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Sources and Toxicities of Phenolic Polychlorinated Biphenyls (OH-PCBs)

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Abstract

Polychlorinated biphenyls (PCBs), a group of 209 congeners that differ in the number and position of chlorines on the biphenyl ring, are anthropogenic chemicals that belong to the persistent organic pollutants (POPs). For many years, PCBs have been a topic of interest because of their bioaccumulation in the food-chain and their environmental persistence. PCBs with fewer chlorine atoms however are less persistent and more susceptible to metabolic attack, giving rise to chemicals characterized by the addition of one or more hydroxyl groups to the chlorinated biphenyl skeleton, collectively known as hydroxylated PCBs (OH-PCBs). In animals and plants this biotransformation of PCBs to OH-PCBs is primarily carried out by cytochrome P-450-dependent monooxygenases. One of the reasons for infrequent detection of lower chlorinated PCBs in serum and other biological matrices is their shorter half-lives, and their metabolic transformation, resulting in OH-PCBs or their conjugates, such as sulfates and glucuronides, or macromolecule adducts. Recent biomonitoring studies have reported the presence of OH-PCBs in human serum. The occurrence of OH-PCBs, the size of this group (there are 837 mono-hydroxyl PCBs alone), their wide spectra of physical characteristics (pKa's and log P's ranging over 5 to 6 orders of magnitude), give rise to a multiplicity of biological effects. Among those are: bioactivation to electrophilic metabolites that can form covalent adducts with DNA and other macromolecules, interference with hormonal signaling, inhibition of enzymes that regulate cellular concentrations of active hormones, and interference with the transport of hormones. New information creates an urgent need for this perspective.

Keywords

PCBs; Hydroxylated PCBs; Phenolic PCBs; Metabolites; Endocrine Disruption; Persistent Organic Pollutants; Biotransformation

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are synthetic organic compounds with the empirical formula $C_{12}H_{10-x}Cl_x$ (x=1–10). There are 209 congeners of PCBs, numbered PCB 1 to PCB 209 based on the number and position of chlorine atoms around the biphenyl rings (Ballschmiter & Zell 1980). Commercial production of PCBs began in the early 1920s, and PCBs were marketed in the US under the trade name Aroclor and with various trade names in other countries (Silberhorn et al. 1990). When added to a material, PCBs impart plastic and fire retardant properties. They are also very good coolants and lubricating agents. For these properties, PCBs were valuable chemicals in the industrial development of the twentieth century. Examples of PCB-containing products are transformers, capacitors, microwave ovens, air conditioners, fluid-cooled motors and electromagnets, electrical light ballasts, hydraulic and heat transfer fluids, switches, voltage regulators, circuit breakers, vacuum pumps, electric cables, inks, lubricants, waxes, flame retardants, adhesives, electrical and thermal insulating materials, pesticides, dyes, paints, asphalts, caulks, sealants and many more (AIHA 2013, ATSDR 2000, 2011, IARC 2013). The commercial production of PCBs was banned in many countries since the late 1970s. However, PCB legacy materials and new materials containing PCBs from pigments continue to be a source of environmental release even today (Hu & Hornbuckle 2010, Hu et al. 2010, Shanahan et al. 2015).

PCBs as a group are persistent organic pollutants (POPs) because they are not readily degraded by the physical and biologic processes in the environment. PCBs are also more soluble in oils, less in water. As a result, they tend to associate with organic materials and to bioaccumulate in fatty tissues of animals in the food web (Janssen et al. 2011). Very high levels of PCBs have been reported in the Inuit people who eat predominately fish and sea mammals (Laird et al. 2013). Average body burden of PCBs in adults living in a community with a history of PCB contamination is as high as 528 ng/g of serum lipid (Pavuk et al. 2014). Although a fish and meat-based diet has been recognized as the primary source of exposure to PCBs, an estimated 6–64% of body burden of PCBs occurs through inhalation exposures (Currado & Harrad 1998). PCBs with four or fewer chlorine atoms are found in many indoor and outdoor environments as a result of temperature-dependent slow evaporation and transport from PCB containing materials (Hu & Hornbuckle 2010, Hu et al. 2010). Other atmospheric sources of PCBs are landfills, electronic waste recycling, and open incineration of plastic wastes (Tue et al. 2013). Generation of atmospheric PCB 11, which was not found in Aroclor mixtures, was a strong indication that newly manufactured materials, like dyes, pigments and paints can also contribute PCBs (Hu et al. 2008). Cities like Chicago and Cleveland (Persoon et al. 2010), and contaminated sites like New Bedford Harbor (Martinez et al. 2017) are major sources of airborne PCBs. PCBs are transported with facility among strata (sediment, water and air). Fluxes of PCBs from sediment to the overlying water (4 kg per year), and from water to air (7 kg per year) have been described for an industrial harbor in East Chicago (Martinez et al 2010b). This site alone contributes about 44 kg per year to Lake Michigan. Airborne PCBs are in dynamic equilibrium and depending on the atmospheric conditions, the flux of PCBs may be from Chicago to Lake Michigan, or the reverse (Hu et al. 2010, Martinez et al. 2010a, Martinez et al. 2010b).

Because PCBs are considered non-degradable chemicals, human biomonitoring for PCB exposure is targeted primarily on the identification of parent PCBs. NHANES surveys still use serum measurement of 42 congeners (PCB 28 and higher chlorinated ones) as an indicator of PCB body burden (CDC 2013). However, a recent human biomonitoring study of mothers and children in Iowa reports the detection of many PCBs including ones that are less chlorinated than PCB 28 (2,4,4'-trichlorobiphenyl) (Koh et al. 2015). In addition, a plethora of metabolism studies now indicate that PCBs in fact are bio-transformed into a variety of metabolites by many life forms including animals, plants and microorganisms.

Since there is no longer intentional commercial production of PCBs, and there is a continuous, but slow biotransformation of many parent PCBs, it is likely that the global stock of PCBs may gradually shift into metabolic forms, although it will be a very slow process. Evidence of PCB metabolism suggests that OH-PCBs are environmentally stable and may be more toxic by some measures.

Physical Properties of OH-PCBs

OH-PCBs are characterized by the addition of one or more hydroxyl groups to the chlorinated biphenyl skeleton. Although 837 mono-hydroxyl PCBs are possible, a much smaller number have been synthesized, characterized and determined in environmental samples. The acid-base dissociation constant (pKa) of all possible mono-OH-PCBs have been calculated with in silico methods and found to range from about 4 to 10 (Grimm et al. 2015b). On this basis, only OH-PCBs pentachloro- and higher would exist as anions in biological systems (Figure 1). Surprisingly, substitution with a hydroxyl group does not greatly alter the lipophilicity (log P) of the PCB (Table 1). OH-PCBs have mean Log P values ranging from 4 to 8, very similar to those of the parent PCBs (Table 1).

Chirality in the OH-PCBs

Nineteen PCB congeners with three or four *ortho* chlorines form atropisomers stable to racemization under physiological conditions. This was demonstrated by isolation of PCB enantiomers (atropisomers) through semi-preparative enrichment, by liquid chromatography and synthesis of diasteriomeric intermediates (Haglund 1996a, b, Püttmann et al. 1989, Püttmann et al. 1986). All nineteen chiral PCBs are present in technical PCB mixtures (Kania-Korwel & Lehmler 2016b), and large quantities of these congeners have been produced across the world (Kania-Korwel & Lehmler 2016b). As a result of their release into the environment, many chiral PCBs are present in wildlife and humans [for in-depth reviews of chiral PCBs see (Kania-Korwel & Lehmler 2016b, a, Lehmler & Robertson 2001, Lehmler et al. 2010)]. Chiral PCBs undergo enantiomeric enrichment in wildlife and humans due to enantioselective biotransformation and other biological processes, a fact that has been used to study the environmental fate and transport of PCBs (Lehmler et al. 2010). Moreover, exposure to chiral PCBs enantioselectively alters the expression of drug metabolizing enzymes in the liver (Püttmann et al. 1990, Püttmann et al. 1989, Rodman et al. 1991) and modulates endpoints in the developmental neurotoxicity of PCBs (Lehmler et al. 2005, Pessah et al. 2009, Yang et al. 2014). Although this has not been shown experimentally, it is likely that the atropisomers of OH-PCBs also cause toxicity in an atropselective manner. Assuming that an OH substituent offers a barrier to rotation approximately equal to that of a

chlorine atom in the *ortho* position, there would be 110 stable mono-hydroxyl PCBs pairs (Table 2). Indeed several of these have been found to be formed (see the discussion below).

Oxidation of PCB congeners by cytochrome P-450 monooxygenases leads to production of OH-PCBs

Cytochrome P-450 monooxygenases are ubiquitous enzymes that are expressed in plants and animals. In humans cytochrome P-450 families 1 and 2 contain major inducible isoforms involved in biotransformation of PCBs. Liver is the main organ for biotransformation reactions, although expression of cytochrome P-450 isoforms has been documented in other organs such as the intestine, brain, and lungs (Stamou et al. 2014, Thelen & Dressman 2009). To understand the mechanism of cytochrome P-450 enzymatic reactions with PCBs, early studies focused on the mono-chlorinated PCBs as substrates (Block & Cornish 1959, Wyndham & Safe 1978). These studies led to the discovery of two oxidative processes cytochrome P-450 enzymes exert. First, the direct process involves the insertion of a hydroxyl group into the PCB. Second, in a more indirect process, an arene oxide intermediate is formed which rearranges to form the OH-PCB (Figure 2). The intermediacy of an arene oxide may allow the movement of neighboring substituents, a process called an NIH (or the 1,2) shift (McLean et al. 1996). Through profiling of all urinary metabolites of PCB 3 (4-monochlorobiphenyl) after *in vivo* exposure in rats, Dhakal *et al.* showed that the formation of arene oxide intermediates is as important as direct insertion because several mercapturic acid metabolites were detected in urine as end products of PCB 3 arene oxide reaction with glutathione (Dhakal et al. 2012). The 4'OH-PCB 3 is the most favorable and major oxidation product of PCB 3 (McLean et al. 1996, Wyndham & Safe 1978). Hydroxylation at the 4' *para* position is in fact the major OH-PCB congener detected in human serum (Koh et al. 2015, Marek et al. 2014, Marek et al. 2013). Incubation of PCB 3 with liver microsomes resulted in the formation of five mono- and three di-hydroxylated PCB 3 metabolites of PCB 3 (McLean et al. 1996). A similar mechanism of the formation of OH-PCBs can be anticipated for higher chlorinated PCBs, although factors like *ortho*-substitution in PCB 126 or isomerism in PCB 95 and PCB 136 may give rise to different OH-PCB forms. Biotransformation of PCBs to OH-PCBs through cytochrome P-450-catalzyed oxidation has also been documented in lower organisms (Kamata et al. 2015) and in plants (Ma et al. 2016, Zhai et al. 2010a, b, 2011b, 2013a).

Chirality in the cytochrome P-450 metabolism of PCBs

Chiral OH-PCBs can be formed by the cytochrome P-450 mediated metabolism of either chiral or prochiral PCBs, thus resulting in 110 possible chiral OH-PCB metabolites (Table 2). Studies examining chiral OH-PCB formation used several model systems including recombinant cytochrome P-450 enzymes (Lai et al. 2011, Lu et al. 2013, Waller et al. 1999a, Warner et al. 2009), liver microsomes (Kania-Korwel et al. 2011b, Kania-Korwel & Lehmler 2013, Schnellmann et al. 1983, Uwimana et al. 2016, Wu et al. 2014a, Wu et al. 2011b), primary rat liver hepatocytes (Vickers et al. 1986) and precision cut liver tissue slices (Wu et al. 2013a, Wu et al. 2013c). These studies demonstrate that chiral PCB congeners, in particular PCB congeners with a 2,3,6-trichloro substitution pattern in one phenyl ring, are metabolized in a congener-specific manner by mammalian cytochrome P-450 enzymes to chiral OH-PCB metabolites. In mammals, CYP2B isoforms, such as rat CYP2B1 (Lu et al.

2013, Waller et al. 1999a, Warner et al. 2009), human CYP2B6 (Warner et al. 2009) and dog CYP2B11 (Waller et al. 1999a), oxidize chiral PCBs with a 2,3,6-trichloro substitution pattern in *meta* position. Based on a recent study with PCB 52 (2,2',5,5'-tetrachlorobiphenyl)(Shimada et al. 2016), human CYP2A6 is likely responsible for the formation of chiral *para* hydroxylated metabolites from chiral PCBs. In addition to the formation of chiral OH-PCBs from chiral parent PCBs, we recently demonstrated that axially prochiral PCB congeners can be metabolized to chiral OH-PCB metabolites (Uwimana et al. 2017). For example, oxidation of the 2,6-dichlorophenyl ring in PCB 51 by CYP2B enzymes in the *meta* position breaks the symmetry of this phenyl ring, thus resulting in a chiral OH-PCB metabolite. The oxidation of both prochiral and chiral PCBs to chiral OH-PCBs is species and congener dependent (Uwimana et al. 2017, Waller et al. 1999b, Wu et al. 2014b). This observation is not surprising given the diversity of expression of cytochrome P-450 enzymes across species. *In vivo* studies also reveal the formation of chiral OH-PCB metabolites in rats (Kania-Korwel et al. 2008c) and mice (Kania-Korwel et al. 2015, Kania-Korwel et al. 2012, Kania-Korwel et al. 2017, Wu et al. 2015) exposed orally or intraperitoneally to a racemic PCB congener. There is also evidence that plants, such as poplar plants, metabolize chiral PCBs to chiral OH-PCB metabolites (Ma et al. 2016, Zhai et al. 2011a).

Similar to the parent compounds, chiral OH-PCBs can be separated using both liquid (Pham-Tuan et al. 2005, Zhai et al. 2013b) and gas chromatographic (GC) techniques (Kania-Korwel et al. 2011a, Kania-Korwel et al. 2008c, Uwimana et al. 2017). To date, enantioselective LC analyses appear to be limited to a smaller number of chiral OH-PCBs. Enantioselective GC analyses using different commercial capillary GC columns containing modified β- or γ-cyclodextrins require the derivatization of the OH-PCBs to the corresponding methylated derivatives, but allow the separation of a considerable number of OH-PCB atropisomer derivatives. Enantioselective GC methods have been used to study the enantioselective metabolism of PCBs to chiral OH-PCBs *in vitro* and *in vivo*. The enantioselective metabolism of PCBs by mammalian cytochrome P-450 enzymes to OH-PCBs is both congener and species dependent (Kania-Korwel et al. 2008b, Uwimana et al. 2017, Wu et al. 2013a, Wu et al. 2014a, Wu et al. 2013c, Wu et al. 2011b). The enantiomeric enrichment of OH-PCBs is not only dependent on their atropselective formation by cytochrome P-450 enzymes, but is also modulated by their further oxidation to dihydroxylated and, most likely, other PCB metabolites. For example, racemic 2,2′,3,5′,6-pentachlorobiphenyl-4-ol and 2,2′,3,5′,6-pentachlorobiphenyl-5-ol, two metabolites of PCB 95, are enantioselectively metabolized by rat CYP2B1 to 3,4-dihydroxy-2,2′,5,5′,6-pentachlorobiphenyl, a chiral catechol metabolite of PCB 95 (Lu et al. 2013).

Only limited information about the absolute configuration of chiral PCB congeners and their metabolites is currently available (Pham-Tuan et al. 2005, Toda et al. 2012). The identification of OH-PCB atropisomers (as methylated derivatives) using GC methods is therefore primarily based on their elution order on the respective enantioselective GC column. Metabolism studies with pure atropisomers of PCB 136 allowed the identification of which OH-PCB atropisomer is formed from which PCB 136 atropisomer in incubations with rat liver microsomes (Figure 3) (Wu et al. 2011a). Based on an analysis of the Chirasil-Dex column, the first (E_1) and second (E_2) eluting atropisomers of 2,2',3,3',6,6'-

hexachlorobiphenyl-5-ol (5-OH-136) are formed from (−)-and (+)-PCB 136, respectively. The atropisomers of the minor metabolite, 2,2',3,3',6,6'-hexachlorobiphenyl-4-ol (4-OH-136), eluting first and second on the Cyclosil-B column, are also formed from (−)- and (+)-PCB 136, respectively. E_2-5-OH-136, formed from (+)-PCB 136, was the major metabolite formed in these rat liver incubations, which is consistent with a significant enrichment of (−)-PCB 136 in these experiments. Similarly, the more rapid oxidation of (+)-PCB 136 to E_2-5-OH-136 is consistent with the enrichment of (−)-PCB 136 observed in incubations of racemic PCB 136 in precision-cut liver tissue slices from phenobarbital pre-treated male and female rats (Wu et al. 2013b). E_2-5-OH-136 is also enriched in the liver of male and female rats exposed intraperitoneally to racemic PCB 136. In contrast, E_1-5-HO-PCB 136 and E_1-4-HO-PCB 136 are formed preferentially in incubations of racemic PCB 136 with liver microsomes or precision-cut liver tissue slices from mice (Wu et al. 2013a, Wu et al. 2014a). In agreement with this finding, mice exposed to racemic PCBs display an enrichment of (+)-PCB 136 in tissues (Kania-Korwel et al. 2010, Kania-Korwel et al. 2008a, Kania-Korwel et al. 2007, Kania-Korwel et al. 2008d, Milanowski et al. 2010a).

Conjugation of OH-PCBs and the biological implications

Biotransformation of xenobiotics often involves conversion of lipophilic compounds to more polar forms. OH-PCBs are Phase I biotransformation products of PCBs. In Phase II biotransformation, phenols are conjugated with functional groups that may result in more polar products that facilitate excretion. The most common conjugation reactions for phenols are glucuronidation and sulfation. A glucuronide metabolite is formed by transfer of a glucuronosyl group from uridine-5'-diphospho-α-D-glucuronic acid (UDPGA) to a phenol in an enzymatic reaction catalyzed by the microsomal UDP-glucuronosyltransferases (UGTs). Similarly, mammalian cytosolic sulfotransferases (SULTs) catalyze formation of sulfate metabolites by transfer of a sulfuryl group from 3'-phosphoadenosine-5'-phosphosulfate (PAPS). OH-PCBs are good substrates for SULT and UGT isoforms *in vitro* (Ekuase et al. 2011, Kester et al. 2000, Liu et al. 2006, Liu et al. 2009, Sacco & James 2005, Tampal et al. 2002). However, some congeners with the phenolic hydroxyl group in an *ortho*-position with respect to the biphenyl ring-junction have been found to hinder conjugation reactions (Ekuase et al. 2011, Tampal et al. 2002). Several phenolic PCBs have been reported to be selective inhibitors of sulfotransferases and UDP-glucuronosyltransferases, and thereby may reduce the sulfation or glucuronidation of other xenobiotics that is required for their elimination (Sacco et al. 2008, van den Hurk et al. 2002, Wang et al. 2006). The reported presence of OH-PCBs in human urine, blood and milk, and their potential inhibitory effects on the sulfotransferases and glucuronosyltransferases, has also been suggested to cause metabolic, endocrine, and developmental defects (James & Ambadapadi 2013, Kester et al. 2000)

Incubation of OH-PCBs with hepatic cytosolic fractions derived from polar bear (*Ursus maritimus*) resulted in formation of sulfate conjugates for 4'-hydroxy-3,3',4,5'-tetrachlorobiphenyl (4'OH-PCB 79), 4'-hydroxy-2,3,3',4,5,5'-hexachlorobiphenyl (4'-OH-PCB 159), 4'-hydroxy-2,3,3',5,5',6-hexachlorobiphenyl (4'-OH-PCB 165) (Sacco & James 2005). There are limited metabolism studies devoted to *in vivo* phase II biotransformations of OH-PCBs. A metabolism study carried out with rats has reported that a majority of PCB 3

metabolites excreted via the urine after inhalation exposure were sulfates (Dhakal et al. 2014). Following intraperitoneal injection of PCB 3 in the rat, some glucuronide metabolite was detected in the urine and bile, but it was minor as compared to either sulfation or glutathione conjugation (Dhakal et al. 2012). More recently, 4-PCB 11 sulfate was detected in the serum of humans who were known to have been exposed to environments containing PCB 11 and other airborne PCBs (Grimm et al. 2017). This provides evidence for both initial hydroxylation of the PCB and subsequent sulfation of the resulting OH-PCB in humans. Interestingly, plants, including poplar trees, can also form sulfated metabolites after exposure to PCBs through their roots (Zhai et al. 2010a). These findings suggest that conjugated metabolites of PCBs are formed in a broad range of species, although the stability and fate of conjugated metabolites of OH-PCBs in the environment have not yet been fully explored.

Although phenolic conjugates provide evidence for the intermediacy of the phenols, it is also clear that sulfatases and β-glucuronidases are capable of catalyzing hydrolysis of sulfate and glucuronide conjugates and would thus provide the basis for a dynamic interchange between OH-PCBs and their conjugates (Parkinson et al. 2012). For example, bisphenol A is a substrate for the estrogen sulfotransferase and the product sulfate ester is a substrate for steroid sulfatase (Stowell et al. 2006). Interestingly, when 4-PCB 11 sulfate was administered to rats by intravenous injection, OH-PCB 11 was detected in urine, suggesting that conversion of this conjugated metabolite of PCB 11 to free phenol was occurring in vivo (Grimm et al. 2015a). Following urinary excretion, however, PCB sulfates may be quite stable. PCB 11 sulfate was stable in rat urine for 24 h at 25 °C (Grimm et al. 2013). Some PCB sulfates were found to be stable in human urine for several days at 25 °C and for more than six weeks at −20 °C (Dhakal et al. 2013). In summary, evidence from various *in vivo* and *in vitro* studies shows that both PCB congeners and their respective sulfate and glucuronide metabolites are converted into OH-PCBs.

OH-PCBs are bioactivated to electrophilic metabolites

Following exposure, PCBs are transferred into the blood circulation and distributed to all organs and tissues (Hu et al. 2014, Milanowski et al. 2010b, Pereg et al. 2001). In the liver and possibly other organs, PCBs are oxidized by cytochrome P-450 with the generation of reactive intermediates, PCB arene oxides. These arene oxides are short lived, very strong electrophiles which may potentially react with macromolecules that have nitrogen and sulfur nucleophilic centers (Amaro et al. 1996). Arene oxides may be detoxified by conjugation with glutathione through the action of glutathione transferases (Dhakal et al. 2012), or disproportionate to the OH-PCB. Alternatively, arene oxides may be detoxified by epoxide hydrolase to a dihydrodiol that can be further metabolized by dihydrodiol dehydrogenase (AKR1C) to the diOH-PCB (McLean et al. 1996). OH-PCBs undergo phase II metabolism by glucuronosyl transferases or sulfotransferases (see above). Alternatively, the OH-PCBs may undergo a second oxidation and the formation of a diOH-PCB which may oxidize to a semiquinone and further to a quinonoid species. PCB quinones (PCB-Q) are another form of bioactivated metabolites. Formation of PCB-Q is favored by the substitution of hydroxyl groups in *ortho* or *para* positions, and are carried out enzymatically by peroxidases (Amaro et al. 1996, Wangpradit et al. 2009). These different metabolites display diverse toxic effects

with strong differences in potency depending on the parent PCB structure. In addition, different tissues with their diverse metabolizing enzyme activities may favor distinct metabolism pathways for PCBs. With the current level of knowledge it is therefore not possible to deduce which pathway is the most predominant leading to a toxic effect.

Toxicity, Genotoxicity and Carcinogenicity of OH-PCBs

PCBs are classified by IARC as Class 1, human carcinogens (Lauby-Secretan et al. 2015). An important question is whether OH-PCBs could play a role in this carcinogenicity, as initiators, promoters or co-carcinogens.

The first step in carcinogenesis is initiation, a genotoxic event. Very few studies exist that evaluated the initiating activity/genotoxicity, of OH-PCBs. Espandiari and coworkers observed that PCB 3, PCB 15, PCB 52 and PCB 77, but not PCB 12 and PCB38, were positive in a modified rat liver foci assay that determines initiating activity of compounds (Espandiari et al. 2003). Using three mono-OH, three di-OH, and two quinone metabolites of PCB 3, they further discovered that only the 4-OH-PCB 3 and the 3,4-quinone of PCB 3 also exhibited this activity (Espandiari et al. 2004). This suggested that PCB 3 and the 4-OH metabolite could be proximate carcinogens and the 3,4-quinone the ultimate carcinogen. The initiating activity of PCB 3 and 4-OH-PCB 3 was further analyzed using the transgenic BigBlue rat model. PCB 3 significantly increased the number of mutations of the lac I reporter gene (Lehmann et al. 2007). 4OH-PCB 3 caused a non-significant doubling of the mutant frequency. The PCB 3 induced mutations were mostly GC→ TA transversions and frameshift mutations, suggesting DNA adduction or oxidative DNA damage as underlying mechanisms (Lehmann et al. 2007). This suggests that an arene-oxide, the precursor of OH-PCBs, could be the genotoxic species, but does not exclude that OH-PCBs are a precursor for a second ultimate carcinogenic metabolite.

To gain insight into the genotoxic activity of the various metabolic products, PCB 3 and seven synthetic metabolites were tested in several genotoxicity assays. Using a V79 cell line which does not contain cytochrome P-450 enzymes, PCB 3 was negative in all assays. Only the quinones induced point mutations, only the 3,4-diOH PCB 3 increased sister chromatid exchanges (SCE), and only the 2,5-diOH PCB 3 produced polyploid cells (Zettner et al. 2007). 4-OH PCB 3 and both the diOH- and the quinonoid metabolites induced chromosome breaks, with an efficiency that increased from 4-OH < diOH- < quinones (Table 3), suggesting that the quinones may be the ultimate clastogenic metabolites. This was confirmed in human promyelocytic leukemia cells, where 2,5-diOH PCB 3 produced DNA strand breaks only if the cellular myeloperoxidase (MPx) was active, while the 2,5-quinone produced strand breaks independently of MPx (Xie et al. 2010). Surprisingly, all three OH-PCB 3s produced chromosome loss in V79 cells although with 10-fold lower efficacy than the diOH and quinoid metabolites (Table 3) (Zettner et al. 2007). This suggests protein binding, but the reactive intermediate is unknown. In fact, PCB 3 and PCB 77 both produced protein adducts in liver nuclei of rats and to a much smaller degree also DNA adducts (Pereg et al. 2001). It is noteworthy that non-genotoxic concentrations of PCB 77 combined with PCB 52, which alone did not produce chromosome damage in human lymphocytes *in vitro*, caused a vastly enhanced cytogenetic effect if combined, and this synergistic effect was also

observed in rat bone marrow cells *in vivo* (Meisner et al. 1992, Sargent et al. 1991, Sargent et al. 1989). Also, several mono- and di-chlorinated PCBs were shown to very efficiently induce micronuclei formation and hprt mutations in hamster V79 cells that express CYP 2E1 and surprisingly the most active congeners were some non-planar tri- and tetra-chlorinated congeners (Liu et al. 2016, Wang et al. 2017, Zhang et al. 2016). V79 cells without CYP or other forms of P-450 were protected from PCB genotoxicity demonstrating that CYP 2E1 may be a predominant enzyme bioactivating PCBs with 1 to 4 chlorines to genotoxins.

The second step in carcinogenesis is promotion. Inhibition of gap junction intercellular communication (GJIC) is believed to be one mechanism of promotion and all non-dioxin-like PCBs and OH-PCBs tested were shown to be positive in an *in vitro* GJIC assay (Hamers et al. 2011, Machala et al. 2004). Hydrophobicity (log P) and molecular volume were found to significantly correlate with potency to down-regulate GJIC. Another marker of tumor-promoting activity, induction of proliferation of confluent liver cells, was positively correlated with the ability of dioxin-like PCBs and OH-PCBs to activate the AhR (Vondracek et al. 2005). Also, lower chlorinated OH-PCBs were shown to have significant estrogenic activity while anti-estrogenic activity was observed with persistent, higher chlorinated and a few lower chlorinated OH-PCB and was negatively correlated with hydrophobicity and molecular volume (Machala et al. 2004). Estrogenic activity was associated with the induction of cervicovaginal tract carcinoma in neonatal female mice exposed to OH-PCB 30 or OH-PCB 61, similar to the effect of DES (Martinez et al. 2005). Thus, lower chlorinated OH-PCBs may be cancer promoters by several mechanisms, inhibition of GJIC, loss of contact inhibition, and estrogenicity. OH-PCBs can readily transfer through the placenta and are found at higher levels in human fetal blood than in maternal blood (Park et al. 2008), raising serious concerns about possible carcinogenic activity of OH-PCBs in humans *in utero*.

OH-PCBs may also act as co-carcinogens by changing the bioavailability of other ultimate carcinogens. Many OH-PCBs are potent activators of the AhR, surpassing the efficacy of the parent PCB (Kamata et al. 2009). Since AhR activation is associated with carcinogenicity, these OH-PCBs may be more dangerous than their parent compounds. OH-PCBs were also shown to inhibit phase II metabolism. Specifically, the inhibition of glucuronosyl transferase by five different OH-PCBs was shown to increase the formation of DNA adducts by benzo(a)pyrene 7,8-dihydrodiol in isolated intact livers (James et al. 2004).

The sheer number of PCBs and their metabolites (837 mono-OH-PCBs alone!) makes it impossible to test even a small percentage of all these compounds for their toxic activity. Efforts are therefore made to use existing data for the development of predictive QSAR models (Niu et al. 2007). Applying such an approach, Ruiz calculated that the probability of mutagenicity was higher for lower chlorinated mono- and di-OH PCBs than with higher chlorinated metabolites (Ruiz et al. 2008). This group also examined the estrogenic activity of OH-PCBs (Ruiz et al. 2016, Ruiz et al. 2013). Analyzing the data of a large multi-center study with 17 in vitro assays and 20 ultra-pure non-dioxin-like PCBs by multivariant toxicity profiling and QSAR modeling revealed that generally smaller, ortho-substituted congeners (5 of 10 with <5 chlorines) were most active, while the second group with less active PCBs contained mostly higher chlorinated congeners (8 of 10 with >4 chlorines), further

highlighting the potential toxicological importance of this usually overlooked group of PCBs, many of which are parent compounds of OH-PCBs (Stenberg et al. 2011).

All these findings indicate that OH-PCBs themselves may not be direct acting carcinogens but may have unexpected toxic and carcinogenic activity through their metabolic precursor or secondary metabolites or by interference with regulatory mechanisms. Our ability to predict which PCB metabolites are active and with which targets awaits further information.

OH-PCBs are endocrine hormone disrupting chemicals by indirect mechanisms

In addition to the roles that PCBs and OH-PCBs play in genotoxicity and carcinogenesis, in part through their hormone-like activity, they have also been shown to be endocrine and metabolic disrupting substances through indirect mechanisms (Gadupudi et al. 2015, Gadupudi et al. 2016a, Gadupudi et al. 2016b, Hamers et al. 2011, Heindel et al. 2015, Kawano et al. 2005, Liu et al. 2006, Ludewig et al. 2008, Machala et al. 2004, Quinete et al. 2014, Robertson & Ludewig 2011, Thayer et al. 2012). Epidemiological studies have shown that children exposed to PCBs in their early life have lower IQ than unexposed children, which may be due to endocrine disruption (Jacobson & Jacobson 1996). While a definitive link between OH-PCBs and these neurodevelopmental effects has not yet been established, several mechanisms for neurotoxicity of selected OH-PCBs have been investigated in vitro (Amano et al. 2010, Londono et al. 2010, Sethi et al. 2017, Shimokawa et al. 2006). Endocrine hormones are essential in growth, development, and sexual orientation. Phenolic metabolites of PCBs have been known as endocrine disrupting chemicals (EDCs) for some time (Kester et al. 2000, Lans et al. 1993, Lans et al. 1994). The effect of a subtle change in the level of hormones during development is very difficult to measure. Therefore, researchers have utilized various *in vitro* models to understand molecular mechanism of toxicity due to EDCs (Borgert et al. 2011). Endocrine hormones such as estradiol, thyroxine and triiodothyronine are small molecules which remain in the circulation bound to carrier proteins. OH-PCBs have been found to interact with and alter the effects of these hormones through various mechanisms. Using porcine pre-pubertal follicle culture, Ptak *et al.*, have reported that there were significant alternations in the secretion of sex hormones by these ovary follicles when exposed to either PCB 3 or OH-PCB 3 (Ptak et al. 2005). Many OH-PCBs are inhibitors of mammalian cytosolic sulfotransferases (SULTs), enzymes that are important in the regulation of cellular concentrations of active estrogens (Kester et al. 2000). In addition to inhibition, some bioactivated OH-PCBs such as biphenyl quinones can modify the catalytic activity of human hydroxysteroid sulfotransferase hSULT2A1, which is responsible for the sulfation of hydroxysteroids such as dehydroepiandrosterone (Qin et al. 2013). Certain hydroxylated metabolites of commonly found non-dioxin-like PCBs have been reported to inhibit aromatase (CYP19) that is necessary for the conversion of androgens to estrogens (Antunes-Fernandes et al. 2011). *In vitro* studies using a yeast-glucocorticoid screening assay have shown that OH-PCBs exhibit anti-glucocorticoid properties by the inhibition of the glucocorticoid receptor (Antunes-Fernandes et al. 2011). OH-PCBs also have the potential to disrupt thyroid hormone homeostasis by inhibition of thyroid hormone sulfation (Schuur et al. 1999, Schuur et al. 1998) and by interfering with the binding of thyroxine to transthyretin (Grimm et al. 2013, Gutleb et al. 2010, Lans et al.

1993). PCB sulfates derived from OH-PCBs also bind with high affinity to transthyretin (Grimm et al., 2015c).

Both OH-PCBs and their sulfated forms in circulation can serve as ligands for human serum albumin, a protein that also functions in the retention and transport of thyroxine with additional functions in binding selected drugs and other molecules (Rodriguez et al. 2016). While the binding of a OH-PCB metabolite to serum albumin may potentially displace drugs, hormones, or other ligands, it is also important to note that the protein may serve as a reservoir for the PCB metabolite and/or facilitate its transfer to tissues that would be subject to toxic effects of the OH-PCBs.

CONCLUSIONS

PCBs are an important class of persistent environmental chemicals which were commercially synthesized for various industrial purposes in the twentieth century. The legacy of PCB pollution has left numerous health questions and concerns. PCBs are converted to OH-PCBs which tend to persist and bioaccumulate in trophic levels. The overall transformation of PCBs into phenols is facilitated by enzymatic biotransformation in animals, plants and other organisms, and potential environmental hydrolysis of sulfated and glucuronidated metabolites back to OH-PCBs. OH-PCBs may exert toxicities through a variety of mechanisms. Among those investigated so far include bioactivation to electrophilic metabolites that can form covalent adducts with DNA and other macromolecules, interference with hormonal signaling through modulations of transcriptional activity of hormone nuclear receptors, inhibition of enzymes that regulate cellular concentrations of of active hormones, and interference with the transport of hormones, through binding to circulating carrier proteins such as transthyretin and albumin. With the imminent global trends in slow biotransformation of bio-accumulated parent PCBs, further research on the mechanisms underlying health effects of OH-PCBs and their metabolites is warranted.

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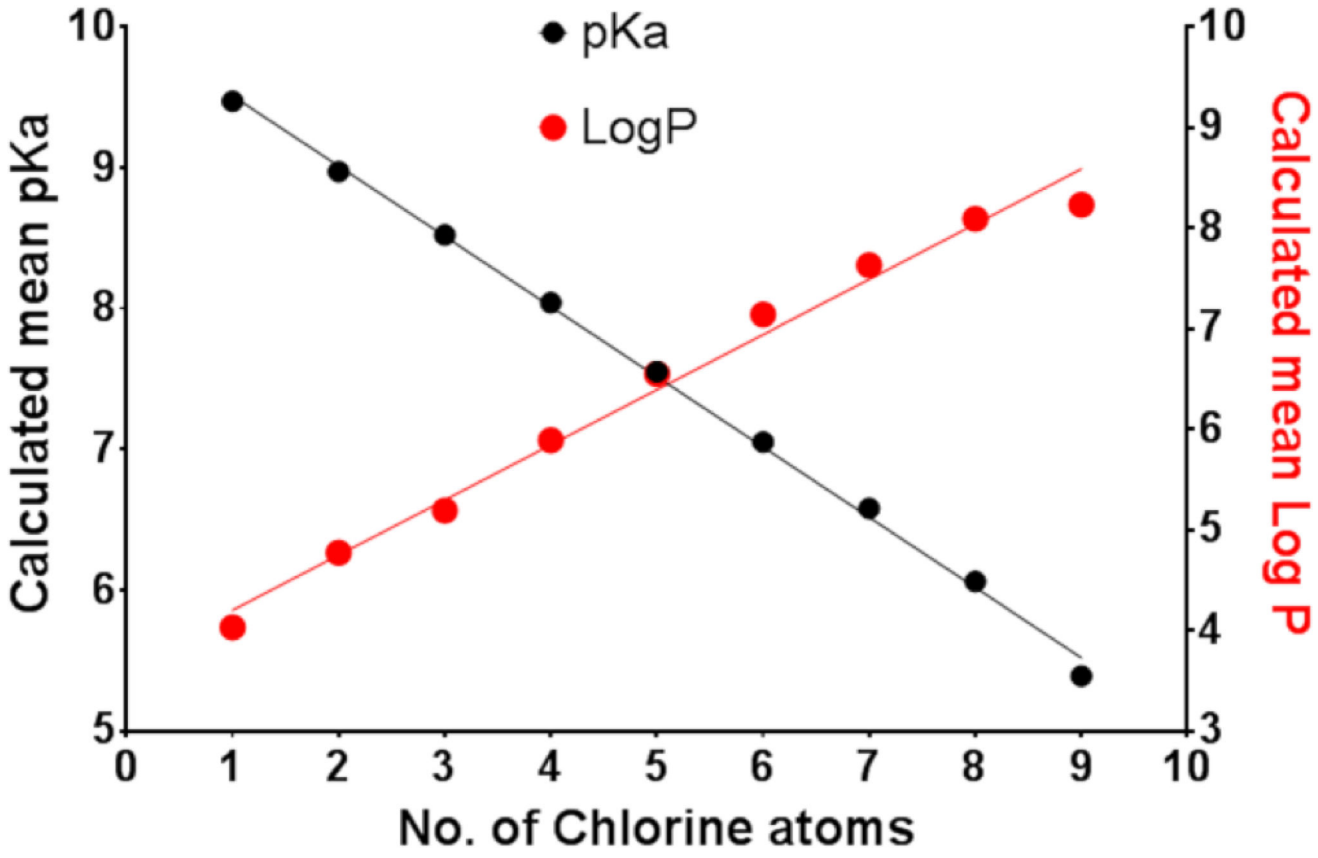

Figure 1. Change in pKa and Log P vs. Chlorine number of hydroxylated polychlorinated biphenyls (PCBs)

Mean pKa and mean Log P values of mono-hydroxyl PCBs were calculated from data provided for each group (please see reference Grimm et al 2015b)

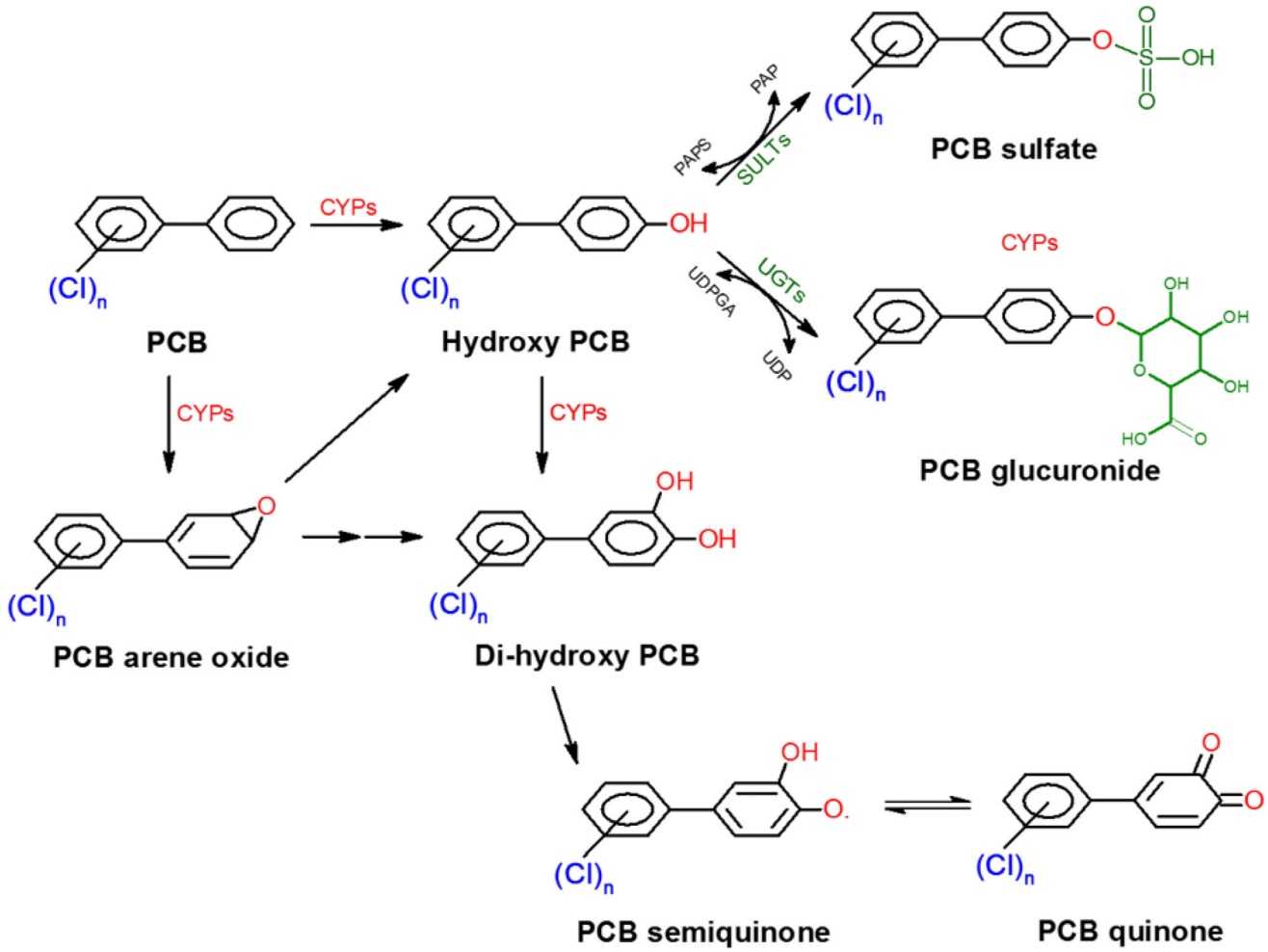

Figure 2. Metabolism of hydroxylated PCBs

The parent PCBs are oxidized in reactions catalyzed by cytochrome P-450 mono-oxygenases (CYPs) to form hydroxylated PCBs. The hydroxylated PCBs may then be conjugated to form PCB sulfates or PCB glucuronides, or metabolized into reactive semi-quinones or quinones.

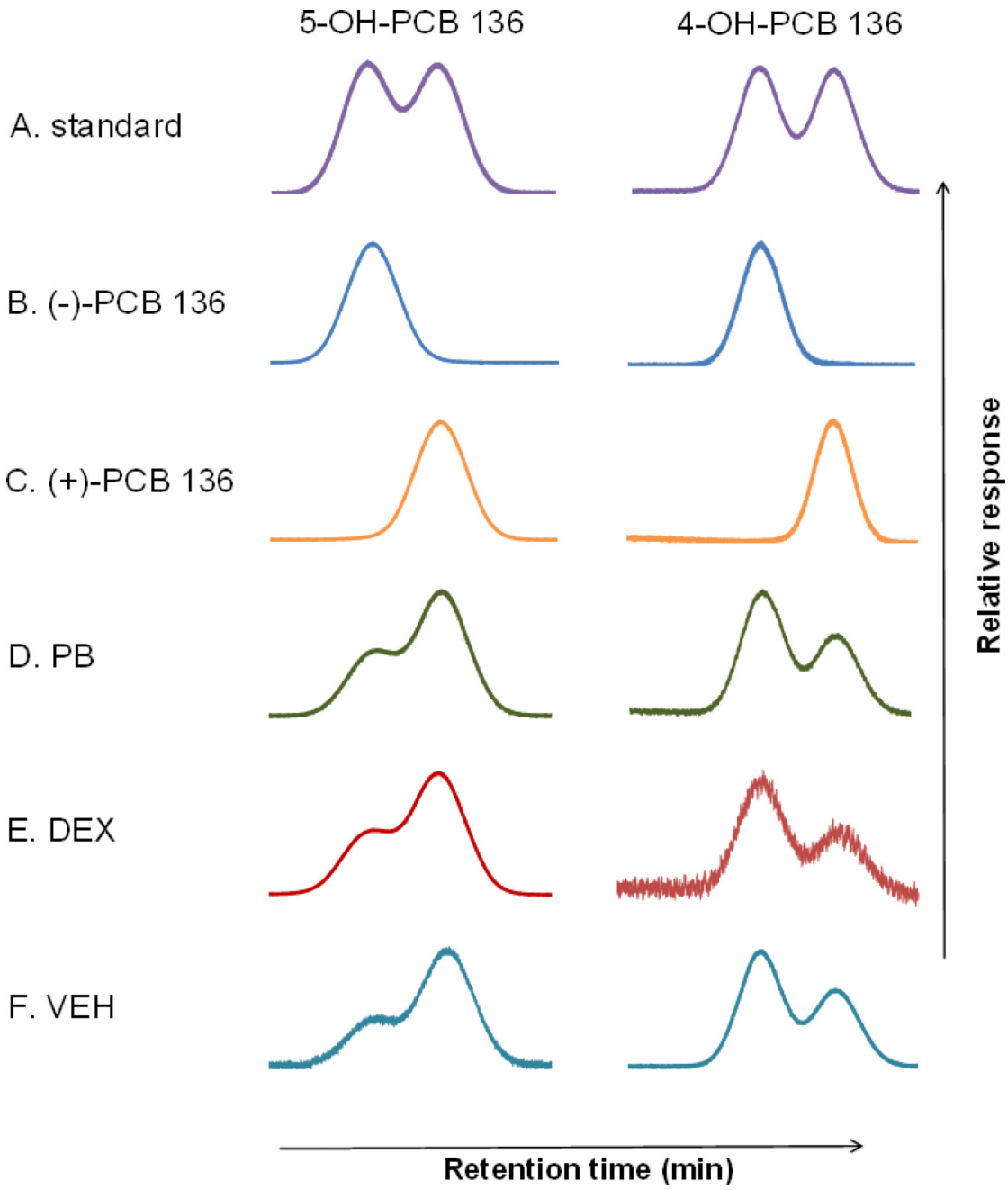

Figure 3.

The atropisomer of 5-OH PCB 136 eluting second on the Chirasil-Dex column (E_2-5-OH-PCB 136) and the atropisomer of 4-OH PCB 136 eluting first on the Cyclosil-B column (E_1-4-OH-PCB) are formed preferentially in incubations of racemic PCB 136 with liver microsomes from rats pretreated with different inducers of cytochrome P-450 enzymes. Based on incubations with pure PCB 136 atropisomers, the first and second eluting atropisomer of both 5-OH-PCB 136 and 4-OH-PCB 136 are formed from (−)-PCB 136 and (+)-PCB 136, respectively. Enantioselective gas chromatograms of (A) racemic 5-OH-PCB 136 and 4-OH-PCB 136 standards; (B) E_1-5-OH-PCB 136 and E_1-4-OH-PCB 136 formed

from (−)-PCB 136; (C) E_2-5-OH-PCB 136 and E_2-4-OH-PCB 136 formed from (+)-PCB 136; and chiral signature of 5-OH-PCB 136 and 4-OH-PCB 136 formed by liver microsomes from rats treated with (D) PB, (E) DEX or (F) VEH. All OH-PCB metabolites were methylated with diazomethane prior to GC analysis. Reprinted with permission from (Wu et al. 2011a). Copyright 2011 American Chemical Society.

Table 1

Number of isomers, pKa and Log P values of mono-hydroxyl PCBs

Log P values of the parent PCBs are presented for comparison. The original sources of pKa and Log P are compiled from (Grimm et al. 2015b). The Log P values of parent PCBs are compiled from (Brodsky & Ballschmiter 1988)

Phenolic Metabolites of	Number of Isomers	pKa OH-PCBs (Mean)[1]	Log P OH-PCBs (Mean)[1]	Log P of parent PCBs (Mean)[2]
Monochlorobiphenyls	19	9.47	4.03	4.51
Dichlorobiphenyls	64	8.97	4.77	5.02
Trichlorobiphenyls	136	8.52	5.19	5.45
Tetrachlorobiphenyls	198	8.04	5.89	5.81
Pentachlorobiphenyls	198	7.55	6.55	6.28
Hexachlorobiphenyls	136	7.05	7.14	6.64
Heptachlorobiphenyls	64	6.58	7.63	6.99
Octachlorobiphenyls	19	6.06	8.09	7.36
Nonachlorobiphenyls	3	5.39	8.23	7.83
Decachlorobiphenyl	0	-	-	8.38
Total	837			

[1] Data compiled from (Grimm et al. 2015b)

[2] Data compiled from (Brodsky & Ballschmiter 1988)

Table 2
All possible mono-OH-PCBs that are chiral

An assumption is that OH hinders rotation/racemization of the PCB atropisomers. This table is based on a similar approach employed by (Püttmann et al. 1989, Püttmann et al. 1986) for PCB atropisomers. The shorthand for the structures is that used in Table 2 of Grimm et al, 2015b. The yellow highlighted panels are duplicates.

Ring Substitution	−2,3,6	−2,3,4,6
−2	6'-0H-PCB 16, 6'-0H-PCB 18, 3'-0H-PCB 19, 2'-0H-PCB 24	6-0H-PCB 41, 4-0H-PCB 45, 6-0H-PCB 48, 3-0H-PCB 50, 2'-0H-PCB 62
−2,3	6-0H-PCB 40, 4'-0H-PCB 44, 3'-0H-PCB 45, 3'-0H-PCB 46, 2'-0H-PCB 59	6-0H-PCB 82, 4-0H-PCB 84, 3'-0H-PCB 88, 6'-0H-PCB 97, 3'-0H-PCB 98, 2'-0H-PCB 109
−2,4	6-0H-PCB 42, 4'-0H-PCB 45, 6'-0H-PCB 49, 3'-0H-PCB 51, 2'-0H-PCB 64	6-0H-PCB 85, 4'-0H-PCB 88, 4-0H-PCB 91, 6-0H-PCB 99, 3-0H-PCB 100, 2'-0H-PCB 115
−2,5	6-0H-PCB 44, 5'-0H-PCB 45, 6-0H-PCB 52, 3'-0H-PCB 53, 6'-0H-PCB 59	6-0H-PCB 87, 5'-0H-PCB 88, 4-0H-PCB 95, 6-0H-PCB 101, 3-0H-PCB 103, 6'-0H-PCB 109
−2,3,4	6'-0H-PCB 82,4'-0H-PCB 84, 6'-0H-PCB 87, 3'-0H-PCB 89, 3'-0H-PCB 91, 2'-0H-PCB 110	6-0H-PCB 128, 4'-0H-PCB 131, 4'-0H-PCB 132, 6'-0H-PCB 138, 3'-0H-PCB 139, 3'-0H-PCB 140, 2'-0H-PCB 158
−2,3,5	6'-0H-PCB 83, 5'-0H-PCB 84, 6'-0H-PCB 92, 3'-0H-PCB 94, 3'-0H-PCB 95, 2'-0H-PCB 113	6-0H-PCB 130, 5'-0H-PCB 131, 4'-0H-PCB 135, 3'-0H-PCB 144, 6'-0H-PCB 146, 3'-0H-PCB 148, 2'-0H-PCB 161
−2,3,6	6'-0H-PCB 84, 6'-0H-PCB 95, 3'-0H-PCB 96	6'-0H-PCB 131, 6-0H-PCB 132, 4-0H-PCB 136, 6'-0H-PCB 144, 3'-0H-PCB 145, 6'-0H-PCB 149, 3'-0H-PCB 150
−2,4,5	5'-0H-PCB 91, 4'-0H-PCB 95, 6-0H-PCB 97, 6'-0H-PCB 101, 3'-0H-PCB 102, 6'-0H-PCB 110	6-0H-PCB 138, 5'-0H-PCB 139, 4'-0H-PCB 144, 4-0H-PCB 149, 6-0H-PCB 153, 3'-0H-PCB 154, 6'-0H-PCB 158
−2,3,4,5	6'-0H-PCB 129, 5-0H-PCB 132, 4-0H-PCB 135, 6'-0H-PCB 141, 3'-0H-PCB 143, 3'-0H-PCB 149, 2'-0H-PCB 164	6'-0H-PCB 170, 5'-0H-PCB 171, 4'-0H-PCB 174, 4'-0H-PCB 175, 6'-0H-PCB 180, 3'-0H-PCB 182, 3'-0H-PCB 183, 2'-0H-PCB 191
−2,3,4,6	6'-0H-PCB 131, 6-0H-PCB 132, 4-0H-PCB 136, 6'-0H-PCB 144, 3'-0H-PCB 145, 6'-0H-PCB 149, 3'-0H-PCB 150	6'-0H-PCB 171, 4'-0H-PCB 176, 6'-0H-PCB 183, 3'-0H-PCB 184

Table 3

Genotoxicity endpoints for PCB 3 and its metabolites

Genotoxic profile of PCB 3 and metabolites, 2-OH, 3-OH and 4-OH PCB 3, 3,4- and 2,5 di OH PCB 3, and 3,4- and 2,5 PCB 3 quinones. Endpoints measured were point mutations, chromosome loss, chromosome breaks, polyploidization, sister chromatid exchanges (SCE) and DNA strand breaks (COMET). Numbers are LOELs (micromolar concentrations). MPx=myeloperoxidase. Table was modified from (Robertson & Ludewig 2011).

Compound	Point mutations	Chromosome loss	Chromosome breaks	Polyploi-dization	SCE	DNA strand break (COMET)
PCB 3	-	-	-	-	-	
2-OH	-	50	-			
3-OH	-	100	-			
4-OH	-	75	75			
3,4-diOH	-	15	25	-	5	
2,5-diOH	-	2.5	5	7.5	-	+ (MPx dependent)
3,4-quinone	0.6	5	15	-	-	
2,5-quinone	0.5	2.5	1	-	-	+ (MPx independent