Chiral Polychlorinated Biphenyl Transport, Metabolism and Distribution - A Review

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Abstract

Chirality can be exploited to gain insight into enantioselective fate processes that may otherwise remain undetected because only biological, but not physical and chemical transport and transformation processes in an achiral environment will change enantiomer compositions. This review provides an in-depth overview of the application of chirality to the study of chiral polychlorinated biphenyls (PCBs), an important group of legacy pollutants. Like other chiral compounds, individual PCB enantiomers may interact enantioselectively (or enantiospecifically) with chiral macromolecules, such as cytochrome P-450 enzymes or ryanodine receptors, leading to differences in their toxicological effects and the enantioselective formation of chiral biotransformation products. Species and congener-specific enantiomer enrichment has been demonstrated in environmental compartments, wildlife and mammals, including humans, typically due to a complex combination of biotransformation processes and uptake via the diet by passive diffusion. Changes in the enantiomer composition of chiral PCBs in the environment have been used to understand complex aerobic and anaerobic microbial transformation pathways, to delineate and quantify PCB sources and transport in the environment, to gain insight into the biotransformation of PCBs in aquatic food webs, and to investigate the enantioselective disposition of PCBs and their methylsulfonyl PCBs metabolites in rodents. Overall, changes in chiral signatures are powerful, but currently underutilized tools for studies of environmental and biological processes of PCBs.

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BRIEF

The occurrence, environmental transport and fate, and toxicological effects of chiral PCBs are highlighted in environmental media and biota.
INTRODUCTION

Chirality is a growing aspect of environmental research. About 25% of agrochemicals are chiral (1), as are a similar proportion of other environmental contaminants. Chirality is significant for several reasons. The enantiomers of a chiral compound rotate polarized light in opposite directions, but otherwise exhibit identical physical and chemical properties. Consequently, environmental physical and chemical processes generally affect both enantiomers identically. However, individual enantiomers may interact differentially with other chiral molecules, such as enzymes or biological receptors, leading to different biological and toxicological effects (2). Hence, determining effects of chiral compounds is confounded by differing concentrations of enantiomers, which may arise from enantioselective biotransformation (e.g., by cytochrome P-450 enzymes or CYPs), exerting differing biological activities. Therefore, chirality must be considered for accurate pollutant exposure and effects assessment (3,4). Moreover, chirality can be exploited to gain insight into enantioselective fate processes that may otherwise remain undetected, because physical and chemical transport and transformation processes will not generally change enantiomer compositions, but biological processes may.

Polychlorinated biphenyls (PCBs) are a well-known class of pollutants. Given their inherent stability, PCBs are difficult to eliminate from environmental matrices, and distinguishing elimination processes in the uncontrolled open environment from a plethora of other fate processes is nontrivial. Because 19 PCB congeners are axially chiral and stable under environmental and instrumental analytical conditions (5), stereoisomer analysis is a useful tool for characterizing biochemical processes affecting PCBs. The atropisomers (stereoisomers of axially-chiral chemicals) of PCBs also show differential toxicity (6-10), thus understanding enantiomer-specific PCB toxicity is of importance.

This review highlights the state of knowledge about chiral PCBs in the environment, with two major foci. First, we show how the enantiomer compositions of PCBs and their chiral metabolites (e.g., methylsulfonyl PCBs or MeSO$_2$-PCBs) in both controlled laboratory experiments and field studies provides deepened insight into their sources, fate, and effects, in matrices as varied as soils and sediments (microbial degradation), aquatic organisms, and mammals, such as rats, mice, and humans. Second, we highlight enantiomer-specific effects of PCBs. These studies demonstrate stereoisomer analysis reveals new features of the fate, transport, and effects of even heavily studied chemicals like PCBs. Throughout, we express enantiomer composition as enantiomer fraction (EF) (11):

\[
EF = \frac{A}{A+B}
\]

where A and B are the (+)- and (-)-enantiomers, respectively, if optical rotation is known, or are the first-eluting and second-eluting enantiomer on a specified enantioselective chromatographic column otherwise.

Enantioselective biodegradation of chiral PCBs in sediments and soils

Although many studies have investigated microbial transformation of PCBs in soils and sediments under both anaerobic and aerobic conditions (12), relatively few studies have considered enantioselective biodegradation, particularly under controlled laboratory conditions. The use of chiral PCBs to understand microbial transformation pathways holds promise for design of bioremediation schemes for dredged sediment removed from sites such as the Hudson River and stored in secure landfills. In situ bioremediation of PCBs has faced a major roadblock, because completely mineralizing mixtures of PCBs containing highly chlorinated congeners requires sequential anaerobic-aerobic processes.
Significantly non-racemic chiral PCB signatures have been observed in sediments from several locations (13-15). Measurements of surface sediments collected from the Hudson River estuary (15) showed non-racemic EFs for CBs 95 and 149. Non-racemic PCB compositions existed (13) in several surface sediments from rivers in the eastern (Hudson River, NY; Housatonic River, MA) and Midwestern US (White River, IN; Fox River, WI) and in sediments from Lake Hartwell, SC, a reservoir and Superfund site heavily contaminated with PCBs. Changes in EF with depth in cores from Lake Hartwell suggested a relationship with concentration and the likelihood of more than one microbial community responsible for dechlorination (14). Racemic EFs have also been measured in samples with low concentrations (ng/g) of total PCBs such as sediment cores from Lake Ontario (14) and surface sediment from the Mohawk River, NY (13).

Evidence of enantioselective microbial transformation in soils is sparser. Non-racemic EFs of CBs 95, 136, and 149 were observed in UK top soils and grass (16-18) and in Canadian soils (19). Microbial transformation may have occurred in soils from one site at surprisingly low concentrations of CBs 95, 136, and 149 at 51, 13, and 107 pg/g, respectively (16), as measured EF values were statistically significantly but not dramatically different from racemic. But as Jamshidi et al. (18) indicated, an enantioselective process clearly occurred. Soils from the Chemical, Metals, and Pesticide (CMP) pits at the Savannah River Site had non-racemic EFs for CBs 84, 91, 95, and 149 at concentrations of 2 μg/g or less total PCBs, implying individual congeners were at concentrations of 1 to 175 ng/g (20,21). Non-racemic EFs for CBs 95 and 136 were found in soils from the area in Toronto, Canada (19), suggesting enantioselective microbial transformation at quite low concentrations (sum of CBs 95, 136, and 149 was <7 ng/g). EFs of CB 95 in Toronto soils favored degradation of the same enantiomer as observed in soil from two UK locations (16); however, EFs of CB 136 in Toronto and UK soils displayed opposite preferences.

To date the only enantiomer-specific aerobic PCB microcosm study is by Singer et al. (22), who tested one tetra-chlorinated congener (CB 45) and three penta-chlorinated congeners (CBs 84, 91, and 95) with five strains of PCB-degrading bacteria. Three co-substrates-biphenyl, cymene, and (S)-carvone-were used with combinations of bacterial strains and PCB congeners. One hexa- (CB 132) and two hepta-chlorinated congeners (CBs 171 and 183) were also tested, but transformation was negligible, as expected (12) under aerobic conditions. Hydroxylated metabolites (HO-PCBs) of the penta-chlorinated congeners, but not CB 45, were observed, with no reports of metabolite enantiomer composition (22).

Singer et al. (22) reported the enantioselectivity of the various strains for the chiral congeners with a “selectivity factor” rather than an EF, rendering comparisons with other studies difficult. The congeners were biotransformed by all strains tested in the order CB 45>84>95>91. Selectivity among the congeners-strain-cosubstrate combinations varied significantly, suggesting a variety of biotransformation pathways were represented by the combinations. However, it was clear gram-negative strains formed similar non-racemic PCB residues, while gram-positive strains formed different enantioselective residues (22). On the other hand, a gram-positive strain with dioxygenase genes similar to gram-negative strains exhibited the gram-negative residue pattern (22). This suggests two different sets of dioxygenases existed, each with its own enantiomer preference. Only one published anaerobic microcosm study that considered biotransformation of chiral PCBs exists (23). The anaerobic microcosms differed from the aerobic cultures used by Singer et al. (22) in several aspects. Sediment from Lake Hartwell served as the inoculum, consisting of a mixed culture. Rather than a congener mixture, single congeners (CBs 132 and 149) were spiked into the anaerobic microcosms. These were selected because the first by-product of reductive dechlorination for each, CBs 91 and 95, respectively, is also chiral. The two spiked compounds, CBs 132 and 149, showed no changes in their racemic EFs as they were dechlorinated, in contrast to results with the aerobic
microcosms (22). However, for both products, CBs 91 and 95, significantly non-racemic EFs were measured as they appeared and were subsequently removed (23). The observation of non-enantioselective dechlorination of the initial congener followed by enantioselective dechlorination of the product generated the hypothesis that two different enzymatic pathways might be responsible. At a later time, enrichment of Hartwell sediment cultures that dechlorinated CB 132 allowed tentative identification of at least two different strains of *Dehalococcoides* (24), supporting the hypothesis.

The implications of the above include confirmation that there are a wide variety of enzymatic processes capable of transforming PCBs under both anaerobic and aerobic conditions in sediments and soils and that there is potential for microbial transformation, in particular at very low PCB concentrations. The growing database of enantiomer-specific data offers potential for modeling approaches to explore the relationship between concentration and biotransformation and the roles of specific enzymes and microorganisms. Chiral PCB congeners thus offer insight into microbial transformation pathways for a notoriously recalcitrant class of contaminants.

**Exploiting Enantiomer Signatures for Source Apportionment**

Enantioselective weathering of PCBs has been used not only to gain insights on microbial degradation in sediments and soils, but also to delineate and quantify sources and transport of contaminants necessary for mitigating human and ecosystem exposure. Conventional approaches to apportionment of PCB sources to a given compartment such as the atmosphere have relied on indirect methods and mathematical modeling (25-27). More recently, researchers have utilized chiral properties of organochlorine pesticides such as α-HCH, heptachlor, and chlordane to distinguish directly between such sources (28,29). Commercial formulations of chiral organochlorines are racemic. As discussed above, enantioselective differences in degradative processes cause EFs in soils to deviate from racemic (16,19,30). Such signatures are preserved on volatilization (31), and once in the atmosphere are unaltered by abiotic chemical and physical removal processes (32).

These facts have been incorporated into a two-source apportionment model quantifying the relative contribution of two sources of a chiral contaminant to an environmental compartment (2) (11):

\[
FC_{S1} = \frac{(EF_{RC} - EF_{S2})}{(EF_{S1} - EF_{S2})}
\]

where \( FC_{S1} \) is the fractional contribution of source matrix 1 to the receiving compartment; \( EF_{RC} \) the receiving compartment EF; and \( EF_{S1} \) and \( EF_{S2} \) the EFs in source matrices 1 and 2 respectively.

This principle was exploited by Asher et al. (15), who observed racemic PCBs in air overlying the Hudson River Estuary at New York City, consistent with an unweathered local urban source to the local atmosphere. In contrast, estuary waters displayed nonracemic CB 95 residues similar to those in Hudson River sediment (13), and correlated to the Hudson River’s water discharge. Using the two-source apportionment model, 85% of CB 95, and by implication other medium molecular weight PCBs, in the estuary was concluded to originate from the Upper Hudson River.

Chirality was also applied for source apportionment by comparing EFs of CBs 95, 136, and 149 in topsoil and outdoor air over a 12 month period at one urban and one rural site in Birmingham, UK (16). In contrast to the conventional view that contemporary concentrations of PCBs in outdoor air were driven by volatile emissions from topsoil (25); EFs in soil and air...
at both sites differed significantly, implying volatile emissions from soil exerted negligible influence on outdoor air. Furthermore, the racemic or near-racemic signatures observed in all outdoor air samples indicated the source(s) to be racemic. Subsequent work extended the comparison of EFs in outdoor air and soil to a further ten UK locations (18). These findings were similar to their earlier study (16). Additionally, EFs of the target PCBs in indoor air from buildings in the same city were racemic. These data, combined with concentrations of PCBs in indoor air that exceed substantially those in outdoor air (33,34), are consistent with emissions from the built environment driving contemporary outdoor air concentrations.

A significant caveat to the above is that PCBs do volatilize from soil, but that at the comparatively low soil concentrations (0.36-13.3 ng ΣPCB/g) detected in the above studies (16,18) such volatilization exerts an impact on enantiomer signatures only in air very close to the soil surface. If this were true, then at higher soil concentrations, one would observe in air as one moved upwards on a vertical transect away from the soil:air interface; an attenuation of concentrations and a progressive shift from the enantiomer signature in soil towards racemic. This was reported for enantiomer signatures of α-HCH (31), and for concentrations of ΣPCB (35), where concentrations in soil were far higher (42 ng α-HCH/g, and between 1.1 and 635 µg ΣPCB/g). The implication, thus, is that volatilization from soil was a far more important source to the atmosphere in the past, and continues to influence the contemporary atmosphere, albeit at most locations only at heights immediately above the soil:air interface.

This latter observation provides a potential explanation for the findings of a study that found enantiomer signatures of CBs 95 and 149 in herbage very similar to those in soil, particularly during warmer sampling periods. One wholly unexpected implication is that the PCBs in vegetation arise principally via vapor phase foliar uptake of PCBs that have volatilized from soil (17).

The examples cited above have considerable policy relevance. In particular, the exploitation of the enantiospecific properties of some PCBs has been crucial in establishing the causal link between the PCB burden in the contemporary built environment and outdoor contamination. This is consistent with the absence of significant decline in concentrations of ΣPCBs in UK indoor air between 1997-1998 and 2003-2004, which remain on average 30-40 times higher than those outdoors (34); and the lack of temporal decline in UK dietary exposure to ΣPCBs measured in 1992, 1997, and 2001 (36). Should these UK data be reflected elsewhere (and there is no reason to suspect otherwise), this suggests future efforts worldwide must focus on reducing the PCB burden in the built environment in applications such as capacitors, transformers, acoustic ceiling tiles, and permanently-elastic construction sealants. This will not only reduce directly exposure via inhalation of indoor air (34) and ingestion of indoor dust (37), but indirectly dietary exposure following reductions in releases to the outdoor environment (38).

Chiral PCBs as a marker of biochemical weathering in aquatic food webs

The distribution of chiral PCBs is enantiomer-specific and species-specific in aquatic organisms (39-49). These results are caused by in vivo biotransformation and/or uptake from prey or the organisms’ surroundings. Because PCBs were released into the environment as racemates, the finding of non-racemic residues in aquatic organisms suggests biotransformation of PCBs in the aquatic food web. This conclusion is significant, as aquatic organisms such as fish and invertebrates, have much lower abundances and activities of CYPs, lower rates of electron transport, and less active monooxygenases than higher organisms (e.g., mammals and birds), and thereby much less capacity to detoxify and biotransform xenobiotic chemicals such as PCBs (50). Thus, chirality is useful as a tool to detect and to gain insight into the biotransformation of PCBs in various aquatic species, ranging from phytoplankton and invertebrates, to fish, to birds, and to aquatic mammals such as cetaceans, seals and polar bears.
PCB atropisomers were almost racemic in phytoplankton and zooplankton collected from Lake Superior (Figure 1) (39), suggesting that these biota at the bottom of the food web have essentially no capability to biotransform PCBs. Similarly, omnivorous copepods (Calanus hyperboreus) collected from the Northwater Polynya in the Canadian Arctic (40) had racemic EF values of CBs 91, 95 and 149. However, EFs for CB 149 of 0.50-0.55 were observed in blue mussels (Mytilus edulis) of the German Bight (41). Nonracemic residues (EFs from 0.35-0.75) of CBs 91, 95, 132, and 183 were also observed in freshwater bivalves (Corbicula) in United States rivers (42). Although EFs of PCBs in phytoplankton, the food source for filter-feeders such as bivalves, were not measured in those studies (41,42), it is likely they were racemic (39). If so, then in vivo enantioselective biotransformation processes in bivalves likely produced the observed residues. Bivalves could not accumulate nonracemic PCBs from their food source, nor was there evidence that the ambient waters and sediment of those sites had nonracemic PCBs. Crayfish (Procambarus sp.) in Lake Hartwell (42) also had nonracemic PCBs, but their source might be sediment, which as noted previously had nonracemic PCBs from microbial reductive dechlorination. Opossum shrimp (Mysis relicta) and amphipods (Diporeia hoyi) had significantly nonracemic residues of CBs 91, 95, 136, 149, 174, 176, and 183 in Lake Superior (39), some of which was attributed to in vivo biotransformation. The capability of mysids to eliminate enantioselectively CBs 91, 95, and to a much lesser extent CB 149 was later confirmed experimentally (46). In summary, enantioselective analyses showed that phytoplankton and zooplankton are unlikely to biotransform PCBs in the aquatic environment. However, larger invertebrates have some PCB biotransformation potential. It should be noted that unlike other chiral organochlorine pollutants (32), enantiomer compositions of dissolved phase PCBs have not been reported with one exception, a heavily contaminated urban site (15). Thus, it must be assumed that no EF changes occurred when phytoplankton bioconcentrated PCBs from the water column.

Prior non-enantioselective analysis has concluded that fish had limited biotransformation ability toward PCBs (50). However, enantiomer-specific and species-specific biotransformation differences of PCBs have also been found in fish, likely from a combination of in vivo biotransformation and uptake from prey. Furthermore, freshwater fish had more nonracemic PCB residues than marine fish. CBs 95, 132, 136, 149, and 174 were racemic or near racemic in the livers of groupers from the northwest African Atlantic Ocean (43). CBs 91 and 149 were also racemic in Arctic cod (Boreogadus saida) from the Canadian Arctic (40); however, a significant EF of 0.463±0.001 (σ) for CB 95 in that species suggested possible biotransformation capacity for that congener, although this conclusion is tentative given low sample size (n=3). Highly nonracemic compositions of CBs 91, 95, 136 and 149 were found in largemouth bass (Micropterus salmoides) and bluegill sunfish (Lepomis macrochirus) in Lake Hartwell (42). Moreover, species-specific differences were found in these two species (Figure 1), despite sample variation in some matrices and some congeners which likely arise from differences in accumulation, distribution, metabolism, and elimination. Lake trout in Lake Superior (39) had similar nonracemic residues of CBs 91, 95 149 and 174 as its major prey, lake herring (Coregonus artedii) and rainbow smelt (Osmerus mordax). This result suggested that these congeners in lake trout mainly accumulated from trophic transfer. In contrast, significant differences in the EFs of CB 136 between lake trout and its prey suggested in vivo enantioselective biotransformation of this congener occurred. The capacity of salmonids to eliminate PCBs enantioselectively was later shown experimentally (46-48), and was affected by chemical (e.g., congener structure) and physiological (e.g., temperature, organism size) factors. Pseudo-first order rate constants could be determined in both field studies (39) and laboratory-based experiments (46-48) based on differences in EFs between predator and prey, and estimations of the time-scale over which biotransformation may have occurred (e.g., organism lifespan). Such rates in field studies would be difficult if not impossible to ascertain without enantioselective analysis.
The higher abundances and activities of CYP isozymes in seabirds and aquatic mammals may enable them to detoxify PCBs more easily than lower trophic level biota. In addition, CYPs are more likely to be induced by higher concentrations of PCBs in high trophic level organisms due to greater biomagnification. Seabirds had higher capabilities for enantioselective biochemical weathering of PCBs than aquatic organisms, as seven species (40) collected from Northwater Polynya had significantly nonracemic enantiomeric compositions of CBs 91, 95 and 149 that were considerably different from the racemic residues in their arctic cod and zooplankton prey. In contrast, near racemic EFs were observed in barn swallows (Hirundo rustica) at Lake Hartwell (42). Bowhead whales (Balaena mysticetus) (44) in arctic waters had nonracemic EFs of PCBs in liver and blubber that differed from zooplankton. Moreover, EFs of CBs 95 and 149 were correlated with specimen length for both males and females, while EFs of CB 91 were correlated with length for males only. This observation suggests that enantioselective accumulation of these three congeners was affected by concentration, age, and an uncharacterized sex-related factor, factors also observed in the stereoselective accumulation of other chlorinated pollutants (51). Likewise, highly nonracemic EFs in ringed seals (Phoca hispida) (40) of the Northwater Polynya that differed from their prey suggested in vivo enantioselective biotransformation. This hypothesis was supported by the observation of nonracemic amounts of the metabolite 3-MeSO$_2$-CB 149 (see (52) for the present nomenclature and (53) for an alternative nomenclature) in ringed seals (54). In contrast, harbor seals (Phoca vitulina) and gray seals (Halichoerus grypus) (45) had nonracemic EF values of CB 149 (EF = 0.58-0.71 and 0.60-0.70, respectively), which also demonstrated species-specific biochemical biotransformation in aquatic mammals.

**Chiral PCBs in human tissues, milk and feces**

Although PCBs are routinely measured in human blood, enantiomeric compositions have not been reported so far. Nonetheless, a few studies have investigated EF values of CBs 95, 132 and 149 in a small number of human tissue samples (55). CBs 95, 132 and 149 were near racemic in Belgian muscle, brain and kidney samples. However, a significant enrichment of these three congeners was observed in liver. EFs have also been reported in human milk (Figure 1) from Germany (56,57), Spain (58) and Switzerland (59). In these studies, (+)-CB 132 was enriched in most samples, with EF values ranging from 0.53 to 0.82. In contrast, CB 149 was near racemic (EF = 0.49-0.63). CB 95 was near racemic in breast milk from Germany and Spain (EF = 0.45-0.60), but showed some enantiomeric enrichment in breast milk from Switzerland (EF = 0.64-0.76). Several other PCBs also showed some enantiomeric enrichment in breast milk from Spain (58).

The extent of enantiomeric enrichment has been employed to investigate excretion of CBs 95 and 149 in healthy volunteers from the UK (17). CB 95 and 149 in the diet and CB 95 in the feces of the volunteers were near racemic, while CB 149 was only detected in two feces samples with a slight enantiomeric enrichment. These results are consistent with the near-racemic compositions observed in human tissues (Figure 1).

**Mammalian toxicokinetics of chiral PCBs**

Understanding the behavior of chiral pollutants in mammals is significant for several reasons. First, mammals have higher CYP levels and activities than aquatic organisms, as previously noted, making biotransformation much easier. In addition, the large body of literature on mammalian toxicokinetics and pharmacokinetics eases interpretation of enantiomer-specific data. Finally, of course, mammalian studies have relevance in terms of addressing human exposure and toxicity.

**Mice**—Several recent studies investigated different chiral PCB congeners (60), route of administration (61), gender (61), dose (62), dietary fat content (63), induction of CYPs (64),
and multidrug resistance transporters (65) as factors influencing the extent of the enantiomeric enrichment in (female) C57Bl/6 mice. Both CBs 84 and 136 underwent enantiomeric enrichment in C57Bl/6 mice, with (+)-CB 84 and (+)-CB 136 enriched in all tissues and excreta (60–64). Intraperitoneal injection of CB 136 produced significantly higher PCB concentrations in blood and tissues (p<0.05), especially liver and brain, than oral administration (Figure 2A) (61). (+)-CB 136 was significantly enriched in most organs, independent of the route of administration; however, more non-racemic CB 136 were found in the oral compared to intraperitoneal treatment groups (Figure 2B). The same study did not show any gender-specific difference in CB 136 concentrations or EF values (61).

A study investigating the dose dependence of the enantiomeric enrichment of CB 136 after oral PCB administration showed that liver concentrations increased with increasing dose after oral PCB administration (Figure 2C), whereas the extent of (+)-CB 136 enrichment decreased with increasing dose (Figure 2D) (62). The same trends were observed in other tissues, blood and feces. These observations indicate a saturation of the processes responsible for enantiomeric enrichment at higher PCB doses, and also suggest that the less pronounced enantiomeric enrichment in animals receiving CB 136 intraperitoneally may be due to higher PCB tissue concentrations. The drastic difference between fecal EF values in mice and humans, discussed previously, suggests differences in biotransformation and/or excretion of PCB atropisomers, a observation with potential human health implications given developmental neurotoxicity of PCBs with multiple ortho substituents (66).

Induction of different CYP enzymes by pretreatment with corn oil alone, β-naphthoflavone (CYP1As), phenobarbital (CYP2Bs) or dexamethasone (CYP2Bs and CYP3As), followed by oral PCB administration, resulted in lower CB 136 concentrations in phenobarbital- and, to a lesser extent, dexamethasone-pretreated animals (Figure 2E) (64), presumably due to the induction of CB 136 metabolizing enzymes. Although (+)-CB 136 was enriched in all tissues (Figure 2F), only EFs in liver of phenobarbital-, dexamethasone- and β-naphthoflavone-pretreated animals was significantly different from animals treated with corn oil vehicle alone. These observations do not suggest a particular CYP subfamily as the cause of the enrichment of (+)-CB 136. Instead, non-specific induction of hepatic enzymes in general seems to be correlated with increased enantiomeric enrichment.

In laboratory studies, PCBs are typically administered with a vehicle containing a comparatively high fat content (e.g., corn oil). To investigate the potential role of the dietary fat content on the disposition of PCBs, CB 136 was administered orally to female mice fed an unrefined (10% fat) or high fat (40% fat) diet (63). As found previously, (+)-CB 136 was enriched in all organs and in feces. The tissue EF values and PCB concentrations were independent of the dietary fat content. In contrast, CB 136 levels and EF values in feces differed between the unrefined diet and high fat diet groups (Figures 2G and 2H) due to differences in the residual fecal fat content. The most intriguing finding of this study was the increase in fecal EF values with time. This suggests that non-absorbed, racemic CB 136 was excreted within the first 24 hours, whereas absorbed CB 136 was excreted into the feces after undergoing an enantiomeric enrichment in vivo. Thus, differences in enantiomeric enrichment can be used to gain further insight into disposition of PCBs.

**Rats**—A few studies have reported an enantiomeric enrichment of PCBs in rats after intraperitoneal administration. While (+)-CB 139 was enriched in the liver of CB 139-treated rats (9), near racemic CB 136 (EF ~ 0.49) was observed in CB 136-treated male and female rats (67). Similarly, intraperitoneal administration of complex PCB mixtures to rats resulted in at most a slight enantiomeric enrichment (68). Similar to mice (61), the comparatively low enantiomeric enrichment in rats is probably due to the intraperitoneal route of administration.
**Binding and metabolism of PCB atropisomers by CYPs**

PCBs are metabolized *in vivo* to hydroxy- and sulfur-containing metabolites (2,69), i.e., CYP enzymes convert these lipophilic compounds to metabolites more easily eliminated from the body, thus preventing the cells from PCB intoxication. However, the PCB metabolites that CYPs produce are often more toxic than the parent molecule itself. Recent studies have shown that CYP enzymes enantioselectively bind and metabolize PCB congeners to hydroxylated metabolites. These processes are thought to cause the enantiomeric enrichment of PCBs observed *in vivo*.

A spectral binding study investigated binding of pure CB 136 atropisomers to P-450 enzymes (70). Microsomes were obtained from mice or rats treated with prototypical inducers of CYPs. Depending on pre-treatment, these microsomes contain predominantly CYP1A (β-naphthoflavone-treated mice and 3-methylcholanthrene-treated rats), CYP2B (phenobarbital-treated animals), and CYP2B/3A enzymes levels (dexamethasone-treated animals), respectively. In addition, high CYP2B enzyme levels were measured in hepatic microsomes from DEX-treated mice.

These microsomes were used to determine the binding of pure CB 136 atropisomers to microsomal CYP enzymes. Binding of racemic CB 136 and its atropisomers to CYP enzymes was greatest in microsomes from phenobarbital-treated animals, and decreased in the order phenobarbital > dexamethasone > β-naphthoflavone/3-methylcholanthrene ~ corn oil (Figure 3A). A significantly larger absorbance change was observed with (+)-CB 136 than with (−)-CB 136 with all four hepatic microsomal preparations in mice and rats, indicating (+)-CB 136 interacted with microsomal CYP enzymes to a greater degree than (−)-CB 136 (Fig. 3B). Finally, binding of CB 136 was inhibited by CYP2B and CYP3A antibodies, indicating involvement of CYP2B. Together these results suggest preferential binding of (+)-CB 136 to CYP enzymes, such as CYP2B and CYP3A, in hepatic microsomes.

*In vitro* CYP-mediated biotransformation of chiral PCBs has been reported recently (71). Human CYP2B6 eliminated CB 45 enantioselectively (EF = 0.44) but not CB 91 (EF = 0.49). In contrast, rat CYP2B1 degraded CBs 45, 84, 91, 95, 136, and 136 enantioselectively, with species-specific, opposite enantiomer preference for CB 45 (EF = 0.82). Each single-congener incubation with rat CYP2B1 produced one HO-PCB metabolite except for CB 95, for which two HO-PCBs were formed. Although metabolites were not identified conclusively, they were likely chiral (see next section). Preferential elimination of (+)-CB 136 (71) contrasted rat microsomal binding experiments (70) and *in vivo* experiments (67), in which stronger binding of (+)-CB 136 to CYPs and near-racemic CB 136 EFs were observed, respectively. However, it should be noted that single purified CYPs were used in the former experiment (71), compared to mixtures in microsomes and *in vivo*.

**Chiral PCB metabolites**

MeSO₂-PCBs and HO-PCBs were detected in fish, birds and mammals including humans (reviewed in (52)). More recently, some of the MeSO₂-PCBs in biota have also been observed to be selectively and strongly retained in liver tissue of mammals, including humans (52). However, the selectivity mechanism is still unknown, although reversible protein binding plays a major role for their retention in liver (72). The most important MeSO₂-PCBs bound in mammalian liver are 3-MeSO₂-2,5,6,2′,3′,4′-hexachlorobiphenyl (abbreviated: 3-MeSO₂-CB 132; see (52)), 3-MeSO₂-2,5,6,2′,4′,5′-hexachlorobiphenyl (3-MeSO₂-CB 149), and 4-MeSO₂-2,5,6,2′,4′,5′-hexachlorobiphenyl (4-MeSO₂-CB 149). The MeSO₂-PCBs formed are persistent and only slightly less hydrophobic than their parent compounds, making them long-lasting environmental contaminants. From a toxicological point, several of the *meta*-MeSO₂-PCBs strongly induce CYPs, such as CYP2B1, CYP2B2, CYP3A2 and CYP2C6 (73,74). Thus,
part of the toxicity of PCBs in the environment may be due to the presence of these metabolites. Furthermore, the main metabolites mentioned above are chiral and, accordingly, enantioselective transformation as well as toxic impacts cannot be excluded.

To gain deepened insight into the enantioselective transformation of atropisomeric PCBs, the research groups of Bergman and Hühnerfuss (52,75) performed a systematic study, which included enantioselective separation of eight MeSO₂-PCB standards (4-MeSO₂-CB 91, 4-MeSO₂-CB 95, 3- and 4-MeSO₂-CB 149, 3- and 4-MeSO₂-CB 132, and 3- and 4-MeSO₂-CB 174) by GC; enantiomer-specific measurement of MeSO₂-PCBs in extracts of rat and human liver as well as rat lung and adipose tissue; enantiomer separation of larger amounts of 3-MeSO₂-CB 132, 3-MeSO₂-CB 149 and 4-MeSO₂-CB 149 and their parent PCBs by enantioselective preparative HPLC for determination of absolute structures; and investigation of enantioselective toxicity of MeSO₂-PCB enantiomers.

As a first step, 18 - 25 mg of each atropisomer of chiral methylsulfonyl- (3- and 4-MeSO₂-CB 149), methylsulfyl (3- and 4-MeS-CB 149) and methoxy-PCBs (4-MeO-CB 149) were isolated, with enantiomeric purities of 95.0-99.9%, by enantioselective HPLC using semi-preparative β-cyclodextrin-based HPLC columns. Thereafter, their absolute structures were determined by electronic circular dichroism and vibrational circular dichroism in combination with quantum chemical calculations (69,76). Rotational angles and absolute configurations were also determined. This study established a sound method for future preparation and absolute structure determination of compounds of the same chemical class, and identified enantioselective GC peaks of environmental sample extracts unequivocally.

Thus far, eight of the ten atropisomeric MeSO₂-PCBs found previously in environmental samples were separated into their enantiomers using a column coated with a 1:1 (w:w) mixture of OV 1701 and heptakis(6-O-tert-butyldimethylsilyl-2,3-di-O-methyl)-β-cyclodextrin (52). 3-MeSO₂-CB 91 was separated using other columns (51,52), while 3-MeSO₂-CB 95 was not studied because no standard was available. In general, MeSO₂-PCBs exert strong interactions with chiral cyclodextrin phases. Because of low maximum temperatures for enantioselective GC columns (468-498 K), retention times were long for all columns studied (52,54,77) e.g., from 50-130 min even for a 10 m column (52), with corresponding peak widths at half-height of 30-110 s. Although baseline separation was observed for most enantiomers and constitutional isomers (52), improving enantiomeric separation of MeSO₂-PCBs would be useful.

Based on this preparatory work, systematic enzymatic transformation processes were investigated using human livers from two women and three men who had died from heart failure or accidents. Only (R)-3-MeSO₂-CB 149 and the second-eluting enantiomer of 3-MeSO₂-CB 132 were encountered in all liver extracts (52). Thus, not only a high congener selectivity was found as previously observed, but also a highly enantioselective liver retention of these MeSO₂-PCBs. None of the MeSO₂-PCBs investigated (52) were detected in the two human lung samples also analysed.

Furthermore, the presence of MeSO₂-PCB atropisomers was determined in liver, lung and adipose tissues of rats exposed to the technical PCB product Clophen A50 (75). In all tissues analysed, especially lung, para-MeSO₂-PCBs were more abundant than meta-derivatives. An excess and dominance of (R)-3-MeSO₂-CB 149 atropisomer in lung extracts was observed. Small amounts of (S)-3-MeSO₂-CB 149 atropisomer were present in lung and adipose tissues but not liver. No significant changes in the enantiomeric excess of 4-MeSO₂-CB 91, 3-MeSO₂-CB 132, 4-MeSO₂-CB 132, 3-MeSO₂-CB 149 and 4-MeSO₂-CB 149 atropisomers were found in lung, liver, or adipose tissues. The results suggest enantioselective formations occurred for both meta- and para-MeSO₂-PCBs. Rat hepatocytes transformed both CB 149...
enantiomers with comparable velocities, thus yielding a nearly racemic metabolite 3-MeSO\textsubscript{2}-CB 149, while subsequent transformation of the 3-MeSO\textsubscript{2}-CB 149 enantiomers by rat hepatocytes led to a drastic transformation of the S-enantiomer. The R-enantiomer remained nearly unaffected, consistent with the above enantiomeric excesses in rat liver extracts. Thus, the dramatic enantiomeric excess of (R)-3-MeSO\textsubscript{2}-CB 149 was caused by enantioselective transformation of this metabolite, not production by the parent CB 149.

Field measurements of chiral MeSO\textsubscript{2}-PCBs have been performed on extracts of grey seal blubber (78) and pelican muscle tissues (79); ringed seals and polar bears (Ursus maritimus) in the Arctic (54); and livers of harbour porpoises (Phocoena phocoena) (77). As with humans, highly enantioselective residues of MeSO\textsubscript{2}-CBs were observed in non-human mammalian tissues (54,77), indicating similar mechanisms for stereoselective binding and/or enrichment of MeSO\textsubscript{2}-CBs are shared among mammals, at least for those species studied.

Compared to MeSO\textsubscript{2}-PCBs, little work has been carried out on chiral HO-PCBs given lack of commercially available standards. However, HO-PCB metabolites of CBs 45, 84, 91, 95, 132, 136, and 149 were synthesized, and enantiomers of several methylated derivatives separated by enantioselective GC (67) albeit with long retention times as with MeSO\textsubscript{2}-PCBs. Male and female rats dosed with racemic CB 136 produced four HO-PCBs with 3-HO-CB 150 and 3-HO-CB 136 formed enantioselectively, whereas parent PCB residues remained racemic (67). Understanding toxicokinetics of pollutant metabolite stereoisomers, as well as parent compounds, will increase greatly our understanding of their environmental fate and effects.

**Toxicity of chiral PCBs**

Pure PCB atropisomers are expected to display enantioselective or even specific biological effects due to differences in pharmacokinetics and interaction with target receptors. Indeed, atropisomers of CBs 88, 139 and 197 had different enzyme inducing properties in chick embryo hepatocytes (10) and immature male Sprague-Dawley rats (8,9). For example, (+)-CB 197 induced total CYP to a larger extent than (-)-CB 197 in cultured chick embryo hepatocytes, whereas the atropisomers of CBs 88 and 139 had equal potencies (10). In contrast, the (+)-atropisomers of these three congeners were more potent at inducing ethoxyresorufin-O-deethylase activity in immature male Sprague-Dawley rats, but only CB 197 atropisomers differed in their potency to induce total CYP, with (+)-CB 197 being more potent (9).

PCB atropisomers can also interfere stereoselectively with intracellular signaling processes and calcium homeostasis, critical processes for normal nervous system function and growth (66). CB 84 atropisomers increased [\textsuperscript{3}H]-phorbol ester binding, a measure of protein kinase C (PKC) translocation from cytosol to the cell membrane, and altered Ca\textsuperscript{2+}-sequestration, as determined by 45Ca\textsuperscript{2+}-uptake by microsomes isolated from adult rat cerebellum, in a concentration-dependent manner (6). However, only the effect on PKC translocation was enantioselective, with lowest observable effect levels of 30 and 50 μM for (+)-CB 84 and (-)-CB 84, respectively.

CB 136 atropisomers enantiospecifically enhanced binding of [\textsuperscript{3}H]-ryanodine to high affinity sites on ryanodine receptors (RyR) (7), a broadly expressed family of microsomal Ca\textsuperscript{2+} channels. While (-)-CB 136 enhanced [\textsuperscript{3}H]-ryanodine to RyR type 1 and type 2 with EC\textsubscript{50}s \textapprox 0.95 μM, (-)-CB 136 was inactive at \textleq 10 μM. Furthermore, (+)-CB 136 induced rapid release of Ca\textsuperscript{2+} from microsomal vesicles by selective sensitization of RyRs, an effect not antagonized by (+)-CB 136, and enhanced the open probability of reconstituted RyR1 channels three-fold by stabilizing open and destabilizing closed conformational states. The enantiospecific effect of (-)-CB 136 was also observable in intact HEK 293 cells expressing RyR1, where (-)-CB 136 but not (+)-CB 136, sensitized responses to caffeine. Together with the observation that a plethora of congener- and species-specific enantioselective biotransformations can result in
nonracemic PCB signatures, the enantiospecificity of CB 136 atropisomers towards RyRs represents a significant health concern for wildlife and human populations exposed to PCBs.

**Implications for Future Research**

Opportunities for exploiting chiral PCBs to improve understanding of fate processes are numerous. Efforts would be facilitated by determination of the absolute configuration of chiral PCBs, such as CBs 91 and 95, and preparation of novel chiral stationary phases with improved life times and better resolution of PCB metabolite enantiomers. The combination of molecular biological techniques and chiral PCBs would elucidate microbial pathways and ecology. Additional work is needed to understand how enantioselectivity operates within food webs to effectively use chiral PCBs as probes for contaminant transfer and ecological processes. To pursue insight into metabolism and toxicology, synthetic strategies for (hydroxylated-)PCB metabolites need development. Building an improved database of enantiomeric signatures, including water and human blood, will expand the use of chiral chemistry.

**Acknowledgments**

This work was supported by grants ES05605, ES013661 and ES012475 from the National Institute of Environmental Health Sciences (HJL), the National Science Foundation (NSF-0828699) (CML), the Canada Research Chairs Program (CSW), and a Natural Sciences and Engineering Research Council of Canada Discovery Grant (CSW).

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*Environ Sci Technol*. Author manuscript; available in PMC 2011 April 15.


Figure 1.
Box plots of EFs of some chiral PCBs in biota. Horizontal line across each panel indicates racemic EF of 0.5. Species and location--A: Sediment (Northwater Polynya) (40); B: Phytoplankton (Lake Superior) (39); C: Zooplankton (Bering-Chukchi-Beaufort Sea) (44); D: Bivalves (Corbicula genus, U.S. rivers) (42); E: Mysis (Mysis relicta, Lake Superior) (39); F: Crayfish (Procambarus sp., Lake Hartwell, SC) (42); G: Carp (Cyprinus carpio, U.S. rivers) (42); H: Sculpins (Cottus genus, U.S. rivers) (42); I: White sucker (Catostomus commersoni, U.S. rivers) (42); G: Watersnake (Nerodia sipedon, Lake Hartwell) (42); K: Bluegill sunfish (Lepomis macrochirus, Lake Hartwell) (42); L: Bass (Micropterus salmoides, Lake Hartwell) (42); M: Lake trout (Salvelinus namaycush, Lake Superior) (39); N: Lake herring (Coregonus
artedii, Lake Superior) (39); O: Rainbow smelt (Osmerus mordax, Lake Superior) (39); P: amphipods (Diporeia hoyi, Lake Superior) (39); Q: Arctic cod (Boreogadus saida, Northwater Polynya) (40); R: Thick-billed murre (Uria lomvia, Northwater Polynya) (40); S: Ivory gull (Pagophila eburnea, Northwater Polynya) (40); T: Bowhead whale blubber (Balaena mysticetus, Bering-Chukchi-Beaufort Sea) (44); U: Ringed seal (Phoca hispida, Northwater Polynya) (40); V: Female mouse liver (65); W: Human breast milk (58).
Figure 2.
The effect of route of administration, dose and fat content in diet on levels and enantiomeric fraction of PCB 136 in mice after oral administration of (±)-PCB 136 (61-64). (A) Levels and (B) enantiomeric fraction in the liver of male and female mice exposed orally or intraperitoneally (61). Dose-dependence of (C) levels and (D) enantiomeric fraction in the liver of female mice (62). (E) Levels and (F) enantiomeric fraction in the liver of mice pretreated with CYP1A (β-naphthoflavone; NF), CYP2B (Phenobarbital; PB) and CYP3A (dexamethasone; DEX) inducers compared to corn oil (vehicle) pretreated animals (64). Time-dependent changes of (G) levels and (H) enantiomeric fraction in the feces of mice fed a regular (10 % fat) or high-fat (37% fat) diet (63). * Different from intraperitoneally exposed mice,
p<0.05; b different from racemic (EF=0.05), p<0.05; c different from lowest dose (2.5 mg/kg body weight), p<0.05; d different from medium dose (10.0 mg/kg body weight), p<0.05; e different from corn oil-treated animals, p<0.05; f different from β-naphthoflavone-treated animals, p<0.05.
Figure 3.
Absorbance changes elicited by increasing concentrations of PCB 136 in mural liver microsomes (70). (A) Effect of the (±)-PCB 136 concentration on the absorbance of microsomes from corn oil, phenobarbital, dexamethasone and β-naphthoflavone-treated mice. (B) Effect of the concentration of (+)- or (-)-PCB 136 on the absorbance of microsomes from phenobarbital-treated mice. For each measurement, (±)-, (+)- or (-)-PCB 136 in DMSO were added to 1 mL sample cuvettes containing 4 nmol P450/mL. Adapted with permission from (70). Copyright 2008 American Chemical Society.