Published in final edited form as:

Environ Sci Pollut Res Int. 2016 February ; 23(3): 2042–2057. doi:10.1007/s11356-015-4150-2.

Chiral Polychlorinated Biphenyls: Absorption, Metabolism and Excretion – A Review

Izabela Kania-Korwel and Hans-Joachim Lehmler*

Department of Occupational and Environmental Health, College of Public Health, The University of Iowa, Iowa City, Iowa

Abstract

Seventy eight out of the 209 possible polychlorinated biphenyl (PCB) congeners are chiral, nineteen of which exist under ambient conditions as stable rotational isomers that are non-superimposable mirror images of each other. These congeners (C-PCBs) represent up to 6% by weight of technical PCB mixtures and undergo considerable atropisomeric enrichment in wildlife, laboratory animals and humans. The objective of this review is to summarize our current knowledge of the processes involved in the absorption, metabolism and excretion of C-PCBs and their metabolites in laboratory animals and humans. C-PCBs are absorbed and excreted by passive diffusion, a process that, like other physicochemical processes, is inherently not atropselective. In mammals, metabolism by cytochrome P450 (P450) enzymes represents a major route of elimination for many C-PCBs. *In vitro* studies demonstrate that C-PCBs with a 2,3,6-trichlorosubstitution pattern in one phenyl ring are readily oxidized to hydroxylated PCB metabolites (HO-PCBs) by P450 enzymes, such as rat CYP2B1, human CYP2B6 and dog CYP2B11. The oxidation of C-PCBs is atropselective, thus resulting in a species and congener-dependent atropisomeric enrichment of C-PCBs and their metabolites. This atropisomeric enrichment of C-PCBs and their metabolites likely plays a poorly understood role in the atropselective toxicity of C-PCBs and, therefore, warrants further investigation.

Keywords

Chirality; enantioselective; biotransformation; oxidation; cytochrome P450 enzyme

Introduction

Seventy eight of the 209 possible polychlorinated biphenyl congeners (PCBs) congeners display axial chirality and exist as rotational isomers, or atropisomers, that are non-superimposable mirror images of each other (Fig. 1) (Lehmler et al. 2009). As with other axially chiral biaryls, a PCB congener is chiral when both phenyl rings have an unsymmetrical substitution pattern relative to the axis formed by the central C-C bond of the biphenyl system. The rotational free energy barriers of PCB congeners with three or four

*To whom correspondence should be addressed: Hans-Joachim Lehmler, The University of Iowa, Department of Occupational and Environmental Health, UI Research Park, #221 IREH, Iowa City, Iowa 52242, Phone: (319) 335-4211, Fax: (319) 335-4290, hans-joachim-lehmler@uiowa.edu.

chlorine substituents *ortho* to the central C-C bond are $\Delta G = 176.6$ kJ/mol and $\Delta G = 246$ kJ/mol, respectively (Harju and Haglund 1999, Schurig and Reich 1998). These free rotational energy barriers are sufficient to prevent a racemization of chiral PCBs with three or four *ortho* chlorine substituents, even at the relatively high temperatures used for gas chromatographic separations (Harju and Haglund 1999). In contrast, atropisomers of chiral PCB congeners with none, one or two *ortho* chlorine substituents readily racemize and, therefore, do not form stable atropisomers under ambient conditions. Out of the 78 chiral PCB congeners, 19 PCB congeners, or C-PCBs, form stable atropisomers under ambient conditions. Thus, when the number of C-PCBs is taken into account, PCBs really make up a class of compounds consisting of 228, and not 209, individual chemicals.

PCBs were manufactured by batch chlorination of biphenyl and used as complex mixtures containing over 150 of the 209 possible PCB congeners (Boonyathumanondh et al. 1995, Driss et al. 1989, Falandysz et al. 2004, Frame 1997, Schulz et al. 1989, Taniyasu et al. 2003). Technical PCB mixtures were sold under many different trade names around the world, for example as Aroclor in the United States or as Kaneclor in Japan (US Environmental Protection Agency 2013). The United States (de Voogt and Brinkman 1989), Slovakia (Holoubek 2006) and Russia (Arctic Monitoring and Assessment Programme 2003) were major manufacturer of PCBs for a large range of consumer and industrial applications, followed by Germany (Fiedler 1997) and Japan (de Voogt and Brinkman 1989). China (Xing et al. 2005) and Poland (Sulkowski et al. 2003) also produced considerable amounts of PCBs. Many other countries, including France and North Korea, produced PCBs; however, total production numbers for these countries are not available. An estimated 500,000 tons of PCBs were manufactured worldwide (estimated based on the following references: (Arctic Monitoring and Assessment Programme 2003, de Voogt and Brinkman 1989, Fiedler 1997, Holoubek 2006, Sulkowski et al. 2003, US Environmental Protection Agency 2013, Xing et al. 2005). In addition, PCBs can be formed inadvertently by industrial processes, which represent a contemporary source of certain PCB congeners. For example, PCBs are present in paint pigments from several manufactures in USA and Japan (Anezaki and Nakano 2014, Hu et al. 2010).

Based on published production data (Arctic Monitoring and Assessment Programme 2003, de Voogt and Brinkman 1989, Fiedler 1997, Holoubek 2006, Sulkowski et al. 2003, US Environmental Protection Agency 2013, Xing et al. 2005) and published congener profiles (Boonyathumanondh et al. 1995, Driss et al. 1989, Falandysz et al. 2004, Frame 1997, Schulz et al. 1989, Taniyasu et al. 2003), C-PCBs constitute 6%, or 29,500 tons, of the total amount of PCB manufactured worldwide. Depending on the level of chlorination, PCB mixtures can contain 0.15–33% by weights of C-PCBs. PCB 95 and PCB 149 are the major C-PCB congeners present in technical PCB mixtures, followed by PCB 132, PCB 174, PCB 84 and PCB 45 (Fig. 2). C-PCBs, such as PCB 95, are also present in paint pigments from several manufactures in USA and Japan (Anezaki and Nakano 2014, Hu et al. 2010). The highest concentration of PCB 95 found in paint pigment was 2.3 ng/g (Anezaki and Nakano 2014). PCB 132 and PCB 149 were also detected in at least one paint pigment samples (Hu and Hornbuckle 2010). PCBs in paint and other building materials, such as caulking and fluorescent light ballasts (US Environmental Protection Agency 2014a, b) contribute to

indoor and outdoor air pollution with PCBs (Klosterhaus et al. 2014, Robson et al. 2010). For example, some C-PCBs (i.e., PCB 84, 91, 95, 132, 149 and 174) have been detected in indoor air in new York City school (Thomas et al. 2012). PCB 95 was found to be one of the highest congeners present in the indoor air, at a concentration of 50 ng/m³. However, diet remains the major route of human PCB exposure (Bergkvist et al. 2014, Chan-Hon-Tong et al. 2013, Kostyniak et al. 2005, Schechter et al. 2010, Su et al. 2012, Voorspoels et al. 2008). Unfortunately, data on C-PCB levels in foodstuff are almost non-existent. For example, a recent study of a wide range of foods from the Dallas metro area reported seven marker PCBs (i.e., PCB 28, 52, 101, 118, 138, 153, and 180), but no C-PCB levels (Schechter et al. 2010).

C-PCBs present in technical PCB mixtures and synthetic C-PCBs are essentially racemic (i.e., are formed as a 1:1 mixture of atropisomers) (Kania-Korwel et al. 2006, Pakdeesusuk et al. 2003). However, pure atropisomers of C-PCBs can be prepared in a multistep synthesis (Püttmann et al. 1989, Püttmann et al. 1986) or obtained after enantioselective separation of the racemic mixture using liquid chromatography (Haglund 1996a, b, Mannschreck et al. 1985, Schurig 2001). The availability of small quantities of pure PCB atropisomers allowed the determination of the optical rotation (i.e., (+)- vs. (–)-enantiomer) (Haglund 1996b); the elution order on different enantioselective gas chromatography columns (Haglund and Wiberg 1996) and the absolute configuration (i.e., *R*- vs. *S*-enantiomer) of some, but not all C-PCBs (Toda et al. 2012). Moreover, the metabolites of C-PCBs are also chiral due to the hindered rotation around the central C-C bond (Nezel et al. 1997), and—as with the parent compounds—can be separated into their atropisomers using liquid chromatography (Pham-Tuan et al. 2005, Zhai et al. 2013) or gas chromatography (Kania-Korwel et al. 2011, Kania-Korwel et al. 2008c). The absolute configuration of several C-PCB metabolites has been determined using a combination of experimental and computational techniques (Pham-Tuan et al. 2005).

C-PCBs can display considerable atropisomeric enrichment in the environment (Lehmler et al. 2009). The atropisomeric enrichment of C-PCBs, especially at higher trophic levels, raises the question if C-PCBs display atropselective biological effects in ecosystems and, ultimately, humans. A particular concern is the developmental neurotoxicity of PCBs, which has been documented in epidemiological (Carpenter 2006, Korrick and Sagiv 2008, Schantz et al. 2003, Seegal 1996, Winneke 2011) and laboratory studies (Mariussen and Fonnum 2006, Seegal 1996, Tilson and Kodavanti 1998). Mechanistic studies suggest that PCB developmental neurotoxicity is mediated by mechanisms independent of aryl hydrocarbon (AhR) activation and likely involves impaired Ca²⁺-signaling (reviewed in: Kodavanti and Curras-Collazo 2010, Mariussen and Fonnum 2006, Pessah et al. 2010)). Recent studies with pure C-PCB atropisomers showed that (–)-PCB 136 activates ryanodine receptors (RyRs) (Pessah et al. 2009), microsomal Ca²⁺ channels that are widely expressed in the mammalian brain and skeletal muscle. Moreover, (–)-PCB 136 enhances dendritic growth in primary cultured rat hippocampal neurons by mechanisms involving RyRs (Yang et al. 2014). In contrast, (+)-PCB 136 lacked activity towards RyRs and had no effect on dendritic growth. PCB 84 also atropselectively impaired Ca²⁺-signaling, as determined using [³H]-phorbol ester binding and microsomal ⁴⁵Ca²⁺-uptake (Lehmler et al. 2005). In addition to

their effects on Ca^{2+} -signaling, C-PCBs atropselectively alter the expression of hepatic xenobiotic metabolizing enzymes, including cytochrome P450 enzymes (Püttmann et al. 1990, Püttmann et al. 1989, Rodman et al. 1991). According to recent experimental evidence, HO-PCBs metabolites of C-PCBs cause muscle dysfunction in cells in culture by mechanisms involving RyRs and, thus, are also toxic (Niknam et al. 2013, Pessah et al. 2006).

The available evidence suggests that exposure to atropisomerically enriched C-PCBs and/or their atropselective disposition plays a poorly understood role in the adverse effects on PCBs in wildlife, laboratory animals and, ultimately, humans. Therefore, the objective of this review is to summarize our current knowledge of the processes involved in the absorption, metabolism and excretion of C-PCBs and their metabolites in laboratory animals and humans. Although C-PCBs and their metabolites are the primary focus of this review, findings from studies of structurally related PCB congeners are discussed when no or limited data for C-PCBs and/or their metabolites are available.

Absorption of C-PCBs

Oral absorption is an important and well understood route for systemic uptake of PCBs following PCB ingestion because diet, in particular fish, represents a major route of human exposure to PCBs (Bergkvist et al. 2014, Chan-Hon-Tong et al. 2013, Kostyniak et al. 2005, Schecter et al. 2010, Su et al. 2012, Voorspoels et al. 2008). To enter the systemic circulation, PCBs must diffuse from the bulk intestinal content to the intestinal wall (for a review of intestinal absorption and biomagnification models see (Kelly et al. 2004)). Since PCBs enter the systemic circulation of several species, including ring doves (Drouillard and Norstrom 2000), dogs (Busbee et al. 1985) and humans (Kuwabara et al. 1979), at rates similar to those observed for dietary lipids, PCBs are thought to move to the intestinal wall dissolved in mixed micelles (i.e., micelles containing bile salts, lipids and lipid digestive products) (Dulfer et al. 1998, Dulfer et al. 1996, Oomen et al. 2001) prior to entering the enterocyte by passive diffusion (Dulfer et al. 1998). As a result, oral absorption of PCBs is highly efficient; however, higher chlorinated congeners are less efficiently absorbed than lower chlorinated ones in most species, including fish (Gobas et al. 1988), ring doves (Drouillard and Norstrom 2000), rats (Matthews and Tuey 1980, Tanabe S. 1981), cows (McLachlan 1993) and humans (Jödicke et al. 1992), probably due to their lower solubility in the interior of mixed micelles (Dulfer and Govers 1995). Although this has not been investigated, the oral absorption efficiency of C-PCBs may, therefore, decrease with increasing degree of chlorination.

Dermal exposure represents an important occupational exposure, especially in workers directly exposed to technical PCB mixtures (Lees et al. 1987). This route of exposure contributes to increased PCB levels in monkeys (Mayes et al. 2002) and humans (Lees et al. 1987, Maroni et al. 1981a, Maroni et al. 1981b), and causes adverse human health effects (Maroni et al. 1981a, Maroni et al. 1981b) due to PCBs' high affinity for skin (Garner et al. 2006). For example, PCB 136, an important C-PCB, readily diffuses into rat skin slices, with almost 90 % of PCB 136 being absorbed by skin slices within 8 hours (Garner et al. 2006). Toxicokinetic studies comparing the dermal vs. oral routes of exposure demonstrate that

dermal exposure increases the time to reach the maximum PCB concentration with increasing degree of chlorination (Garner et al. 2006). In contrast, the maximum PCB concentration in blood occurs within 3–4 hours after oral administration of PCBs to rats, independent of the PCB congener. This is due to the retention of higher chlorinated and more lipophilic PCB congeners in fat depots of the skin.

There is currently growing concern about inhalation as a route of PCB exposure, especially in susceptible populations such as children, because of PCBs presence in indoor and outdoor air (Currado and Harrad 1998, Currado and Harrad 2000, Harrad et al. 2006, Hu et al. 2012a, Jamshidi et al. 2007, Robson and Harrad 2004). The observation that certain C-PCBs have considerable concentrations in indoor air from US schools represents a particular public health concern (Thomas et al. 2012). Recent nose-only inhalation studies demonstrate the PCBs, including some C-PCBs, are absorbed in the lung and distributed systemically in rats (Hu et al. 2010, Hu et al. 2012b). The uptake of PCBs in the lung appears to be highly efficient, with a 99.8% pulmonary uptake of an inhaled, radiolabeled PCB reported in rats (Hu et al. 2014). Together, these studies demonstrate that inhalation is an important, but currently overlooked route of exposure to PCBs.

Experimental evidence suggests that the absorption of PCB, including C-PCBs, following oral, dermal and inhalation exposure occurs by passive diffusion and, thus, should not be atropselective. It is possible that, in addition to passive diffusion, active transport processes play a role in the absorption of C-PCB in the gastrointestinal tract and, thus, contribute to the atropisomeric enrichment of C-PCBs observed *in vivo*; however, animal studies do not support the hypothesis that PCBs are substrates for active transporters, such as multidrug resistance transporters (Milanowski et al. 2010, Tampal et al. 2003). It is more likely that atropselective metabolism at the site of PCB absorption contributes to the atropisomeric enrichment of C-PCBs. Indeed, skin is able to rapidly metabolize C-PCBs, such as PCB 136 (Garner et al. 2006). Moreover, the intestine, lung and other extrahepatic tissues express P450 enzymes in different species (Ding and Kaminsky 2003, Renaud et al. 2011) and, thus, may be an important site of atropselective PCB metabolism. Further studies are therefore needed to assess the contribution of extrahepatic tissues to the disposition of C-PCBs.

Metabolism of C-PCBs to HO-PCBs

Structure-activity relationships and HO-PCB profiles of C-PCB metabolism

PCBs are typically thought of as persistent organic pollutants; however, many PCB congeners, including C-PCBs, are biotransformed through complex, species-dependent metabolic pathway to hydroxylated, methylsulfonylated, sulfated, glucuronidated and other metabolites (James 2001, Letcher et al. 2000) (Fig 3). A convenient nomenclature for PCB metabolites has been proposed by Maervoet et al and, as shown for PCB 136 in Fig 3, will be used throughout this manuscript (Maervoet et al. 2004). The oxidation of C-PCBs, in particular those with a 2,3,6-trichloro substitution pattern in one phenyl ring, to HO-PCBs has been studied extensively using recombinant enzymes (Lu et al. 2013, Lu and Wong 2011, Waller et al. 1999, Warner et al. 2009), hepatic microsomes (Kania-Korwel et al. 2011, Kania-Korwel and Lehmler 2013, Schnellmann et al. 1983, Wu et al. 2014, Wu et al. 2011), isolated hepatocytes (Vickers et al. 1986) and, liver, hippocampus and skin slices

(Garner et al. 2006, Wu et al. 2013a, Wu et al. 2013b) obtained from mammalian species. To the best of our knowledge, the oxidation of C-PCBs in non-mammalian species, such as amphibians, fish or avian species, has not been investigated to date. Indirect evidence, for example from toxicokinetic studies, suggests that C-PCBs are also subject to biotransformation in many non-mammalian species.

Detailed kinetic studies for the oxidation of C-PCBs by P450 enzymes have been reported only for racemic PCB 136 in pooled human liver microsomes (HLMs; pool of three) (Schnellmann et al. 1983) and rat hepatocytes (Vickers et al. 1986). Schnellmann et al. reported that PCB 136 is metabolized by HLMs with a K_m of 20.2 mM and a V_{max} of 19.3 pmoles/min/nmoles P450 (Schnellmann et al. 1983). Metabolism studies with PCB 136 in isolated rat hepatocytes suggested the presence of two different metabolism reactions (Vickers et al. 1986). The high affinity reaction had an apparent K_m of 0.48 μ M and a V_{max} of 3.7 pmoles/min/mg hepatocyte. The low affinity reaction had an apparent K_m of 645 μ M and a V_{max} of 367 pmoles/min/mg hepatocyte. Several recent studies using pure PCB 132 and 136 atropisomers suggest that the respective atropisomers interfere with each other's metabolism by CYP2B1 (Lu et al. 2013, Lu and Wong 2011), and further studies of the enzyme kinetics of pure PCB atropisomers are needed.

A simplified outline for the metabolism of PCB 136 is shown in Fig. 3. Briefly, the oxidation reaction occurs either by direct insertion of oxygen into an aromatic C-H bond (Preston et al. 1983) or via an epoxide intermediate (Forgue and Allen 1982, Forgue et al. 1979). The 4,5-epoxide intermediate of C-PCBs with a 2,3,6-trichloro substitution pattern can rearrange to form both 4- or 5-hydroxylated PCB metabolites. In addition, a 1,2-shift (or NIH-shift) (Guroff et al. 1967, Jerina and Daly 1974) of the 3-chloro substituent can lead to the formation of HO-PCBs with a 2,4,6-trichloro-3-hydroxy substitution pattern as minor metabolites (Kania-Korwel et al. 2012, Kania-Korwel et al. 2011, Wu et al. 2013a, Wu et al. 2014). Studies with recombinant P450 enzymes demonstrate that CYP2B isoforms, such as rat CYP2B1 (Lu et al. 2013, Waller et al. 1999, Warner et al. 2009), human CYP2B6 (Warner et al. 2009) and dog CYP2B11 (Waller et al. 1999), are involved in the formation of *meta* hydroxylated C-PCBs. The P450 isoforms responsible for the formation of *para* hydroxylated metabolites of C-PCBs have not been identified to date. Many of the mono- and dihydroxylated metabolites of C-PCBs reported *in vitro* are also detected *in vivo* (Table 1).

Species differences in the metabolism rates and metabolite profiles have been observed in many studies investigating the P450 enzyme-mediated oxidation of C-PCBs to HO-PCBs (Ohta et al. 2013, Ohta et al. 2005, Waller et al. 1999, Warner et al. 2009, Wu et al. 2014). Several factors contribute to these differences, for example species-differences in the structure of relevant P450 enzymes (Waller et al. 1999) and the hepatic P450 enzyme and P450 isoform composition (Lewis et al. 1998). Studies with liver microsomes reveal that 5-HO-PCB 136 is formed by liver microsomes with a rank order of rat > dog ~ guinea pig ~ hamster > human > monkey ~ mouse ~ rabbit (Fig. 4) (Wu et al. 2014). In the same study, 4-HO-PCB 136 is formed in the order human > monkey > rabbit ~ rat > dog ~ guinea pig ~ mouse ~ hamster. 5-HO-PCB 136 is the major metabolite formed in HLMs (pool from 50 individual donors), with the 5-HO-PCB 136/4-HO-PCB 136 metabolite ratio ranging from

0.4:1 to 0.8:1. An earlier study reports a different metabolite profile in incubations with HLMs from a pool of three donors, with 4-HO-PCB 136 being the major metabolite (5-HO-PCB 136/4-HO-PCB 136 metabolite ratio = 1.3) (Schnellmann et al. 1983). The differences in the HO-PCB profiles are likely due to differences in the hepatic P450 enzyme composition. Consistent with this explanation, Ohta et al. report that the formation of HO-PCBs by individual HLMs depends on the P450 isoform composition and correlates with hepatic CYP2B6 activity (Ohta et al. 2013).

In addition to species differences, mutations of a P450 isoform can affect the rates and regioselectivity of the oxidation of C-PCBs. A study with *E. coli*-expressed rat CYP2B1 and dog CYP2B11 mutants demonstrates that the metabolism of PCB 136 to 5-HO-PCB 136 by P450 mutants is slower compared to the wild type P450 enzyme (Waller et al. 1999). The I480V mutant is an exception and increases the 5-HO-PCB 136 formation approximately 1.7-fold compared to the CYP2B1 wild type. Interestingly, CYP2B1 mutant I114V and the CYP2B11 mutant L363V display an altered regioselectivity and also form 4-HO-PCB 136. The differences in the metabolite profiles and the altered metabolism rates in the CYP2B1 and CYP2B11 mutants can be attributed to a different orientation adopted by PCB 136 in the active site of the respective mutant. These observations are important because human CYP2B6, the human orthologue of CYP2B1 and CYP2B11 is a highly polymorphic enzyme (Zanger et al. 2007). Similar to chlorpyrifos (Crane et al. 2012), C-PCB metabolism rates and metabolite profiles may therefore depend on the CYP2B6 genotype. Since PCB 136 binds atropselectively to rat microsomal CYP2B enzymes (Kania-Korwel et al. 2008b), site mutants of P450 enzymes may also metabolize PCB 136 atropisomers with different selectivity compared to the wild type enzyme. Further studies are needed to assess if chiral signatures of PCBs and HO-PCBs are affected by P450 polymorphisms.

Atropselective oxidation of C-PCBs and their metabolites

Several studies using recombinant P450 enzymes (Lu et al. 2013, Warner et al. 2009), liver microsomes (Kania-Korwel et al. 2011, Kania-Korwel and Lehmler 2013, Wu et al. 2014, Wu et al. 2011) or liver tissue slices (Wu et al. 2013a, Wu et al. 2013b) demonstrate that PCBs are atropselectively metabolized to HO-CPBs by P450 enzymes, thus resulting in an atropisomeric enrichment of PCBs and HO-PCBs in the microsomal incubation (Table 2). The atropselective biotransformation of C-PCBs to HO-PCB metabolites by recombinant rat and human cytochrome P450 enzymes has been studied in some detail (Lu et al. 2013, Warner et al. 2009). Incubations of individual, racemic PCB congeners or a mixture of several C-PCBs with rat CYP2B1 show preferential transformation of E₂-PCB 45, (–)-PCB 84, E₁-PCB 91, E₂-PCB 95, (–)-PCB 132, (+)-PCB 136 and (–)-PCB 149. The same direction of atropisomeric enrichment is observed in rat liver microsomal incubations (Kania-Korwel et al. 2011, Kania-Korwel and Lehmler 2013, Wu et al. 2011) and, for PCB 136, in rat liver tissue slices (Wu et al. 2013b). At the same time, the formation of HO-PCB metabolites by rat P450 enzymes is atropselective, thus resulting in an atropisomeric enrichment of the HO-PCBs. Although all C-PCBs investigated are atropselectively metabolized by rat P450 enzymes to HO-PCBs, the PCB metabolism rates and HO-PCB formation rates are highly congener specific, which may, at least in part, explain the

differences in the extent and direction of the atropisomeric enrichment of different C-PCBs in wildlife (Lehmler et al. 2009).

The chiral signatures observed in *in vitro* studies predict the direction of the atropisomeric enrichment of C-PCBs *in vivo*. Specifically, the observation that (+)-PCB 136 is more rapidly biotransformed by CYP2B1 is in agreement with a study showing an enantiomeric enrichment of (–)-PCB 136 in male and female Sprague-Dawley rats (Kania-Korwel et al. 2008c). Moreover, the atropisomeric enrichment of E₂-PCB 95 in the *in vitro* metabolism experiments is consistent with the atropisomeric enrichment of PCB 95 observed in rats treated with an Aroclor 1254 mixture (Kania-Korwel et al. 2006). Similarly, *in vitro* metabolism studies (Wu et al. 2013a) accurately predict the direction of the atropisomeric enrichment of PCB 136 (Kania-Korwel et al. 2010, Kania-Korwel et al. 2008a, Kania-Korwel et al. 2007, Kania-Korwel et al. 2008d, Milanowski et al. 2010) and PCB 95 (Kania-Korwel et al. 2012, Kania-Korwel et al. 2010, Milanowski et al. 2010) in mice *in vivo*. Incubations with human CYP2B6 result in biotransformation of PCBs 45, 91 and 132; however, only the biotransformation of PCBs 45 and 132 is enantioselective. Interestingly, (–)-PCB 132 is more rapidly biotransformed by CYP2B6, which is consistent with the enrichment of (+)-PCB 132 in human liver (Chu et al. 2003) and breast milk (Bordajandi et al. 2008).

Hepatic microsomes prepared from male Sprague-Dawley rats pretreated with phenobarbital (PB, CYP2B inducer) were used to identify which HO-PCB atropisomers form which PCB 136 atropisomer based on their gas chromatographic elution order (Fig. 5) (Wu et al. 2011). (–)-PCB 136 elutes first on Chirasil-Dex and Cyclosil-B columns (Kania-Korwel and Lehmler 2013). Both HO-PCB metabolites of (–)-PCB 136 also elute first (E₁) on both enantioselective columns. Vice versa, metabolites formed from (+)-PCB 136 corresponded to the second eluting atropisomer (E₂) of 5-HO-PCB 136 and 4-HO-PCB 136. In parallel incubations with racemic PCB 136, the second elution 5-HO-PCB 136 atropisomer (E₂-5-136; Chirasil-Dex column) and the first eluting 4-HO-PCB 136 atropisomer (E₁-4-HO-PCB 136; Cyclosil-B column) are enriched in microsomal incubations using racemic PCB 136, independent of the microsomal formulation, PCB 136 concentration and time point analyzed. These observations demonstrate that the metabolism of (+)-PCB 136 to E₂-5-HO-PCB 136 in microsomal incubations is faster compared to the metabolism of (–)-PCB 136 to E₂-5-HO-PCB 136. Since 5-HO-PCB 136 is the major metabolite, this observation is consistent with an atropisomeric enrichment of (–)-PCB 136 in *in vitro* (Lu et al. 2013, Wu et al. 2013b, Wu et al. 2011) and *in vivo* metabolism studies in rats (Kania-Korwel et al. 2008c).

Several recent metabolism studies demonstrate that the atropselective formation of HO-PCBs is also species dependent (Kania-Korwel et al. 2008b, Wu et al. 2013a, Wu et al. 2014, Wu et al. 2013b, Wu et al. 2011). For example, both 5-HO-PCB136 and 4-HO-PCB136 are atropselectively formed by liver microsomes from humans, dogs, monkeys, guinea pigs, mice, hamsters and rabbits (Fig. 6). While the extent of the atropisomeric enrichment of both HO-PCB 136 metabolites differs significantly between species, the direction of the atropisomeric enrichment is similar in many species. Specifically, E₂-5-HO-PCB136 and E₂-4-HO-PCB136 are formed preferentially from racemic PCB 136 in

incubations using liver microsomes from different, toxicologically relevant species. Both metabolites are formed from (+)-PCB 136. The mice is a notable exception, with E₁-5-HO-PCB136 and E₁-4-HO-PCB136 formed preferentially *in vitro* (Wu et al. 2013a, Wu et al. 2014). In agreement with this finding, mice display an enrichment of (+)-PCB 136 in all tissues and excreta (Kania-Korwel et al. 2010, Kania-Korwel et al. 2008a, Kania-Korwel et al. 2007, Kania-Korwel et al. 2008d, Milanowski et al. 2010).

Atropselective metabolism of HO-PCBs

HO-PCBs themselves can be further metabolized to a myriad of different metabolites (Fig. 3). For example, P450 enzymes can oxidize monohydroxylated C-PCBs to dihydroxylated C-PCBs (Lu et al. 2013, Waller et al. 1999). Studies with recombinant rat CYP2B1 reveal that the formation of 4,5-HO-PCB 95 from 5-HO-PCB 95 and 4-HO-PCB 95 is both regio- and atropselective (Fig. 7) (Lu et al. 2013). Specifically, the 5-HO-PCB 95 regioisomer has a higher relative metabolism rate than the 4-HO-PCB 95 regioisomer. Moreover, the extent, but not the direction of the atropisomeric enrichment of 4,5-HO-PCB 95 are different between 5-HO-PCB 95 and 4-HO-PCB 95, with a more pronounced enrichment of 4,5-HO-PCB 95 observed in incubations with 5-HO-PCB 95. In contrast, an *in vivo* disposition study in mice reports near racemic chiral signatures for 4,5-HO-PCB 95 (Kania-Korwel et al. 2012). As shown in Fig. 3, dihydroxylated C-PCBs may subsequently undergo autooxidation and enzymatic oxidation to PCB quinones (Amaro et al. 1996). Afterwards, the reactive quinone intermediates can react with cellular nucleophiles in a non-enzymatic chlorine displacement reaction (Song et al. 2009). In addition to the corresponding PCB 136 epoxides, the proposed PCB 136 quinone intermediate may contribute to the microsomal protein binding observed in HLM incubations with racemic PCB 136 (Schnellmann et al. 1983).

There is some experimental evidence that C-PCBs are metabolized to glucuronide conjugates in rats. For example, the disposition of chiral catechol PCB metabolites differs between Wistar and Gunn rats (Haraguchi et al. 2004). Specifically, the dihydroxylated metabolites are more persistent in Gunn than Wistar rats, most likely due to the uridine diphosphate glucuronosyltransferases (UGT) deficiency in Gunn rats. There is also evidence that PCB 136 glucuronides accumulate in the fetal compartment of pregnant rats (Lucier et al. 1978). Moreover, a growing number of *in vitro* and *in vivo* studies demonstrate that HO-PCBs can be further metabolized to PCB sulfates; however, the formation of C-PCB sulfates *in vivo* has not been investigated to date. It is also unknown if the metabolism of HO-PCBs to sulfate or glucuronide conjugates, or other metabolites, is atropselective. Unfortunately, analytical standards and straightforward analytical methods for studies of C-PCB sulfate or glucuronide conjugates *in vitro* or *in vivo* are currently not available.

Atropselective formation of methylsulfone metabolites of C-PCBs

In addition to the direct insertion of an oxygen atom in the *meta* position, PCBs can also form reactive PCB epoxide intermediates that undergo glutathione conjugation, followed by stepwise transformation to mercapturic acid and, ultimately, methylsulfone derivatives of PCBs (MeSO₂-PCBs) (Bakke et al. 1982, Letcher et al. 2000). *Para*-substituted MeSO₂-PCBs, such as 4-MeSO₂-PCB 91, 4'-MeSO₂-PCB 132 and 4-MeSO₂-PCB 149, are typically

present at higher concentrations in the liver, lung and adipose tissues of wildlife and laboratory animals than the corresponding *meta*-substituted MeSO₂-PCBs (Karasek et al. 2004, Larsson et al. 2002). Like the parent C-PCBs, MeSO₂-PCB metabolites and the respective intermediates also display axial chirality, can be separated into the respective atropisomers (Pham-Tuan et al. 2005), and undergo atropisomeric enrichment *in vivo*. Some chiral MeSO₂-PCB congeners, such as 4-MeSO₂-PCB 149, 5-MeSO₂-PCB 149, 4'-MeSO₂-PCB 149 and 5'-MeSO₂-PCB 132, display atropisomeric enrichment in human postmortem liver (Ellerichmann et al. 1998) and wildlife tissues (Karasek et al. 2004, Karasek et al. 2007, Larsson et al. 1999, Larsson et al. 2002). The atropisomeric enrichment of *para*-substituted MeSO₂-PCBs is typically not as pronounced as of *meta*-substituted MeSO₂-PCBs (Ellerichmann et al. 1998, Larsson et al. 1999). In addition to these environmental studies, the *R*-enantiomer of 4'- and 5'-MeSO₂-PCB 132 is formed almost atropselectively in male rats following single oral administration of racemic PCB 132 (Norström et al. 2006). The processes resulting in the atropisomeric enrichment of MeSO₂-PCBs are poorly understood. Limited evidence demonstrating the atropselective biotransformation of PCB 149 to 5-*S*-MeSO₂-PCBs 149 by rat hepatocytes suggests a role of P450 enzymes in the atropisomeric enrichment of MeSO₂-PCBs *in vivo* (Hühnerfuss et al. 2003). Moreover, the toxicological relevance of the atropisomeric enrichment of MeSO₂-PCBs remains unexplored.

Excretion of C-PCBs and their metabolites

Feces and urine are only minor routes of excretion for parent PCBs. For example, in the case of PCB 136 only a small percentage of the parent compound is excreted with feces and urine in the rat (Birnbaum 1983), dog (Sipes et al. 1982) and monkey (Sipes et al. 1982). Instead, fecal and urinary excretion of PCB 136 occurs primarily in form of its metabolites (Birnbaum 1983, Sipes et al. 1982). Two processes contribute to the fecal excretion of PCBs. A small percentage of unabsorbed PCBs are excreted within the first 24 hours after PCB administration in rats (Tanabe S. 1981) and mice (Kania-Korwel et al. 2008a). The contribution of unabsorbed PCBs to fecal PCB levels is directly related to the fecal fat content, with higher fecal fat contents increasing the PCB partitioning capacity of feces and, thus, fecal PCB levels (Drouillard 2003, Gobas et al. 1988). A second relevant process is the intestinal elimination of the parent PCB, which also is related to the fecal fat content. Biliary excretion of PCB 136 apparently does not contribute to the excretion of the parent compound (Sipes et al. 1982). These excretion processes occur by passive diffusion and, therefore, are not atropselective. Although PCBs apparently are not substrates for active transporters, such as multidrug resistance transporters (Milanowski et al. 2010, Tampal et al. 2003), in *in vivo* studies, the possibility that atropselective active transport processes contribute to the excretion of C-PCBs cannot be completely ruled out. Moreover, it is currently unknown if active transporters are involved in the excretion of PCB metabolites.

Several studies have investigated the enantiomeric enrichment of C-PCBs in feces. Mice fed a high fat diet excreted near racemic PCB 136 in the feces within the first 24 hours after oral administration of a bolus dose of racemic PCB 136 (Kania-Korwel et al. 2008a). Twenty four hours after PCB administration, a significant enrichment of (+)-PCB 136 was observed in the feces from the same animals due to the elimination of PCB 136 from the systemic

circulation. The fact that little-to-no enantiomeric enrichment was observed within the first 24 hours is due to the 10- to 100-times higher levels of unabsorbed PCBs in feces, which mask the chiral signatures of the PCB eliminated from the systemic circulation. Similarly, Norström and coworkers reported that the enantiomeric enrichment of PCB 132 in feces collected over a four day period from male Wistar rats was much less pronounced compared to tissue EF values due to non-absorbed PCB 132 (Norström et al. 2006). Chiral signatures of hydroxylated C-PCBs and their conjugates in excreta have not been investigated to date.

Harrad et al. investigated the enantiomeric enrichment of PCBs 95 and 149, two environmentally relevant PCB congeners, in feces from volunteers eating a diet containing trace levels of racemic PCBs 95 and 149 (Harrad et al. 2006). Only a slight enantiomeric enrichment of PCB 95 was observed in two out of ten feces samples. PCB 149 was only detected in one feces sample. This is a surprising observation because, based on microsomal metabolism studies with HLMs (Wu et al. 2014), disposition studies in laboratory animals (Kania-Korwel et al. 2008a, Norström et al. 2006) and chiral signatures in human liver (Chu et al. 2003) and breast milk (Glausch et al. 1995) samples, a more pronounced atropisomeric enrichment would be expected in human feces. Therefore, unabsorbed PCBs are one possible explanation for the near racemic PCBs 95 and 149 residues in the study by Harrad and co-workers.

Conclusion

C-PCBs represent an important group of PCBs that are thought to play an important role in the adverse effects of PCBs, in particular in PCB developmental neurotoxicity. An intriguing, yet unanswered question is how the atropselective disposition of C-PCBs and their metabolites affects adverse outcomes in laboratory animals and humans. Specific questions that warrant future attention include:

- The absorption of C-PCBs in the gastrointestinal tract, lung and skin occurs by passive transport processes and, thus, makes no contribution to the atropisomeric enrichment of C-PCBs observed *in vivo*. However, it is currently unclear if atropselective metabolism at the site of absorption or in other, extrahepatic tissues contributes to the atropisomeric enrichment of C-PCBs.
- Hepatic P450 enzymes atropselectively metabolize C-PCBs to HO-PCBs and other metabolites. However, only limited information about the P450 isoforms involved in the atropselective metabolism of C-PCBs is currently available. Moreover, it is unknown how P450 polymorphisms affect the atropselective metabolism of C-PCBs.
- *In vitro* studies demonstrate that pure PCB atropisomers can interfere with each other's metabolism. Further studies are needed to assess how exposure to complex mixtures affects the atropselective disposition of C-PCBs *in vivo*. Such studies will make a fundamental contribution to our understanding of the chiral signatures observed in wildlife and human samples.
- C-PCBs can be metabolized via the corresponding HO-PCBs or PCB epoxides to a myriad of other metabolites (Fig. 3). Similar to other PCB congeners, chiral HO-

PCBs can be further oxidized to dihydroxylated metabolites and/or metabolized to sulfate or glucuronide conjugates. Moreover, PCB epoxides can form glutathione conjugates that are converted via the mercapturic acid pathway to chiral methylsulfonyl PCBs. Unfortunately, only limited information about the phase II metabolites of C-PCBs is available. Furthermore, it is unknown which atropselective biological processes play a role in the formation, distribution and elimination of these phase II metabolites.

- Active transport processes may play a role in atropselective distribution and elimination of C-PCBs and their metabolites and, therefore, warrant further investigation.
- Metabolites of C-PCBs, such as their HO-PCB and MeSO₂-PCB metabolites, are potentially toxic themselves. Because C-PCB metabolites are also chiral, future studies need to assess if—like the parent C-PCBs—their toxicity is atropselective.
- Authentic standards and validated analytical methods are typically not available to systematically address the above mentioned questions. Additional efforts are needed to develop novel synthetic strategies for C-PCB metabolites and established methods for their atropselective analysis in different biological matrices.

Acknowledgments

The authors would like to acknowledge support through grants from the National Institute for Environmental Health Sciences/National Institutes of Health (ES05605, ES012475, ES013661 and ES017425) where their own work is cited.

References

- Amaro AR, Oakley GG, Bauer U, Spielmann HP, Robertson LW. Metabolic activation of PCBs to quinones: Reactivity toward nitrogen and sulfur nucleophiles and influence of superoxide dismutase. *Chem Res Toxicol*. 1996; 9:623–629. [PubMed: 8728508]
- Anezaki K, Nakano T. Concentration levels and congener profiles of polychlorinated biphenyls, pentachlorobenzene, and hexachlorobenzene in commercial pigments. *Environ Sci Pollut Res*. 2014; 21:998–1009.
- Arctic Monitoring and Assessment Programme. [Accessed October 14, 2014] PCB in the Russian Federation: Inventory and proposals for priority remedial actions. Executive summary of the report of phase 1: Evaluation of the current status of the problem with respect to environmental impact and development of proposals for priority remedial actions of the multilateral cooperative project on phase-out of PCB use, and management of PCB-contaminated wastes in the Russian Federation. 2003. <http://www.amap.no/documents/doc/pcb-in-the-russian-federation-inventory-and-proposals-for-priority-remedial-actions/797>
- Bakke JE, Bergman AL, Larsen GL. Metabolism of 2,4',5-trichlorobiphenyl by the mercapturic acid pathway. *Science*. 1982; 217:645–7. [PubMed: 6806905]
- Bergkvist C, Kippler M, Larsson SC, Berglund M, Glynn A, Wolk A, Aakesson A. Dietary exposure to polychlorinated biphenyls is associated with increased risk of stroke in women. *J Intern Med*. 2014; 276:248–259. [PubMed: 24428778]
- Birnbaum LS. Distribution and excretion of 2,3,6,2',3',6'- and 2,4,5,2',4',5'-hexachlorobiphenyl in senescent rats. *Toxicol Appl Pharmacol*. 1983; 3:262–272. [PubMed: 6414105]
- Boonyathumanondh R, Watanabe S, Laovakul W, Tabucanon M. Development of a quantification methodology for polychlorinated biphenyls by using Kanechlor products as the secondary reference standard. *Fresenius J Anal Chem*. 1995; 352:261–267.

- Bordajandi LR, Abad E, Gonzales MJ. Occurrence of PCBs, PCDD/Fs, PBDEs and DDTs in Spanish breast milk: Enantiomeric fraction of chiral. PCBs Chemosphere. 2008; 70:567–575. [PubMed: 17727913]
- Busbee DL, Yoo J-SH, JON, Joe CO. Polychlorinated biphenyl uptake and transport by lymph and plasma components. Proc Soc Exper Biol Med. 1985; 179:116–122. [PubMed: 3921971]
- Carpenter DO. Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. Rev Environ Health. 2006; 21:1–23. [PubMed: 16700427]
- Chan-Hon-Tong A, Charles M-A, Forhan A, Heude B, Sirot V. Exposure to food contaminants during pregnancy. Sci Total Environ. 2013; 458–460:27–35.
- Chu S, Covaci A, Schepens P. Levels and chiral signatures of persistent organochlorine pollutants in human tissues from Belgium. Environ Res. 2003; 93:167–176. [PubMed: 12963401]
- Crane AL, Klein K, Zanger UM, Olson JR. Effect of CYP2B6*6 and CYP2C19*2 genotype on chlorpyrifos metabolism. Toxicology. 2012; 293:115–122. [PubMed: 22281205]
- Currado GM, Harrad S. A comparison of polychlorinated biphenyl concentrations in indoor and outdoor air and the potential significance of inhalation as a human exposure pathway. Environ Sci Technol. 1998; 32:3043–3047.
- Currado GM, Harrad S. Factors influencing atmospheric concentrations of polychlorinated biphenyls in Birmingham, U.K. Environ Sci Technol. 2000; 34:78–82.
- de Voogt, P.; Brinkman, U. Production, properties and usage of polychlorinated biphenyls. In: Kimborough, RD.; Jensen, A., editors. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier Science Publishers; Amsterdam: 1989. p. 3-43.
- Ding X, Kaminsky LS. Human extrahepatic cytochromes P450: Function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. Annu Rev Pharmacol Toxicol. 2003; 43:149–173. [PubMed: 12171978]
- Driss MR, Sabbah S, Bouguerra ML. High-resolution gas chromatography of PCBs [polychlorobiphenyls]. Analysis of commercial mixtures of Phenochlor Analisis. 1989; 17:252–8.
- Drouillard KG, Norstrom RJ. Dietary absorption efficiencies and toxicokinetics of polychlorinated biphenyls in ring doves following exposure to Aroclor mixtures. Environ Toxicol Chem. 2000; 19:2707–2714.
- Drouillard KGN, RJ. The influence of diet properties and feeding rates on PCB toxicokinetics in the ring dove. Arch Environ Contam Toxicol. 2003; 44:97–106. [PubMed: 12434224]
- Dulfer WJ, Govers HAJ. Solubility and micelle-water partitioning of polychlorinated biphenyls in solutions of bile salt micelles. Chemosphere. 1995; 30:293–306. [PubMed: 7874475]
- Dulfer WJ, Groten JP, Govers HA. Effect of fatty acids and the aqueous diffusion barrier on the uptake and transport of polychlorinated biphenyls in Caco-2 cells. J Lipid Res. 1996; 37:950–61. [PubMed: 8725148]
- Dulfer WJ, Govers HAJ, Groten JP. Kinetics and conductivity parameters of uptake and transport of polychlorinated biphenyls in the Caco-2 intestinal cell line model. Environ Toxicol Chem. 1998; 17:493–501.
- Ellerichmann T, Bergman A, Franke S, Hühnerfuss H, Jakobsson E, König WA, Larsson C. Gas chromatographic enantiomer separations of chiral PCB methyl sulfones and identification of selectively retained enantiomers in human liver. Fres Environ Bull. 1998; 7:244–257.
- Falandysz J, Taniyasu S, Flisak M, Swietojanska A, Horii Y, Honari N, Yamashita N. Composition of chlorobiphenyl congeners in the Chlorofen formulation. Roczniki Panstw Zakl Hig. 2004; 55:313–324. [PubMed: 19097581]
- Fiedler, H. Polychlorinated biphenyls (PCB): Uses and environmental releases. Proceedings of the Subregional Awareness Raising Workshop on Persistent Organic Pollutants (POPs); St. Petersburg, Russian Federation. 1997.
- Forgue ST, Preston BD, Hargraves WA, Reich IL, Allen JR. Direct evidence that an arene oxide is a metabolic intermediate of 2,2',5,5'-tetrachlorobiphenyl. Biochem Biophys Res Commun. 1979; 91:475–83. [PubMed: 42397]
- Forgue ST, Allen JR. Identification of an arene oxide metabolite of 2,2',5-5'-tetrachlorobiphenyl by gas chromatography-mass spectroscopy. Chem Biol Interact. 1982; 40:233–45. [PubMed: 6805966]

- Frame GM. A collaborative study of 209 PCB congeners and 6 Aroclors on 20 different HRGC columns. 2 Semi-quantitative Aroclor congener distributions Fresenius. *J Anal Chem.* 1997; 357:714–722.
- Garner CE, Demeter J, Matthews HB. The effect of chlorine substitution on the disposition of polychlorinated biphenyls following dermal administration. *Toxicol Appl Pharmacol.* 2006; 216:157–67. [PubMed: 16784763]
- Glausch A, Hahn J, Schurig V. Enantioselective determination of chiral 2,2',3,3',4,6'-hexachlorobiphenyl (PCB 132) in human milk samples by multidimensional gas chromatography/electron capture detection and by mass spectrometry. *Chemosphere.* 1995; 30:2079–2085. [PubMed: 7620847]
- Gobas FAPC, Muir DCG, Mackay D. Dynamics of dietary bioaccumulation and fecal elimination of hydrophobic organic chemicals in fish. *Chemosphere.* 1988; 17:943–962.
- Guroff G, Daly JW, Jerina DM, Renson J, Witkop B, Udenfriend S. Hydroxylation-induced migration: the NIH shift. Recent experiments reveal an unexpected and general result of enzymatic hydroxylation of aromatic compounds. *Science.* 1967; 157:1524–1530. [PubMed: 6038165]
- Haglund P. Enantioselective separation of polychlorinated biphenyl atropisomers using chiral high performance liquid chromatography. *J Chromatogr.* 1996a; 724:219–228.
- Haglund P. Isolation and characterization of polychlorinated biphenyl (PCB) atropisomers. *Chemosphere.* 1996b; 32:2133–2140.
- Haglund P, Wiberg K. Determination of the gas chromatographic elution sequences of the (+) and (–) enantiomers of stable enantiomeric PCBs on Chirasil-Dex. *J High Resol Chromatogr.* 1996; 19:373–376.
- Haraguchi K, Kato Y, Koga N, Degawa M. Metabolism of polychlorinated biphenyls by Gunn rats: identification and serum retention of catechol metabolites. *Chem Res Toxicol.* 2004; 17:1684–1691. [PubMed: 15606145]
- Haraguchi K, Kato Y, Koga N, Degawa M. Species differences in the tissue distribution of catechol and methylsulphonyl metabolites of 2,4,5,2',5'-penta- and 2,3,4,2',3',6'-hexachlorobiphenyls in rats, mice, hamsters and guinea pigs. *Xenobiotica.* 2005a; 35:85–96. [PubMed: 15788370]
- Haraguchi K, Koga N, Kato Y. Comparative metabolism of polychlorinated biphenyls and tissue distribution of persistent metabolites in rats, hamsters, and guinea pigs. *Drug Metab Dispos.* 2005b; 33:373–380. [PubMed: 15608140]
- Harju MT, Haglund P. Determination of the rotational energy barriers of atropisomeric polychlorinated biphenyls. *Fresenius J Anal Chem.* 1999; 364:219–223.
- Harrad S, Ren J, Hazrati S, Robson M. Chiral signatures of PCBs 95 and 149 in indoor air, grass, duplicate diets and human faeces. *Chemosphere.* 2006; 63:1368–1376. [PubMed: 16289232]
- Holoubek, I. The national implementation plan for the implementation of the Stockholm Convention in the Czech Republic. TOCOEN; Brno: 2006.
- Hu D, Hornbuckle KC. Inadvertent polychlorinated biphenyls in commercial paint pigments. *Environ Sci Technol.* 2010; 44:2822–7. [PubMed: 19957996]
- Hu D, Lehmler HJ, Martinez A, Wang A, Hornbuckle KC. Atmospheric PCB congeners across Chicago. *Atmos Environ.* 2012a; 44:1550–1557.
- Hu X, Adamcakova-Dodd A, Lehmler H-J, Hu D, Kania-Korwel I, Hornbuckle KC, Thorne PS. Time course of congener uptake and elimination in rats after short-term inhalation exposure to an airborne polychlorinated biphenyl (PCB) mixture. *Environ Sci Technol.* 2010; 44:6893–900. [PubMed: 20698547]
- Hu X, Adamcakova-Dodd A, Lehmler HJ, Hu D, Hornbuckle K, Thorne PS. Subchronic inhalation exposure study of an airborne polychlorinated biphenyl mixture resembling the Chicago ambient air congener profile. *Environ Sci Technol.* 2012b; 46:9653–62. [PubMed: 22846166]
- Hu X, Adamcakova-Dodd A, Thorne PS. The fate of inhaled (14)C-labeled PCB11 and its metabolites in vivo. *Environ Int.* 2014; 63:92–100. [PubMed: 24275706]
- Hühnerfuss H, Bergman A, Larsson C, Peters N, Westendorf J. Enantioselective transformation of atropisomeric PCBs or of their methylsulfonyl metabolites by rat hepatocytes? *Organohalogen Compd.* 2003; 62:265–268.

- James, MO. Polychlorinated biphenyls: Metabolism and metabolites. In: Larry, W.; Robertson, LGH., editors. PCBs: Recent Advances in Environmental Toxicology and Health Effects. The University Press of Kentucky; Lexington, KY: 2001. p. 35-46.
- Jamshidi A, Hunter S, Hazrati S, Harrad S. Concentrations and chiral signatures of polychlorinated biphenyls in indoor and outdoor air and soil in a major UK conurbation. *Environ Sci Technol*. 2007; 41:2153–2158. [PubMed: 17438756]
- Jerina DM, Daly JW. Arene oxides. New aspect of drug metabolism. *Science*. 1974; 185:573–582. [PubMed: 4841570]
- Jödicke B, Ende M, Helge H, Neubert D. Fecal excretion of PCDDs/PCDFs in a 3-month-old breast-fed infant. *Chemosphere*. 1992; 25:1061–1065.
- Kania-Korwel I, Garrison AW, Avants JK, Hornbuckle KC, Robertson LW, Sulkowski WW, Lehmler H-J. Distribution of chiral PCBs in selected tissues in the laboratory rat. *Environ Sci Technol*. 2006; 40:3704–3710. [PubMed: 16830530]
- Kania-Korwel I, Shaikh N, Hornbuckle KC, Robertson LW, Lehmler H-J. Enantioselective disposition of PCB 136 (2,2',3,3',6,6'-hexachlorobiphenyl) in C57BL/6 mice after oral and intraperitoneal administration. *Chirality*. 2007; 19:56–66. [PubMed: 17089340]
- Kania-Korwel I, Hornbuckle KC, Robertson LW, Lehmler HJ. Influence of dietary fat on the enantioselective disposition of 2,2',3,3',6,6'-hexachlorobiphenyl (PCB 136) in female mice. *Food Chem Toxicol*. 2008a; 46:637–644. [PubMed: 17950514]
- Kania-Korwel I, Hrycay EG, Bandiera S, Lehmler H-J. 2,2',3,3',6,6'-Hexachlorobiphenyl (PCB 136) atropisomers interact enantioselectively with hepatic microsomal cytochrome P450 enzymes. *Chem Res Toxicol*. 2008b; 21:1295–1303. [PubMed: 18494506]
- Kania-Korwel I, Vyas S, Song Y, Lehmler HJ. Gas chromatographic separation of methoxylated polychlorinated biphenyl atropisomer. *J Chromatogr A*. 2008c; 1207:146–154. [PubMed: 18760792]
- Kania-Korwel I, Xie W, Hornbuckle KC, Robertson LW, Lehmler H-J. Enantiomeric enrichment of 2,2',3,3',6,6'-hexachlorobiphenyl (PCB 136) in mice after induction of CYP enzymes. *Arch Environ Contam Toxicol*. 2008d; 55:510–517. [PubMed: 18437444]
- Kania-Korwel I, El-Komy MHME, Veng-Pedersen P, Lehmler HJ. Clearance of polychlorinated biphenyl atropisomers is enantioselective in female C57Bl/6 mice. *Environ Sci Technol*. 2010; 44:2828–2835. [PubMed: 20384376]
- Kania-Korwel I, Duffel MW, Lehmler HJ. Gas chromatographic analysis with chiral cyclodextrin phases reveals the enantioselective formation of hydroxylated polychlorinated biphenyls by rat liver microsomes. *Environ Sci Technol*. 2011; 45:9590–9596. [PubMed: 21966948]
- Kania-Korwel I, Barnhart CD, Stamou M, Truong KM, El-Komy MH, Lein PJ, Veng-Pedersen P, Lehmler HJ. 2,2',3,5',6-Pentachlorobiphenyl (PCB 95) and its hydroxylated metabolites are enantiomerically enriched in female mice. *Environ Sci Technol*. 2012; 46:11393–11401. [PubMed: 22974126]
- Kania-Korwel I, Lehmler HJ. Assigning atropisomer elution orders using atropisomerically enriched polychlorinated biphenyl fractions generated by microsomal metabolism. *J Chromatogr A*. 2013; 1278:133–144. [PubMed: 23347976]
- Karasek L, Hajslova J, Rosmus J, Huehnerfuss H. Enantioselective gas chromatographic separation of methylsulfonyl PCBs in seal blubber, pelican muscle and human adipose tissues. *Organohalogen Compd*. 2004; 66:408–412.
- Karasek L, Hajslova J, Rosmus J, Hühnerfuss H. Methylsulfonyl PCB and DDE metabolites and their enantioselective gas chromatographic separation in human adipose tissues, seal blubber and pelican muscle. *Chemosphere*. 2007; 67:S22–227. [PubMed: 17215020]
- Kato S, McKinney JD, Matthews HB. Metabolism of symmetrical hexachlorobiphenyl isomers in the rat. *Toxicol Appl Pharma*. 1980; 53:389–398.
- Kelly BC, Gobas FAPC, McLachlan MS. Intestinal absorption and biomagnification of organic contaminants in fish, wildlife and human. *Environ Toxicol Chem*. 2004; 23:2324–2336. [PubMed: 15511095]

- Klosterhaus S, McKee LJ, Yee D, Kass JM, Wong A. Polychlorinated biphenyls in the exterior caulk of San Francisco Bay Area buildings, California, USA. *Environ Int.* 2014; 66:38–43. [PubMed: 24518434]
- Kodavanti PRS, Curras-Collazo MC. Neuroendocrine actions of organohalogens: Thyroid hormones, arginine vasopressin, and neuroplasticity. *Front Neuroendocrinol.* 2010; 31:479–496. [PubMed: 20609372]
- Korrick SA, Sagiv SK. Polychlorinated biphenyls, organochlorine pesticides and neurodevelopment. *Curr Opin Pediatr.* 2008; 20:198–204. [PubMed: 18332718]
- Kostyniak P, Hansen L, Widholm J, Fitzpatrick R, Olson J, Helferich J, Kim K, Sable H, Seegal R, Pessah I, Schantz S. Formulation and characterization of an experimental PCB mixture designed to mimic human exposure from contaminated fish. *Toxicol Sci.* 2005; 88:400–411. [PubMed: 16177234]
- Kuwabara K, Yakushiji T, Watanabe I, Yoshida S, Yoyama K, Kunita N. Increase in the human blood PCB levels promptly following ingestion of fish containing PCBs. *Bull Environ Contam Toxicol.* 1979; 21:273–278. [PubMed: 109143]
- Larsson C, Ellerichmann T, Franke S, Athanasiadu M, Hühnerfuss H, Bergman A. Enantiomeric separation of chiral methylsulphonyl PCB congeners in liver and adipose tissue from rats dosed with A50. *Organohalogen Compd.* 1999; 40:427–430.
- Larsson C, Ellerichmann T, Hühnerfuss H, Bergman A. Chiral PCB methyl sulfones in rat tissues after exposure to technical PCBs. *Environ Sci Technol.* 2002; 36:2833–2838. [PubMed: 12144255]
- Lees PSJ, Corn M, Breyse PN. Evidence for dermal absorption as the major route of body entry during exposure of transformer maintenance and repairmen to PCBs. *Am Ind Hyg Assoc J.* 1987; 48:257–264. [PubMed: 3107363]
- Lehmler H-J, Robertson LW, Garrison AW, Kodavanti PRS. Effects of PCB 84 enantiomers on [3H] phorbol ester binding in rat cerebellar granule cells and 45Ca^{2+} -uptake in rat cerebellum. *Toxicol Lett.* 2005; 156:391–400. [PubMed: 15763638]
- Lehmler HJ, Harrad SJ, Hühnerfuss H, Kania-Korwel I, Lee CM, Lu Z, Wong CS. Chiral polychlorinated biphenyl transport, metabolism and distribution: A review. *Environ Sci Technol.* 2009; 44:2757–2766. [PubMed: 20384371]
- Letcher, RJ.; Klasson-Wehler, E.; Bergman, A. Methyl sulfone and hydroxylated metabolite of polychlorinated biphenyls. In: Passivirta, J., editor. *The handbook of environmental chemistry - New types of persistent halogenated compounds.* Springer-Verlag; Berlin Heidelberg; 2000. p. 315-359.
- Lewis DF, Ioannides C, Parke DV. Cytochromes P450 and species differences in xenobiotic metabolism and activation of carcinogen. *Environ Health Perspect.* 1998; 106:633–41. [PubMed: 9755138]
- Lu Z, Wong CS. Factors affecting phase I stereoselective biotransformation of chiral polychlorinated biphenyls by rat cytochrome P-450 2B1 isozyme. *Environ Sci Technol.* 2011; 45:8298–305. [PubMed: 21863805]
- Lu Z, Kania-Korwel I, Lehmler HJ, Wong CS. Stereoselective formation of mono- and dihydroxylated polychlorinated biphenyls by rat cytochrome P450 2B1. *Environ Sci Technol.* 2013:12184–92. [PubMed: 24060104]
- Lucier GW, McDaniel OS, Schiller CM, Matthews HB. Structural requirements for the accumulation of chlorinated biphenyl metabolites in the fetal rat intestine. *Drug Metab Dispos.* 1978; 6:584–90. [PubMed: 30609]
- Maervoet J, Covaci A, Schepens P, Sandau CD, Letcher R. A reassessment of the nomenclature of polychlorinated biphenyl (PCB) metabolites. *Environ Health Perspect.* 2004; 112:291–294. [PubMed: 14998742]
- Mannschreck A, Pustet N, Robertson LW, Oesch F, Puttmann M. Enantiomers of polychlorinated-biphenyls semipreparative enrichment by liquid-chromatography. *Liebigs Ann Chem.* 1985:2101–2103.
- Mariussen E, Fonnum F. Neurochemical targets and behavioral effects of organohalogen compounds: an update. *Crit Rev Toxicol.* 2006; 36:253–89. [PubMed: 16686424]

- Maroni M, Colombi A, Arbosti G, Cantoni S, Foa V. Occupational exposure to polychlorinated biphenyls in electrical workers. II Health effects. *Br J Ind Med*. 1981a; 38:55–60. [PubMed: 6451237]
- Maroni M, Colombi A, Cantoni S, Ferioli E, Foa V. Occupational exposure to polychlorinated biphenyls in electrical workers. I Environmental and blood polychlorinated biphenyls concentrations. *Br J Ind Med*. 1981b; 38:49–54. [PubMed: 6781529]
- Matthews HB, Tuey DB. The effect of chlorine position on the distribution and excretion of four hexachlorobiphenyl isomers. *Toxicol Appl Pharmacol*. 1980; 53:377–388. [PubMed: 6770494]
- Mayes BA, Brown GL, Mondello FJ, Holtzclaw KW, Hamilton SB, Ramsey AA. Dermal absorption in rhesus monkeys of polychlorinated biphenyls from soil contaminated with Aroclor 1260. *Regul Toxicol Pharmacol*. 2002; 35:289–295. [PubMed: 12202044]
- McLachlan MS. Mass balance of polychlorinated biphenyls and other organochlorine compounds in a lactating cow. *J Agric Food Chem*. 1993; 41:474–80.
- Milanowski B, Lulek J, Lehmler H-J, Kania-Korwel I. Assessment of disposition of chiral polychlorinated biphenyls in female mdr 1a/b knockout versus wild-type mice using multivariate analyses. *Environ Int*. 2010; 36:884–892. [PubMed: 19923000]
- Nezel T, Müller-Plathe F, Müller MD, Buser H-R. Theoretical considerations about chiral PCBs and their methylthio and methylsulfonyl metabolites being possibly present as stable enantiomers. *Chemosphere*. 1997; 35:1895–1906.
- Niknam Y, Feng W, Cherednichenko G, Dong Y, Joshi SN, Vyas SM, Lehmler HJ, Pessah IN. Structure-activity relationship of selected meta- and para-hydroxylated non-dioxin like polychlorinated biphenyls: from single RyR1 channels to muscle dysfunction. *Toxicol Sci*. 2013; 136:500–13. [PubMed: 24014653]
- Norström K, Eriksson J, Haglund J, Silvani V, Bergman A. Enantioselective formation of methyl sulfone metabolites of 2,2',3,3',4,6'-hexachlorobiphenyl in rat. *Environ Sci Technol*. 2006; 40:7649–7655. [PubMed: 17256508]
- Ohta C, Haraguchi K, Kato Y, Koga N. In vitro metabolism of 2,2',3,4',5,5',6-heptachlorobiphenyl (CB187) by liver microsomes from rats, hamsters and guinea pigs. *Xenobiotica*. 2005; 35:319–330. [PubMed: 16019954]
- Ohta C, Haraguchi K, Kato Y, Endo T, Koga N. Species difference in the metabolism of 2, 2', 3, 4', 5, 5'-hexachlorobiphenyl (CB146) by animal and human liver microsomes. *Fukuoka Igaku Zasshi*. 2013; 104:161–169. [PubMed: 23858795]
- Oomen AG, Tolls J, Kruidenier M, Bosgra SSD, Sips AJAM, Groten JP. Availability of polychlorinated biphenyls (PCBs) and lindane for uptake by intestinal Caco-2 cells. *Environ Health Perspect*. 2001; 109:731–737. [PubMed: 11485873]
- Pakdeesusuk U, Jones WJ, Lee CM, Garrison AW, O'Niell WL, Freedman DL, Coates JT, Wong CS. Changes in enantiomeric fractions (EF) during microbial reductive dechlorination of PCB 132, PCB 149, and Aroclor 1254 in Lake Hartwell sediment microcosms. *Environ Sci Technol*. 2003; 37:1100–1107. [PubMed: 12680661]
- Pessah IN, Hansen LG, Albertson TE, Garner CE, Ta TA, Do Z, Kim KH, Wong PW. Structure-activity relationship for noncoplanar polychlorinated biphenyl congeners toward the ryanodine receptor-Ca²⁺ channel complex type 1 (RyR1). *Chem Res Toxicol*. 2006; 19:92–101. [PubMed: 16411661]
- Pessah IN, Lehmler HJ, Robertson LW, Perez CF, Cabrales E, Bose DD, Feng W. Enantiomeric specificity of (–)-2,2',3,3',6,6'-hexachlorobiphenyl toward ryanodine receptor types 1 and 2. *Chem Res Toxicol*. 2009; 22:201–207. [PubMed: 18954145]
- Pessah IN, Cherednichenko G, Lein PJ. Minding the calcium store: Ryanodine receptor activation as a convergent mechanism of PCB toxicity. *Pharmacol Ther*. 2010; 125:260–85. [PubMed: 19931307]
- Pham-Tuan H, Larsson C, Hoffmann F, Bergman A, Fröba M, Hühnerfuss H. Enantioselective semipreparative HPLC separation of PCB metabolites and their absolute structure elucidation using electronic and vibrational circular dichroism. *Chirality*. 2005; 17:266–280. [PubMed: 15844204]

- Preston BD, Miller JA, Miller EC. Non-arene oxide aromatic ring hydroxylation of 2,2',5,5'-tetrachlorobiphenyl as the major metabolic pathway catalyzed by phenobarbital-induced rat liver microsomes. *J Biol Chem.* 1983; 258:8304–11. [PubMed: 6408087]
- Püttmann M, Oesch F, Robertson LW, Mannschreck A. Characteristics of polychlorinated biphenyl (PCB) atropisomers. *Chemosphere.* 1986; 15:2061–2064.
- Püttmann M, Mannschreck A, Oesch F, Robertson L. Chiral effects in the induction of drug-metabolizing enzymes using synthetic atropisomers of polychlorinated biphenyls (PCBs). *Biochem Pharmacol.* 1989; 38:1345–1352. [PubMed: 2495802]
- Püttmann, M.; Arand, M.; Oesch, F.; Mannschreck, A.; Robertson, LW. Chirality and the induction of xenobiotic-metabolizing enzymes: Effects of the atropisomers of the polychlorinated biphenyl 2,2',3,4,4',6-hexachlorobiphenyl. In: Frank, H.; Holmstedt, B.; Testa, B., editors. *Chirality and Biological Activity*. Alan R. Liss, Inc; New York: 1990. p. 177-184.
- Renaud HJ, Cui JY, Khan M, Klaassen CD. Tissue distribution and gender-divergent expression of 78 cytochrome P450 mRNAs in mice. *Toxicol Sci.* 2011; 124:261–77. [PubMed: 21920951]
- Robson M, Harrad S. Chiral PCB signatures in air and soil: implications for atmospheric source apportionment. *Environ Sci Technol.* 2004; 38:1662–1666. [PubMed: 15074672]
- Robson M, Melymuk L, Csiszar SA, Giang A, Diamond ML, Helm PA. Continuing sources of PCBs: the significance of building sealants. *Environ Int.* 2010; 36:506–13. [PubMed: 20452025]
- Rodman LE, Shedlofsky SI, Mannschreck A, Puttmann M, Swim AT, Robertson LW. Differential potency of atropisomers of polychlorinated biphenyls on cytochrome P450 induction and uroporphyrin accumulation in the chick embryo hepatocyte culture. *Biochem Pharmacol.* 1991; 41:915–922. [PubMed: 1901208]
- Schantz SL, Widholm JJ, Rice DC. Effects of PCB exposure on neuropsychological function in children. *Environ Health Perspect.* 2003; 111:357–376. [PubMed: 12611666]
- Schecter A, Colacino J, Haffner D, Patel K, Opel M, Papke O, Birnbaum L. Perfluorinated compounds, polychlorinated biphenyl, and organochlorine pesticide contamination in composite food samples from Dallas, Texas. *Environ Health Perspect.* 2010; 118:796–802. [PubMed: 20146964]
- Schnellmann R, Putnam C, Sipes I. Metabolism of 2,2',3,3',6,6'-hexachlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl by human hepatic microsomes. *Biochem Pharmacol.* 1983; 32:3233–3239. [PubMed: 6416258]
- Schulz DE, Petrick G, Duinker JC. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography- electron capture detector. *Environ Sci Technol.* 1989; 23:852–859.
- Schurig V, Reich S. Determination of the rotational barriers of atropisomeric polychlorinated biphenyls (PCBs) by a novel stopped-flow multidimensional gas chromatographic technique. *Chirality.* 1998; 10:316–320.
- Schurig V. Separation of enantiomers by gas chromatography. *J Chromatogr.* 2001; 906:275–299.
- Seegal RF. Epidemiological and laboratory evidence of PCB-induced neurotoxicity. *Crit Rev Toxicol.* 1996; 26:709–37. [PubMed: 8958469]
- Sipes IG, Slocumb ML, Chen HS, Carter DE. 2,3,6,2',3',6'-Hexachlorobiphenyl: distribution, metabolism, and excretion in the dog and the monkey. *Toxicol Appl Pharmacol.* 1982; 62:317–24. [PubMed: 6800067]
- Song Y, Wagner BA, Witmer JR, Lehmler HJ, Buettner GR. Nonenzymatic displacement of chlorine and formation of free radicals upon the reaction of glutathione with PCB quinones. *Proc Natl Acad Sci U S A.* 2009; 106:9725–30. [PubMed: 19497881]
- Su G, Liu X, Gao Z, Xian Q, Feng J, Zhang X, Giesy JP, Wei S, Liu H, Yu H. Dietary intake of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) from fish and meat by residents of Nanjing, China. *Environ Int.* 2012; 42:138–143. [PubMed: 21764134]
- Sulkowski WW, Kania-Korwel I, Robertson LW, Szafran B, Lulek J. Polychlorinated biphenyls production in Poland. *Fresenius Environ Bull.* 2003; 12:152–157.
- Sundström G, Jansson B. The metabolism of 2,2',3,5',6-pentachlorobiphenyl in rats, mice and quails. *Chemosphere.* 1975; 4:361–370.

- Tampal NM, Robertson LW, Srinivasan C, Ludewig G. Polychlorinated biphenyls are not substrates for the multidrug resistance transporter-1. *Toxicol Appl Pharmacol.* 2003; 187:168–177. [PubMed: 12662900]
- Tanabe SNY, Tatsukawa R. Absorption efficiency and biological half-life of individual chlorobiphenyls in rats treated with Kanechlor products. *Agric Biol Chem.* 1981; 45:717–716.
- Taniyasu S, Kannan K, Holoubek I, Ansorgova A, Horii Y, Hanari N, Yamashita N, Aldous KM. Isomer-specific analysis of chlorinated biphenyls, naptalenes and dibenzofurans in Delor: polychlorinated biphenyl preparations from former Czechoslovakia. *Environ Poll.* 2003; 126:169–178.
- Thomas, K.; Xue, J.; Williams, R.; Jones, P.; Whitaker, D. Polychlorinated biphenyls (PCBs) in school buildings: Sources, environmental levels, and exposures. United States Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory; 2012.
- Tilson HA, Kodavanti PR. The neurotoxicity of polychlorinated biphenyls. *Neurotoxicology.* 1998; 19:517–25. [PubMed: 9745906]
- Toda M, Matsumura C, Tsurukawa M, Okuno T, Nakano T, Inoue Y, Mori T. Absolute configuration of atropisomeric polychlorinated biphenyl 183 enantiomerically enriched in human samples. *J Phys Chem A.* 2012:9340–9346. [PubMed: 22917060]
- US Environmental Protection Agency. [Accessed October 14, 2014] Aroclor and other PCB mixtures. 2013. <http://www.epa.gov/epawaste/hazard/tsd/pcbs/pubs/aroclor.htm>
- US Environmental Protection Agency. [Accessed September 28, 2014] PCB-containing fluorescent light ballasts (FLBs) in school buildings. A guide for school administrators and maintenance personnel. 2014a. <http://www.epa.gov/osw/hazard/tsd/pcbs/pubs/ballasts.htm>
- US Environmental Protection Agency. [Accessed September 28, 2014] PCBs in caulk in older buildings. 2014b. <http://www.epa.gov/epawaste/hazard/tsd/pcbs/pubs/caulk/index.htm>
- Vickers AEM, Sipes G, Brendel K. Metabolism-related spectral characterization and subcellular distribution of polychlorinated biphenyl congeners in isolated rat hepatocytes. *Biochem Pharmacol.* 1986; 35:297–306. [PubMed: 3080006]
- Voorspoels S, Covaci A, Neels H. Dietary PCB intake in Belgium. *Environ Toxicol Pharmacol.* 2008; 25:179–182. [PubMed: 21783856]
- Waller SC, He YA, Harlow GR, He YQ, Mash EA, Halpert JR. 2,2',3,3',6,6'-hexachlorobiphenyl hydroxylation by active site mutants of cytochrome P450 2B1 and 2B11. *Chem Res Toxicol.* 1999; 12:690–699. [PubMed: 10458702]
- Warner NA, Martin JW, Wong CS. Chiral polychlorinated biphenyls are biotransformed enantioselectively by mammalian cytochrome P-450 isozymes to form hydroxylated metabolites. *Environ Sci Technol.* 2009; 43:114–121. [PubMed: 19209593]
- Winneke G. Developmental aspects of environmental neurotoxicology: lessons from lead and polychlorinated biphenyls. *J Neurol Sci.* 2011; 308:9–15. [PubMed: 21679971]
- Wu X, Pramanik A, Duffel MW, Hrycay EG, Bandiera SM, Lehmler HJ, Kania-Korwel I. 2,2',3,3',6,6'-Hexachlorobiphenyl (PCB 136) is enantioselectively oxidized to hydroxylated metabolites by rat liver microsomes. *Chem Res Toxicol.* 2011; 24:2249–2257. [PubMed: 22026639]
- Wu X, Duffel M, Lehmler H-J. Oxidation of polychlorinated biphenyls by liver tissue slices from phenobarbital-pretreated mice is congener-specific and atropselective. *Chem Res Toxicol.* 2013a; 26:1642–1651. [PubMed: 24107130]
- Wu X, Kania-Korwel I, Chen H, Stamou M, Dammanahalli KJ, Duffel M, Lein PJ, Lehmler HJ. Metabolism of 2,2',3,3',6,6'-hexachlorobiphenyl (PCB 136) atropisomers in tissue slices from phenobarbital or dexamethasone-induced rats is sex-dependent. *Xenobiotica.* 2013b; 43:933–947. [PubMed: 23581876]
- Wu X, Kammerer A, Lehmler H-J. Microsomal oxidation of 2,2',3,3',6,6'-hexachlorobiphenyl (PCB 136) results in species-dependent chiral signatures of the hydroxylated metabolites. *Environ Sci Technol.* 2014; 48:2436–2444. [PubMed: 24467194]
- Xing Y, Lu Y, Dawson RW, Shi Y, Zhang H, Wang T, Liu W, Ren H. A spatial temporal assesment of pollution from PCBs in China. *Chemosphere.* 2005; 60:731–739. [PubMed: 15964056]
- Yang D, Kania-Korwel I, Ghogha A, Chen H, Stamou M, Bose DD, Pessah IN, Lehmler HJ, Lein PJ. PCB 136 atropselectively alters morphometric and functional parameters of neuronal

connectivity in cultured rat hippocampal neurons via ryanodine receptor-dependent mechanisms. *Toxicol Sci.* 2014;379–92. [PubMed: 24385416]

Zanger UM, Klein K, Saussele T, Blievernicht J, Hofmann HM, Schwab M. Polymorphic CYP2B6: molecular mechanisms and emerging clinical significance. *Pharmacogenomics.* 2007; 8:743–59. [PubMed: 17638512]

Zhai G, Wu X, Lehmler H-J, Schnoor J. Atropisomeric determination of chiral hydroxylated metabolites of polychlorinated biphenyls using HPLC-MS. *Chem Cent J.* 2013; 7:183. [PubMed: 24360245]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1.

Structure of the two rotational PCB 136 isomers (or atropisomers) that are non-superimposable mirror images of each other. Note: Although the optical rotation (Haglund 1996b) and the elution orders of both atropisomers on different enantioselective gas chromatography columns (Haglund and Wiberg 1996) have been established, the absolute configuration of PCB 136 (and most other C-PCBs) has not been established.

Fig. 2.

Total production numbers for C-PCBs by (A) country and (B) PCB congener. Data are based on the available production data for the United States, Germany, Russia, Japan, Slovakia and Poland and published PCB congener profiles for the respective technical mixtures (Arctic Monitoring and Assessment Programme 2003, de Voogt and Brinkman 1989, Fiedler 1997, Holoubek 2006, Sulkowski et al. 2003).

Fig. 3. Simplified metabolism scheme of PCB 136 atropisomers (only one atropisomer is shown for clarity reasons)

PCB 136 can be oxidized by direct insertion of oxygen into a *meta* C-H bond or form epoxide intermediates. PCB 136 epoxides can either rearrange to form HO-PCB, undergo 1,2-shift to form 3-HO-PCB 150, from an dihydrodiol epoxide and react with cellular nucleophiles, such as glutathione (GSH). HO-PCBs can be further oxidized to dihydroxylated metabolites, such as 4,5-HO-PCB 136. Alternatively, mono- and dihydroxylated PCB 136 metabolites may undergo phase II metabolism to glucuronide or sulfate conjugates. Similar to other dihydroxylated PCB metabolites, 4,5-HO-PCB 136 can likely be oxidized via a semiquinone radical to PCBs quinones, which subsequently will react with cellular nucleophiles via a chlorine displacement reaction. Glutathione conjugates formed from PCB 136 epoxides will be metabolized in several steps to the corresponding methylsulfonyl metabolites. Metabolites shown in black have been detected in *in vitro*

and/or *in vivo* studies, whereas experimental evidence suggests that metabolites shown in blue are likely formed in mammals. For details regarding the PCB metabolites nomenclature see Maervoet et al. (2004). Glc, glucuronide; GS, glutathione residue; GST, glutathione transferase; Nuc, cellular N- or S-nucleophile; P450, cytochrome P450 enzyme; SULT, sulfotransferase; UGT, UDP glucuronosyl transferase.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 4.

The P450 enzyme-mediated formation of 5-HO-PCB 136 and 4-HO-PCB 136 from PCB 136 is highly species dependent (Wu et al. 2014). Liver microsomes prepared from different species were incubated with racemic PCB 136 and relative rates of metabolism, adjusted for total P450 content in the respective microsomal preparation, were determined for 5-HO-PCB 136 and 4-HO-PCB 136. Different letters indicate significant differences in the relative rates of metabolism for 5-HO-PCB 136 or 4-HO-PCB 136 ($p < 0.05$). Data represents the mean $\pm$ one standard deviation of three microsomal incubations. For details regarding the PCB metabolites nomenclature see Maervoet et al. (2004). Reprinted with permission from (Wu et al. 2014). Copyright 2014, American Chemical Society.

Fig. 5.

Metabolism studies with pure PCB 136 atropisomers demonstrate that, relative to (A) the racemic standard, (B) (-)-PCB 136 is metabolized by microsomal P450 enzymes to the first eluting atropisomers (E_1) of 5-HO-PCB 136 and 4-HO-PCB 136, whereas (C) (+)-PCB is metabolized to the second eluting atropisomers (E_2) of 5-HO-PCB 136 and 4-HO-PCB 136 (Wu et al. 2011). Microsomal incubations of racemic PCB 136 result in the preferential formation on E_2 -5-HO-PCB 136 and E_1 -4-HO-PCB 136 using rat liver microsomes from male rats pretreated with (D) phenobarbital (PB), (E) dexamethasone (DEX), or (F) corn oil alone (VEH). Microsomal incubations were optimized for protein, time and NADPH concentration and performed with 50 μ M (-), (+)- or racemic PCB 136 at 37 °C for 10 min. Atropisomers of 5-HO-PCB 136 and 4-HO-PCB 136 were separated on commercially available. Chirasil-Dex and Cyclosil-B columns, respectively, as described previously

(Kania-Korwel et al. 2008c). For details regarding the PCB metabolites nomenclature see Maervoet et al. (2004). Reprinted with permission from (Wu et al. 2011). Copyright 2011, American Chemical Society.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 6.

The direction and extent of the atropselective metabolism of PCB 136 by cytochrome P450 enzymes is highly species dependent (Wu et al. 2014). Representative chromatograms of (A) 5-HO-PCB 136 and (B) 4-HO-PCB 136 (analyzed as the corresponding methoxy derivatives) formed by incubations of liver microsomes from several toxicologically relevant species, including microsomes from humans (pooled HLMs), dogs, monkeys, guinea pigs, mice, hamsters and rabbits. The racemic composition of the respective authentic standard is shown for comparison. 5-HO-PCB 136 and 4-HO-PCB 136 were separated on Chirasil-Dex and Cyclosil-B columns, respectively. For details regarding the PCB metabolites nomenclature see Maervoet et al. (2004). Reprinted with permission from (Wu et al. 2014). Copyright 2014, American Chemical Society.

Fig. 7.

Monohydroxylated metabolites of C-PCBs, such as 4-HO-PCB 95 and 5-HO-PCB 95, are oxidized stereoselectively to dihydroxylated metabolites, such as 4,5-HO-PCB 95, by recombinant rat CYP2B1 (Lu et al. 2013). The different biotransformation rates result in an atropisomeric enrichment of the parent HO-PCB congener and the 4,5-HO-PCB 95 metabolites. (A1) Biotransformation activities of 4-HO-PCB 95 and 5-HO-PCB 95; (A2) enantiomeric fractions of 4-HO-PCB 95 and 5-HO-PCB 95; (B1) formation activities of 4,5-HO-PCB 95 from 4-HO-PCB 95 and 5-HO-PCB 95; and (B2) enantiomeric fraction of 4,5-HO-PCB 95 formed from 4-HO-PCB 95 and 5-HO-PCB 95, respectively, in incubation of rat CYP2B1 after a 60 min. incubation time. Values are the mean $\pm$ standard deviation of

three replicates. * $p < 0.05$, ** $p < 0.01$, unpaired, one-tailed t-test. For details regarding the PCB metabolites nomenclature see Maervoet et al. (2004). Reprinted with permission from (Lu et al. 2013). Copyright 2013, American Chemical Society.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Hydroxylated PCB metabolites detected in different animal species. The major metabolite is shown in bold.

PCB congener	Species	Tissue	Metabolites	Reference
PCB 95	Quail ^{II}	Feces	3'-OH-PCB 95	(Sundström and Jansson 1975)
			4'-OH-PCB 95[#]	
			4-OH-PCB 95	
			5-OH-PCB 95	
			3',4-OH-PCB 95	
			3',5-OH-PCB 95	
			4,4'-OH-PCB 95	
			4',5-OH-PCB 95	
	Mouse ^{II}	Feces	3'-OH-PCB 95	(Sundström and Jansson 1975)
			4'-OH-PCB 95	
			4-OH-PCB 95	
			5-OH-PCB 95[#]	
			3',4-OH-PCB 95	
			4',5-OH-PCB 95	
	Rat ^{II}	Feces	3'-OH-PCB 95	(Sundström and Jansson 1975)
			4'-OH-PCB 95	
			4-OH-PCB 95	
			5-OH-PCB 95[#]	
			3',4-OH-PCB 95	
			3',5-OH-PCB 95	
			4,4'-OH-PCB 95	
PCB 132	Ddy mice (male) ^{\$}	Liver, serum	4-OH-PCB 132	(Haraguchi et al. 2005a)
			5-OH-PCB 132^{&}	
			4,5-OH-PCB 132	
	Wistar rats (male) ^{\$}	Liver, serum	4-OH-PCB 132	(Haraguchi et al. 2005a)
			5-OH-PCB 132^{&}	
			4,5-OH-PCB 132	
	Wistar rats (male) [°]	Liver, serum	nd	(Haraguchi et al. 2004)
	Gunn rats (male) [*]	Liver, serum	4,5-OH-PCB 132	(Haraguchi et al. 2004)
	GoldenSyrian hamster (male) ^{\$}	Liver, serum	4-OH-PCB 132	(Haraguchi et al. 2005a)
			5-OH-PCB 132 ^{&}	
			4,5-OH-PCB 132	
	Hartley guinea pigs (male) ^{\$}	Liver, serum	4-OH-PCB 132	(Haraguchi et al. 2005a)

PCB congener	Species	Tissue	Metabolites	Reference
5-OH-PCB 132				
4,5-OH-PCB 132 [@]				
PCB 136	Sprague-Dawley rats (male)	Feces	4-OH-PCB 136	(Kato et al. 1980)
	Sprague-Dawley rats (female, male)	Liver, blood	4-OH-PCB 136	(Kania-Korwel et al. 2008c)
			5-OH-PCB 136	
			4,5-OH-PCB 136	
PCB 149	Wistar rats (male) [°]	Liver, serum	nd	(Haraguchi et al. 2005b)
	Gunn rats (male) [*]	Liver, serum	4,5-OH-PCB 149	(Haraguchi et al. 2004)
	Hartley guinea pigs (male) [°]	Serum	nd	(Haraguchi et al. 2005b)
	GoldenSyrian hamster (male) [°]	Serum	4,5-OH-PCB 149	(Haraguchi et al. 2005b)

^{II} According to the manuscript, PCB 95 “was given to the animals in peanut oil solution (ca. 20 mg/ml). The rats and mice were allowed to ingest the solution freely for some time ... Quails were given the solution (ca. 0.5 ml/animal) by stomach tube...”;

[#] Major metabolite based on relative peak area.;

^{\$} PCB 132 (19 mg/kg b.w.) dissolved in Panacete 810 (5 mL/kg b.w.) was administered intraperitoneally. Animals were euthanized 4 days after PCB administration and levels of PCB metabolites were determined in liver, lung, adipose tissue and serum;

[&] Major metabolite in serum;

[°] Kanechlor 500 (100 mg/kg b.w.) dissolved in corn oil was administered intraperitoneally. Animals were euthanized 5 days after PCB administration and levels of PCB metabolites were determined in liver, lung, and serum;

^{*} Animals received a single intraperitoneal injection of a mixture of PCB 101 (112 mg/kg b.w.) and Kanechlor 500 (100 mg/kg b.w.) in Panacete 810 (5 mL/kg b.w.) and were euthanized 4 days after PCB exposure;

[@] Major metabolite in serum and liver, with serum levels > liver levels; nd = not detected. For details regarding the PCB metabolites nomenclature see Maervoet et al. (2004).

Table 2
Direction of the atropisomeric enrichment of HO-PCBs formed from C-PCBs with a 2,3,6-trichloro substitution pattern.

Species	Tissue samples	Parent compound	1,2 shift product	<i>meta</i> (4-) hydroxylated PCB	<i>para</i> -(5-) hydroxylated PCB	<i>meta-para</i> -(4,5-) dihydroxylated PCB	Reference
Rat	Liver	PCB 136	E ₂ ^a	nd	E ₂ ^{a,+}	nd	(Kania-Korwel et al. 2008c)
		PCB 91	E ₁ ^b	nr	E ₁ ^a	nr	
	Liver microsomes	PCB 95	E ₂ ^c	E ₂ ^d	0.33 ^a	nr	(Kania-Korwel et al. 2011)
		PCB 132	E ₁ ^c	nr	0.31 ^a	nr	
		PCB 149	E ₁ ^e	na	0.65 ^a	na	
Rat	Liver microsomes	PCB 136	nd	E ₁ ^{a,1}	E ₂ ^{a,+}	nr	(Wu et al. 2011)
Mouse	Liver	PCB 95	nd	E ₂ ^c	E ₁ ^a	E ₂ ^d	(Kania-Korwel et al. 2012)
	Blood		nd	E ₂ ^c	nd	racemic	
		PCB 91	nd		E ₂ ^a		
		PCB 95	nd		E ₂ ^a		
Rat	CYP2B1	PCB 132	nd	nd	E ₁ ^a	nd	(Lu et al. 2013)
		PCB 136	nd		E ₁ ^{a,-}		
		PCB 149	na	nd	E ₂ ^a	na	
Rat	Liver tissue slices	PCB 136	nd	E ₁ ^{e,-}	E ₂ ^{a,+}	nr	(Wu et al. 2013b)
		PCB 91		E ₂ ^d	E ₂ ^d		
		PCB 95			E ₁ ^a		
Mouse	Liver tissue slices	PCB 132			E ₂ ^d		(Wu et al. 2013a)
		PCB 136		E ₁ ^{e,-}	E ₁ ^{a,-}		
		PCB 149		na	E ₁ ^d	na	
Human	Liver microsomes	PCB 136		E ₂ ^{a,+}	E ₂ ^{a,+}		(Wu et al. 2014)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Species	Tissue samples	Parent compound	1,2 shift product	<i>meta</i> (4+) hydroxylated PCB	<i>para</i> -(5-) hydroxylated PCB	<i>meta-para</i> -(4,5-) dihydroxylated PCB	Reference
Dog				E ₂ ^a ,+	E ₂ ^a ,+		
Monkey				E ₂ ^a ,+	E ₂ ^a ,+		
Guinea pig				E ₂ ^a ,+	E ₂ ^a ,+		
Mouse				E ₁ ^a ,–	E ₁ ^a ,–		
Hamster				E ₂ ^a ,+	racemic		
Rabbit				E ₂ ^a ,+	E ₂ ^a ,+		

^a Chirasil-Dex

^b GTA

^c BGB

^d BDM

^e Cyclosil-B enantioselective column.

– formed from (–)-PCB136,

+ formed from (+)-PCB 136, see Figure 5. na – authentic standard not available, nd – below detection limit, nr – not resolved.