structure (Fig. 2) in producing dioxinlike toxic effects has been recognized for some time (8). The chemical reactivity (9) associated with this structural feature is the ability to undergo stacking-type molecular interactions with other planar aromatic ring systems such as the heme system in hemoproteins. Such stacking interactions between aromatic ring systems have also been referred to as a charge-transfer or donor-acceptor interaction and can be viewed in a simplistic sense as a "Velcro-type" interaction in which the planar rings facilitate the "sticking together" of the two pieces. It should be pointed out here that the term "coplanar" PCB as widely used in this context is a misnomer in the sense that all PCBs are basically non-coplanar in nature (8). The important distinguishing property of the non-ortho-substituted PCBs is that the increase in the conformational enthalpy (ΔH) required for the non-ortho-substituted biphenyl to achieve a coplanar state is sufficiently offset (outweighed) by the favorable change in binding free energy (ΔG) associated with formation of a stacking biphenyl-receptor complex. All ortho-substituted PCBs are likely to undergo binding interactions in which a coplanar state is never fully achieved.

**Cleft-type Interactions and Lateral Chlorine Substitution**

Lateral (meta, para substitutions) chlorination (Fig. 2) has also been recognized (10–12) as an important structural feature in PCB binding interactions and toxicity. Halogen atoms like chlorine contain many electrons and are thus highly polarizable. Many appropriately placed polarizability interactions in a protein-binding site can be quite effective in binding a PCB molecule. The most polarizable chlorines are those contained in the lateral positions of the PCB molecule. This can be envisioned as a molecular cleft-type interaction between the highly polarizable lateral halogen atoms and the hydrophobic interior of the cleft provided by amino acid side chains that converge on the halogen substituents. Similar binding interactions could involve chlorines in nonlateral (ortho substitutions) positions of the molecule, but there is not clear evidence that such interactions are important in PCB binding and toxicity (3). An additional factor associated with the polarizable chlorines is when the number and positions of chlorines are favorable to driving a preferred polarizability vector in the molecule (8). This is the case in the coplanar PCBs with lateral substitutions, where the preferred orientation is about the longest axis (through the para, para' positions) of the molecule. This type of preferred polarizability can facilitate the stacking interactions referred to earlier by strengthening the charge-transfer interaction or in simple, nonscientific terms, making it "stickier" (7). Both stacking and polarizability interactions might also be possible for certain stable metabolites of PCBs such as their hydroxylated metabolites.

**Areas of High Electron Density and Oxidative Metabolism**

A third type of structural feature that may be important in leading to a different type of PCB toxicity is the availability of vicinal unsubstituted positions in the molecule (such as vacant meta, para positions) that can provide sites for oxidative metabolism to occur (13). In this case the chemical reactivity in the molecule that may control the rate and regiospecificity of oxidative attack involves areas of high electron density such as may occur at opposite ends of the polarization vector described above. Because reactive intermediary metabolites can be formed in such a process, it is possible that reactive metabolites can lead to covalently bound residues with biomolecules. Stable metabolites can have chemical reactivity of their own and lead to similar or different kinds of binding and toxicity as seen with the parent PCBs (14). Other types of metabolites and metabolic process-
es are possible and may be important in the expression of certain forms of toxicity such as the reported association of chronic respiratory effects with methylsulfone metabolites of certain PCB congeners (3).

Although other structural features and reactivities of PCBs are possible, they have been less studied in an SAR framework. For example, certain pure phenobarbital (PB)-type inducing PCBs with ortho substitutions cause hepatomegaly and exhibit activity as promoters in short-term bioassays for carcinogenesis (3). The structural basis for these activities is not clear, but they may be more a function of specific substructural features than overall PCB structure consistent with the many diverse structures known to act as PB-type inducers.

Reactivity Models and Predictions

Stacking Models

In attempting to understand the relationship between PCB structure, chemical reactivity, and biological activity, theoretical models for relevant biological activities based on PCB molecular parameters have been sought (7). A planar (or energetically favorable coplanar) aromatic ring system is the primary structural feature that is found in all known high-affinity ligands for the Ah (or dioxin) receptor, which include both halogenated and nonhalogenated aromatic compounds. Several lines of evidence (7, 15–17) now suggest that an important molecular property determining receptor binding and associated responses for these compounds is electron affinity. The dependence on this property can best be explained in terms of compound electron acceptance in a charge-transfer or stacking-type receptor model. Such a model also permits one to readily visualize steric hindrance to binding, as is the case for the ortho-substituted PCBs (Fig. 3), and variations in molecular size (e.g., compare PCBs with halogenated naphthalenes). The somewhat flexible but reasonably coplanar dioxin molecule shows the closest stacking followed by the non-ortho and ortho-substituted PCBs. In general, the stacking model predicts increased binding for the class of chlorinated dioxins with increased chlorination, but this trend has not been observed in in vitro experimental binding studies, possibly reflecting solubility differences. There is evidence (18) for such a trend in in vivo absorption and tissue distribution studies in the rat. It is important to realize that a favorable, close stacking can occur in some aromatic compounds that are not totally coplanar but have a sterically accessible planar face, as in certain chlorinated diphenyl ethers (Fig. 2 and 3). This consideration bears directly on the structural relationship of PCBs to

Figure 3. Space-filled stacking models comparing coplanar (3,3',4,4',5,5'−, upper left) and non-coplanar (2,3,4,4',5,5', upper right) PCBs with dioxin (lower left) and chlorinated diphenyl ether (2,3',4,4',5,5', lower right) interactions with porphine ring system (note separation distance and planar geometrical extent of stacking interaction involving planar faces).

Figure 4. Molecular surface representation of 3,3',4,4',5,5'−hexachlorobiphenyl (from docking, red) and thyroxine complex (from X-ray, green) with binding domain (blue) of human protein prealbumin [compare contacts with protein surface and importance of lateral chlorines in filling binding pockets of thyroxine; see Rickenbacher et al. (17)].
Another structural determinant of binding dioxin in Cleft-type Models role and interaction with this family of proteins has potential biological consequences as both agonists and antagonists. do not hallucinate.

Estrogen-Active Analog Model

While the stacking and cleft-type models have both pointed to a structural relationship of PCBs to thyroid hormones, other work (22) has suggested a structural relationship to steroid hormones, particularly estrogens. The steroidal estrogens are relatively rigid, polycyclic molecules with pronounced asymmetry, and their binding to the estrogen receptor generally reflects a high degree of stereospecificity. Several classes of nonsteroidal synthetic estrogens have been based on phenyl-substituted hydrocarbons (23) because of the structural relationship to the phenolic A ring in estradiol. Hydroxylated PCBs are often found as metabolites of the parent PCBs (19). In this regard, we theorized that certain hydroxylated PCBs (Fig. 5) that were also conformationally restricted due to ortho substitution might be effective in binding the estrogen receptor. This proved to be the case in that the most active PCB analogues where those that combined a para-hydroxyphenyl ring with some degree of ortho substitution. However, it is not clear if there is some unique property of the hydroxyl group (such as hydrogen bonding) or if any other similar-sized group (such as chlorine) could be substituted. It is possible that increased ortho chlorines in the PCB structure might also provide important hydrophobic bulk structure in binding the estrogen receptor, but there was not any direct evidence for this. In additional analysis of these data (unpublished results), the solubility/lipophilic properties of the PCB are also proving to be important.

Although ortho substitution in PCBs can significantly lower the dioxinlike toxicity, it can potentially have the opposite effect on any estrogen-receptor-mediated toxicity. This PCB active analog modeling approach to estrogen receptor binding has implications for understanding any associated toxicity. As was suggested for other PCB activities and dioxin and thyroxine equivalents, it is possible to express this PCB activity in terms of estradiol equivalents.

Possible Relationships to Toxic Endpoints

Relationship to Chlorinated Diphenyl Ethers

As mentioned earlier, the chlorinated diphenyl ethers (CDEs) are an important test case for the structural requirements for dioxin like toxicity for PCBs. CDEs contain two phenyl rings as do PCBs but for energetic reasons are unlikely to achieve coplanar states in their binding interactions, even in the absence of ortho substitution. Any dioxinlike toxicity and associated Ah receptor binding are more likely to reflect molecular interactions involving non-coplanar states, especially with ortho-chlorine substituents. The main structural difference in the two classes of compounds is the ether oxygen bridge (C–O–C angle of 120°) contained in the CDEs. In addition to serving as structurally related model compounds for PCBs, the CDEs are also of interest as potential environmental contaminates, such as their association with used transformer fluid (24). For both PCBs and CDEs, chlorine substitutions provide steric constraint that defines the minimum energy conformation of the aromatic rings.

However, the effects on overall conformational properties and structure can be quite different in the context of having an accessible planar face for a stacking-type interaction to occur as postulated for the dioxin-receptor-binding interaction. In the case of PCBs, any degree of ortho substitution substantially raises the energy barrier to internal rotation about the pivot bond (25), and it is likely that ortho-PCBs interact with the dioxin receptor in their non-planar minimum energy conformations (26). Therefore, to the extent that toxicity is truly mediated by coplanar conformers, ortho-PCBs are likely to be relatively non-

Figure 5. Wire- and space-filled models comparing estrogenic PCB analog (4-OH, 2',4',6', bottom) with estradiol (top) (molecules aligned by common phenolic ring).
toxic. Lethality in guinea pigs may be an example (10) of this because the nearly isosteric 3,3',4,4',5,5'-hexachlorobiphenyl is about 1/100 as toxic as dioxin (reflecting the energy cost to achieve a coplanar state) and the structurally related 2,3,3',4,4',5'-heptachlorobiphenyl is relatively nontoxic at significantly larger (>10-fold) doses.

In the case of CDEs, the effects of *ortho* substitution on dioxin-receptor-binding are likely to have an enhancing effect. *Ortho* substituents provide desirable minimum-energy conformations (Fig. 3) because the rings now approach a mutually perpendicular relationship with an accessible planar face for entering into stacking interactions. An additional enhancing effect is realized because there is less energy cost (more favorable binding free energy from freezing out rotatable bonds) required to position the more rigid binding groups. A similar effect of *ortho* substituents in hydroxylated PCBs in enhancing estrogen receptor binding was previously described (22). Although other binding modes are possible, they are somewhat less favored energetically, but in general also seriously limit the accessibility of the other phenyl ring system in entering into other binding interactions such as those involving lateral substituents.

Recent work (27,28) has shown interesting differences in the structure–activity relationships for CDEs as compared to PCBs. The evidence suggests that both classes of compounds can produce dioxin-like toxic effects mediated by binding to the dioxin receptor. For example, non-*ortho*-but laterally substituted CDEs were less toxic than their mono-*ortho*-substituted analogues, and similar results were also observed for their enzyme-inducing potencies. Active congeners were also shown to include CDEs with multiple *ortho*-substitutions. These results are consistent with the anticipated enhancing effects of *ortho* substitution in CDEs on dioxin receptor binding through a stacking molecular mechanism as previously predicted (7).

In addition, an interesting result was found (28) in certain highly chlorinated CDEs that contain multiple *ortho* substitutions, especially in both rings. The potentials were shown to be similar to those found (27) for the non-*ortho*- and mono-*ortho*-substituted compounds previously studied. It is apparent that high degrees of *ortho* substitution in CDEs do not significantly diminish their Ah-receptor-mediated responses as seen for the PCBs. It is proposed that in these cases increased conformational restriction is brought about by additional steric interactions between the ring systems. The favored conformers would place the adjacent *ortho*-chlorine in the plane of the stacking ring (see Figure 3), which could influence the nature of the stacking interaction. Highly chlorinated diphenyl ethers, especially with high degrees (three or four *ortho*-chlorines) of *ortho* substitution, would increase the polarizability and steric rigidity of the system, both expected to further enhance a stacking interaction. It is anticipated that the stacking ring would be the most highly substituted ring, although this is not always clear (e.g., when both rings contain the same number of chlorines). However, high degrees of *ortho* substitution would not only lead to chlorine atoms positioned in the stacking plane but also to close through-space interactions of *ortho*-chlorine atoms with the face of the adjacent aromatic ring system (Fig. 6). This latter effect can be relieved somewhat in the absence of *meta*-chlorine substituents that buttress the adjacent *ortho* substituent (29). Both the in-plane and through-space intramolecular effects would weaken any intermolecular stacking interaction. Nevertheless, it is anticipated that the structure–activity relationships for these types of molecules would be complex and difficult to interpret. *Ortho* substituents in CDEs can have both qualitatively and quantitatively different effects on molecular structure and reactivity as compared to the effects seen in the PCBs.

The available SAR data on CDEs support the involvement of a conformationally restricted diphenyl system with an accessible planar face that can participate in stacking interactions with protein binding sites. This type of analysis of CDEs and model for active compounds further support the proposal (20) for a stacking type interaction in dioxin receptor binding and should be helpful in guiding the selection of other congeners to test for the ability to produce dioxinlike toxicity. The potential toxicity of members of this class of chlorinated aromatic hydrocarbons needs further attention because hexa- to decachlorodiphenyl ethers, all of which are likely to have high degrees of *ortho* substitution (wider range of active congeners possible), have been identified (30) in human adipose samples at concentrations as high as 2000 pg/g, and they are known contaminants of commercial products such as technical-grade pentachlorophenol (31). The close structural relationship of active CDEs to thyroid hormones is also of considerable interest and compatible with the possible thymoermetic properties (7) of these compounds. Clearly, in the context of this stacking model for binding, the non-*ortho*-substituted congeners do not take on special significance and, other things being equal, may have reduced activity relative to their *ortho*-substituted counterparts.

Preliminary quantitative structure–activity relationship (QSAR) studies (Wallner C, and McKinney J, unpublished observations) with the CDEs based on the stacking-model concept look promising in understanding the dioxin-receptor-mediated responses of these chemicals. In a recent teratogenicity study (32) with nine CDEs in mice, 2,2',4,5,6-penta- and 2,3,3',4,5,6-tetrachlorodiphenyl ether resulted in the loss of litters at relatively low doses. Both of these CDEs contain one di-*ortho*-substituted ring, and the other has lateral (*meta*, *para*) substituents, but they are not fully *ortho* substituted to maximize the steric...
Research Advances • PCBs as hormonally active structural analogues

interactions shown in Figure 6. These results appear to be consistent with the structural requirements of active congeners predicted by our models.

Relationship to Thyroid Hormones

What emerges from such analysis of SAR results is that these structurally related compounds (PCBs and CDEs) can participate in similar molecular recognition processes in their potential interactions with specific molecular sites in biological systems in which the nature, accessibility, and polarizability of planar (or coplanar) face interactions can vary. The obvious next step in this type of analysis is to compare these molecular recognition processes with those that have been well documented (33,34) for thyroid hormone analogues as natural halogenated diphenyl ether systems of considerable biological importance. The SAR literature for thyroid-hormone-binding proteins points to two basic types of binding proteins: those that bind 3,5,3′-triiodo-L-thyronine (T₃) in preference to 1-thyroxine (T₄) and those that bind T₄ in preference to T₃. Interestingly, the SARs for the T₄ binding proteins seem to depend more on the structural properties of the inner (tyrosyl) ring, whereas the SARs for the T₃ binding proteins depend more on the outer (phenolic) ring properties (Fig. 2). In this regard, it is interesting to note that the nuclear receptors for thyroid hormone show considerable dependence of binding on a sterically constrained inner ring brought about by the 3,5,3′- (ortho) substituents. We have further shown (29) that these results can be reasonably explained using a donor-acceptor or stacking aromatic ring model as previously described. In this model framework it is not surprising that biphenyl thyroid hormone analogues (which lack an ether oxygen bridge) are inactive (33) because they basically represent worse-case ortho-substituted PCBs. Binding ligands for the triiodothyronine (T₃) nuclear receptor and the dioxin receptor may share common molecular recognition factors in the expression of their binding activities.

In contrast, the lateral arrangement of the ortho-diiodophenolic structure in the outer ring of thyroid hormones is an important feature characterizing the binding of all three major transport proteins (globulin, prealbumin, and albumin), and is the sole major binding feature for albumin. In fact, the T₄ binding proteins appeared to be ideally suited to bind lateral substituted chlorinated aromatic compounds such as the lateral (meta, para) substituted PCBs. Molecular modeling and experimental studies (10,11) now provide considerable support for this hypothesis. The best binders (four- to eightfold better than T₄) have only lateral chlorine substituents, and additional ortho-chlorines do not lower binding much (two- to threefold better than T₄). One lateral chlorine (as in the 2,4,6-pattern) is sufficient for appreciable (about half T₄) binding activity. When no chlorines (biphenyl) or all (2,2′,6′,6′) ortho positions are filled, the compounds show no binding activity. As persistence in the environment and in biological tissues usually equates to a high degree of lateral substitution, it is anticipated that most, if not all, PCB residues found in human tissues are likely to effectively bind this family of proteins. It is also known (10,11,13,35) that hydroxy PCBs can bind this family of proteins, which suggests that certain PCB hydroxylated metabolites could produce the same or similar responses associated with binding. Similar SAR results (11) were shown for a T₄ nuclear-binding protein (12) and the microsomal deiodinase (36) in liver tissue. Other proteins that may give similar results include tyrosine hydroxylase and the dopamine receptor.

An important consideration here is that ortho chlorination in PCBs does not significantly affect this binding activity. One indirect effect of ortho substitution on this binding activity may be to effectively inhibit binding to the T₄ family of binding proteins that presumably require a stacking interaction. Therefore, any biological responses associated with this "laterality" type of binding activity (as in the prealbumin interaction model) could be elicited by a much larger number of PCB congeners of environmental concern because most are ortho substituted to some degree. The recent finding of PCB neurotoxicity (37) with certain ortho but lightly substituted congeners may fall into this category. Biochemical thyroid status at the cellular level has been shown (38) to depend not only on the concentration of free T₄ but also on the concentration of prealbumin. Prealbumin is synthesized in the brain (as well as the liver), where it plays a major role in transport of thyroid hormone to the central nervous system (39).

Relationship to Retinoids

Another factor that may bear on some of the toxicological properties of PCBs relates to evidence (40) suggesting a close association between thyroid hormone and retinoid transport, metabolism, and gene transcription. For example, prealbumin binds to retinol-binding protein, and this serves as both a transport and protective mechanism for the smaller protein (41). Earlier findings (42) of prealbumin depletion in rat sera of dioxin-treated animals coupled with findings (McKinney JD et al. unpublished observations) of a parallel increase in retinol-binding protein and retinol are consistent with this view and further support a role for thyroid-hormone-binding proteins in the mechanism of toxicity of these compounds. SAR studies (43) on the effects of PCBs on retinoid concentrations in serum and tissues indicate that there are at least two classes of PCBs (i.e., the coplanar PCBs that affect hepatic, renal, and serum retinoids and other ortho-substituted PCBs that only decrease serum retinol). It is not clear whether these effects are secondary to the effects of PCBs on thyroid hormone metabolism. These effects may also reflect differences in preferences for the two classes of thyroid-hormone-binding proteins described earlier.

Relationship to Estradiol

Another class of hydroxylated PCBs (presumed metabolites) have been shown (22) to bind specifically to the mouse uterine estrogen receptor and as such represent potential agonists/antagonists ligands for certain estrogen receptor functions. As described earlier, this is believed to be associated with a close match of the phenolic ring with the A-ring in estradiol and enhanced steroidlike structure brought about by the ortho substituents. Developmental and reproductive toxicity are possible outcomes of interactions of this type with steroid hormone receptors. For example, reproductive impairment in marine mammals has been directly attributed to feeding on PCB-contaminated fish (44). The potential human health effects of exposure to ortho-substituted PCBs and their hydroxylated metabolites should be considered in this light.

Conclusions

From a structural chemistry point of view, there are at least two distinct classes of PCBs: the non-ortho- and ortho-substituted congeners. The important distinguishing feature of the non-ortho-substituted PCBs is the energetic accessibility of the coplanar state that can facilitate stacking interactions such as may occur in a receptor-binding domain. The effects of ortho substitution on PCB reactivity are less clear, but they can include three basic types: hindrance to stacking interactions, conformational restriction and more rigid steroidlike structure, and increased carbon–chlorine bond polarizability in nonlateral positions. Lateral chlorine substituents can be important in enhancing the binding properties of both classes of PCBs through polarizability interactions.

For the non-ortho-substituted (coplanar) congeners, most, if not all, of the important toxic effects we know about appear to be mediated by binding to the dioxin or Ah receptor. A simple stacking
interaction model involving planar faces is offered as a way to visualize the Ah-receptor-binding process, but it must be viewed as an oversimplification of a complex binding interaction. Further support for the stacking model is derived from analysis of the important structural features of the more active dioxinlike chlorinated diphénylethers. The activity of the chlorinated diphénylethers also provides support for the thyromimetic properties of these classes of compounds. The prealbumin interaction model is offered as a way to visualize the regulatory interactions in the Ah receptor. The estrogen active analog modeling approach offers one way of understanding the importance of ortho substituents and conformational restriction in receptor binding. For the ortho-substituted congeners, the link to specific toxic endpoints are not well established, and it is likely that multiple mechanisms are involved. To some extent, quantitative differences in responses appear to be due to variations in the degree of coplanarity or conformational restriction (both dependent on degrees of ortho substitution) or accessibility of planar faces and laterality that exists among the PCB congeners. Agonist and antagonist effects may also depend on variations and combinations of the important reactivity properties (kinds and amount) among the congeners. There appear to be some areas of overlap between the two PCB classes such as the weak dioxinlike properties of certain mono-ortho-substituted PCBs, but the significance of this is not fully understood. As ortho substitution in biphenyls is expected to lower their dioxinlike binding properties in biological systems, one might anticipate differences in their toxicokinetic properties based on the degree of ortho substitution (45).

As suggested earlier, PCBs may interfere with endocrine function in various ways. Their ability to directly mimic natural hormones (both as potentially potent and persistent agonists and antagonists) including recognition of their specific binding sites is emerging as a guiding concept in the case of the thyroid and estrogenic steroid hormones. Indirect effects might include alterations in the receptor number or affinity of other hormones involved in multihormonal regulating systems. For example, there is evidence (46) for a direct effect of thyroid hormone on the hepatic synthesis of estrogen receptors. The antiestrogenic action of dioxin (47) and possibly coplanar PCBs (48) in downregulating estrogen receptors could be related to their potential thyromimetic properties. Toxicity could result from overor underexpression of normal hormonal responses that may be mediated by both receptor and nonreceptor protein and associated DNA interactions. For example, recent work (49) suggests that the human estrogen receptor structural gene contains a DNA sequence that binds activated mouse and human Ah receptor.

The potential for reproductive and developmental toxicity (50) is of particular concern. Thyroid hormones are known to be essential for brain development (51), and many investigators (52) have provided evidence demonstrating effects of hormones on immature brain cell maturation, myellogenesis, protein and nucleic acid metabolism, and electrical activity of the growing brain. In addition, there are a number of well-documented examples of the regulation of single and multiple clusters of genes through multiple hormonal interactions (53). Thyroidal, adrenal, and gonadal hormones are all necessary for the normal differentiation of the nervous system and involved in the ultimate expression of the target genes in several of these multihormonal regulating systems. Because humans are exposed to mixtures of PCB congeners, it is likely that there are complex interactive biological effects. Understanding the biological properties of PCBs and how they are determined by the molecular structure are important research objectives. Perhaps more direct molecular biological approaches would be helpful in elucidating the involvement of both receptor and nonreceptor protein-mediated responses. In turn, such mechanistic insight should be helpful in reducing the scientific uncertainty associated with health hazard/risk assessments associated with human exposures to PCBs and related compounds.

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• Letter of invitation from the prospective host.

• Agreement to release the applicant from the home institution for the duration of the exchange.

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• Statement concerning the provision of 50 percent of financial support by European sources. Non-EORTC member country candidates must continue at full salary at the home institution for the duration of the exchange.

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Volume 102, Number 3, March 1994 297

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