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EFFECTS OF THIOL ANTIOXIDANTS ON THE ATROPSELECTIVE OXIDATION OF 2,2',3,3',6,6'-HEXACHLOROBIPHENYL (PCB 136) BY RAT LIVER MICROSOMES

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Abstract

Chiral polychlorinated biphenyl (PCB) congeners, such as PCB 136, are atropselectively metabolized to various hydroxylated PCB metabolites (HO-PCBs). The present study investigates the effect of two thiol antioxidants, glutathione and *N*-acetyl-cysteine (NAC), on profiles and chiral signatures of PCB 136 and its HO-PCB metabolites in rat liver microsomal incubations. Liver microsomes prepared from rats pretreated with phenobarbital were incubated with PCB 136 (5 μ M) in the presence of the respective antioxidant (0–10 mM), and levels and chiral signatures of PCB 136 and its HO-PCB metabolites were determined. Three metabolites, 5-136 (2,2',3,3',6,6'-hexachlorobiphenyl-5-ol), 4-136 (2,2',3,3',6,6'-hexachlorobiphenyl-4-ol) and 4,5-136 (2,2',3,3',6,6'-hexachlorobiphenyl-4,5-diol), were detected in all incubations, with 5-136 being the major metabolite. Compared to microsomal incubations without antioxidant, levels of 4,5-136 increased with increasing antioxidant concentration, whereas levels of PCB 136 and both mono-HO-PCBs were not affected by the presence of either antioxidant. PCB 136, 4-136 and 5-136 displayed significant atropisomeric enrichment; however, the direction and extent of the atropisomeric enrichment was not altered in the presence of an antioxidant. Because 4,5-136 can either be conjugated to a sulfate or glucuronide metabolite that is readily excreted or further oxidized a potentially toxic PCB 136 quinone, the effect of both thiol antioxidants on 4,5-136 formation suggests that disruptions of glutathione homeostasis may alter the balance between both metabolic pathways and, thus, PCB 136 toxicity *in vivo*.

Keywords

Glutathione; NAC; enantioselective; PCB 136; hepatic microsomes; cytochrome P450 enzymes; biotransformation

Introduction

Historic uses of PCBs, for example as coolants, lubricants and dielectric fluid, have resulted in widespread environmental contamination and continue to contribute to human PCB

exposures via dietary sources, especially contaminated fish (Kostyniak et al. 2005, Schecter et al. 2010). PCBs were also used in building materials, for example as light ballasts and caulking, and are major sources of current PCB contamination in older buildings. Recent studies demonstrate that PCBs are formed inadvertently by industrial processes and are contaminants in indoor paint (Anezaki & Nakano 2013, Hu & Hornbuckle 2010), thus contributing to indoor air contamination of old and new buildings, including schools (Thomas et al. 2012). PCB congeners with multiple *ortho* chlorine substituents represent a particular concern because of their prevalence in susceptible populations, such as school children (Megson et al. 2013) or individuals with genetic risk factors for neurodevelopmental disorders (Mitchell et al. 2012). Moreover, human epidemiological data and laboratory studies suggest a negative association between developmental exposure to environmental PCBs and measures of neurophysiological function in infancy and childhood (Mariussen & Fonnum 2006). Taken together, these studies demonstrate that PCBs remain a current and significant human health risk.

Several neurotoxic PCB congeners with three or four *ortho* substituents and their HO-PCB metabolites are chiral because they exist as stable rotational isomers, or atropisomers, that are non-superimposable mirror images of each other (Lehmler et al. 2010). Chiral PCB congeners are atropselectively oxidized to HO-PCBs by P450 enzymes, thus resulting in atropisomerically enriched PCBs and HO-PCBs (Kania-Korwel et al. 2012, Kania-Korwel & Lehmler 2015b, Lu et al. 2013, Wu et al. 2013a, Wu et al. 2014, Wu et al. 2011). The oxidation of PCBs to HO-PCBs can occur via a reactive PCB epoxide (Forgue & Allen 1982, Forgue et al. 1979) that subsequently rearranges to an HO-PCB or reacts with cellular nucleophiles (e.g. glutathione). Alternatively, HO-PCBs can be formed by direct insertion of an oxygen atom into an aromatic C-H bond (Preston et al. 1983). HO-PCB metabolites can be conjugated to form glucuronide (Sacco & James 2004, Tampal et al. 2002) or sulfate metabolites (Dhakal et al. 2012, Liu et al. 2006, Sacco & James 2005). Alternatively, HO-PCBs can be further metabolized by P450 enzymes to dihydroxy and, subsequently, quinone metabolites (Amaro et al. 1996). The quinone metabolites can react with proteins or other cellular nucleophiles, such as thiol antioxidants, and are thought to play an important role in the toxicity of PCBs (Grimm et al. 2015). It is likely that metabolism steps involving the reaction of reactive PCB metabolites are atropselective and, thus, affect metabolite profiles and chiral signatures of PCB metabolites, including HO-PCBs; however, the stereoselectivity of the oxidation of PCBs in the presence of antioxidants or cellular nucleophiles has not been investigated to date.

Several studies have reported that some PCBs and their metabolites alter cellular glutathione homeostasis, and that NAC co-treatment can attenuate adverse effects in cells in culture and *in vivo* following PCB exposure (Lai et al. 2010, Slim et al. 2000, Srinivasan et al. 2001, Zhu et al. 2013). The present study examines if the presence of both thiol antioxidants in microsomal incubations affects HO-PCB metabolite profiles and chiral signatures of HO-PCB metabolites. A microsomal formulation prepared from rats pretreated with phenobarbital (Wu et al. 2011), a classical inducer of CYP2B enzymes, was used because chiral PCBs are metabolized by CYP2B enzymes (Lu et al. 2013, Warner et al. 2009) to RyR-active *meta* hydroxylated metabolites (Niknam et al. 2013). PCB 136 was selected for

this exploratory study because of its environmental relevance (Kania-Korwel & Lehmler 2015a, Lehmler et al. 2010), its well investigated *in vitro* and *in vivo* metabolism (Birnbaum 1983, Schnellmann et al. 1983, Wu et al. 2013a, Wu et al. 2014, Wu et al. 2011) and its effect on cellular targets implicated in PCB developmental neurotoxicity (Pessah et al. 2009, Yang et al. 2014).

Materials and Methods

Chemicals and Reagents

PCB 136, 2,2',3,3',6,6'-hexachlorobiphenyl-4-ol (4-136), 2,2',3,3',6,6'-hexachlorobiphenyl-5-ol (5-136), 4,5-dimethoxy-2,2',3,3',6,6'-hexachlorobiphenyl (methylated derivative of 2,2',3,3',6,6'-hexachlorobiphenyl-4,5-diol; 4,5-136) and 2,2',3',4,6,6'-hexachloro-3-methoxybiphenyl (methylated derivative of 2,2',3',4,6,6'-hexachlorobiphenyl-3-ol; 3-150) were synthesized as described previously (Waller et al. 1999). The chemical structures and the abbreviations of the HO-PCB metabolites are shown in Figure 1A. Surrogate recovery standards (2,3,4',5,6-pentachlorobiphenyl, PCB 117; 2',3,3',4,5,5'-hexachlorobiphenyl-4-ol, 4-159) and internal standard (2,2',3,4,4',5,6,6'-octachlorobiphenyl, PCB 204) were purchased from Accustandard (New Haven, CT). NAC and tetrabutylammonium sulfite were purchased from Fisher Scientific (Pittsburg, PA, USA). L-Glutathione and β -nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Pesticide grade *n*-hexane, 2-propanol and methyl *tert*-butyl ether (MTBE) were purchased from Fisher Scientific (Pittsburg, PA, USA).

Animal treatment and microsome preparation

The preparation and characterization of the microsomes used in this study has been described previously (Wu et al. 2011). In short, microsomes were prepared by differential centrifugation from eight week old male Sprague-Dawley rats (Harlan, Inc., Indianapolis, IN, USA) pretreated with intraperitoneal injections of phenobarbital (102 mg/kg b.w./day) in saline for three consecutive days. Aliquots of the microsomes were stored at -80°C . 7-Ethoxyresorufin-*O*-deethylase (CYP1A), benzyloxyresorufin-*O*-debenzylase (CYP2B) and 7-benzyloxyquinoline debenzilation (CYP3A) activities have been reported previously and were 4-, 100- and 2-fold higher compared to activities of control microsomes from saline pretreated animals (Wu et al. 2011).

Microsomal incubations with PCB 136 in the presence of antioxidants

Incubation mixtures consisted of 0.1 M phosphate buffer (pH = 7.4), 3 mM magnesium chloride, 0.5 mg/mL microsomal protein, 0.5 mM NADPH (Wu et al. 2011) and various concentrations of glutathione (0 to 10 mM) or NAC (0 to 10 mM). These antioxidant concentrations were selected based on an earlier metabolism study with hexachlorobenzene (van Ommen et al. 1986). Mixtures were preincubated for 5 min at 37°C in a shaking water bath. A solution of PCB 136 in DMSO (10 μL) was added to give a concentration of 5 μM PCB 136 in a final volume of 2 mL. Earlier studies demonstrated that a considerable percentage of PCB 136 is oxidized to HO-PCBs at this PCB 136 concentration, thus allowing the simultaneous determination of chiral signatures for the parent PCB and its HO-

PCB metabolites (Wu et al. 2011). The incubations were continued at 37 °C for 5 min and stopped by adding 0.5 N NaOH (2 mL), followed by heating each sample at 90 °C for 10 min. Incubations were performed in triplicate for each concentration of glutathione or NAC. Control incubations were performed in parallel and included: (1) blank incubations and incubations without PCB 136 to determine background contamination with OH-PCBs and their methylated derivatives, and (2) incubations without NADPH, without microsomes or with heat inactivated microsomes to control for any abiotic transformation of PCB 136. No metabolites were observed in these control samples.

Extraction and separation of PCB 136 and its hydroxylated metabolites

The extraction of PCB 136 and its hydroxylated metabolites was performed following a published method without separation of PCB 136 from its metabolites (Wu et al. 2011). Briefly, surrogate recovery standards (PCB 117, 500 ng; 4-159, 274 ng) were added to each sample after heat inactivation. Solvents blank were extracted in parallel. The samples were acidified with 6 M HCl (1 mL) and extracted with 2-propanol (3 mL) and hexane-MTBE (5 mL, 1:1, v/v). The top organic layer was transferred to a different vial and the aqueous layer was washed with hexane (3 mL). The combined organic layer was washed with aqueous KCl (1%, 3 mL). The KCl phase was reextracted with hexane (3 mL), and the combined organic phases were concentrated to ~1 mL under a gentle stream of nitrogen. Five drops of methanol were added to each sample and the hydroxylated metabolites were derivatized with diazomethane in diethyl ether (0.5 mL) as described earlier (Wu et al. 2011). The samples were subjected to a sulfur clean-up step, followed by treatment with concentrated sulfuric acid using a published method (Kania-Korwel et al. 2007). The internal standard PCB 204 (200 ng) was added to each sample before the gas chromatographic analysis.

Gas chromatographic determinations

The analysis of PCB 136 and the HO-PCB metabolites (as the corresponding methylated derivatives) has been described elsewhere (Wu et al. 2011). All analyses employed an Agilent 7890A gas chromatograph equipped with a ⁶³Ni-μECD detector. The total amounts of PCB 136 and metabolites were initially determined on a DB-1 MS capillary column (60 m × 0.25 mm ID × 0.25 μm film thickness; Agilent, Santa Clara, CA, USA) without separation of atropisomers. The injector and detector temperatures were 280 and 300 °C, respectively. The temperature program was as follows: 100 °C for 1 min, 5 °C/min to 250 °C, hold for 20 min, 5 °C/min to 280 °C, and hold for 3 min. The flow rate of the carrier gas (helium) was 3 mL/min. Levels of PCB 136 and its HO-PCB metabolites were calculated using PCB 204 as internal standard and were not adjusted for the recovery of the surrogate recovery standards.

Subsequently, PCB 136 and 5-136 atropisomers were separated on a Chirasil-Dex capillary column (CD column, 25 m × 0.25 mm ID × 0.25 μm film thickness; Varian, Palo Alto, CA, USA). The first (E₁) and second eluting (E₂) atropisomers of PCB 136 correspond to (–)-PCB 136 and (+)-PCB 136, respectively (Haglund & Wiberg 1996). E₁-5-136 and E₂-5-136 are formed from (–)- and (+)-PCB 136, respectively (Wu et al. 2011). 4-136 atropisomers were separated on a Cyclosil-B capillary column (CB column, 30 m × 0.25 mm ID × 0.25 μm film thickness; Agilent, Santa Clara, CA, USA). E₁-4-136 and E₂-4-136 are formed from

(-)- and (+)-PCB 136, respectively (Wu et al. 2011). The atropisomers of 4,5-136 could not be separated on either enantioselective column. The temperature program for the separation of the PCB and HO-PCB atropisomers was as follows: 15 °C/min from 50 to 145 (160)°C, hold for 410 (360) min, 15 °C/min to 200 °C, and hold for 10 min on the CD (CB) column. The injector and detector temperatures were kept at 250 °C. The flow rate of the carrier gas (helium) was 3 mL/min. The enantiomeric fraction (EF) values for PCB 136, 5-136 and 4-136 were determined as $EF = \text{Area } E_2 / (\text{Area } E_1 + \text{Area } E_2)$.

Quality Assurance and Quality Control

The ^{63}Ni - μECDs used for the analysis of PCB 136 and its metabolites were linear up to concentrations of 1,000 ng/mL for all analytes investigated ($R^2 > 0.999$). The recoveries of PCB 117 and 4-159 were 94 ± 2 % (range: 86 to 99 %) and 93 ± 8 % (range: 84 to 101%), respectively. The limits of detection for PCB 136, 5-136, 4-136 and 4,5-136 were 50, 2.8, 4.3 and 1.7 ng, respectively (n=5). The resolution (R_s) of PCB 136, 5-136 and 4-136 atropisomers were 0.882 (CD column), 0.737 (CD column) and 0.667 (CB column), respectively. The EF values for the racemic standards of PCB 136, 5-136 and 4-136 on the respective enantioselective column were 0.520 ± 0.021 , 0.493 ± 0.002 and 0.514 ± 0.029 , respectively.

Statistical analysis

The data are presented as the mean $\pm$ SD (standard deviation). Differences in metabolites profiles, percentage change relative to incubation with glutathione or NAC, EF values of PCB 136, 5-136 and 4-136 were tested with two-sample, two-tailed Student's t-test. Comparisons were considered to be significant for $p < 0.05$.

Results

Effect of glutathione on microsomal PCB 136 metabolism

Glutathione is an important cellular nucleophile that readily reacts with reactive PCB metabolites, such as PCB epoxides formed by P450 enzymes. Approximately 62 to 65% of PCB 136 (5 μM , 10 nmol) were recovered as HO-PCB 136 metabolites after a 5 min. incubation time from microsomal incubations containing 0–10 mM glutathione (Table 1). Average mass balances ranged from 96–107%. The *meta* hydroxylated metabolites, 5-136, was the major metabolite in all incubations (88 to 96 % of the sum of HO-PCBs ($\Sigma\text{HO-PCBs}$)), independent of the glutathione concentration. In addition, the *para* hydroxylated metabolite, 4-136, and a vicinal dihydroxylated metabolite, 4,5-136, were detected as minor metabolites with $> 3\%$ of the $\Sigma\text{HO-PCBs}$. No 1,2-shift product was detected in any microsomal incubation. Levels of 4,5-136 increased compared to incubations without glutathione with increasing glutathione concentrations, whereas levels of unmetabolized PCB 136 as well as levels of 5-136 and 4-136 were essentially not affected by the presence of glutathione (Figure 1B). As a result, the HO-PCB metabolite profiles changed significantly in incubations at glutathione concentrations 0.1 mM (Table 1).

NAC effect on PCB 136 metabolism

NAC is a precursor of glutathione and an important ROS scavenger of clinical interest. We therefore investigated the effect of NAC on the metabolism of PCB 136. Overall, the effects of NAC co-treatment on PCB 136 metabolism were comparable to the effects seen with glutathione, with 62 to 67% of PCB 136 recovered as HO-PCB 136 metabolites from microsomal incubations containing 0–10 mM NAC (Table 1). Average mass balances ranged from 99–111%. Three metabolites, 5-136, 4-136 and 4,5-136, were observed, with 5-136 being the major metabolite (>87% of the Σ HO-PCBs). The 1,2 shift product was not detected under any of the conditions investigated. As with experiments using glutathione as antioxidant, levels of 4,5-136 increased with increasing concentration of NAC (Figure 1C), with 12 % of the Σ HO-PCBs being 4,5-136 at 10 mM NAC. At the same time, levels of PCB 136, 5-136 and 4-136 were not altered in the presence of NAC. The HO-PCB 136 metabolite profiles, expressed as percent of Σ HO-PCBs, were significantly different from the metabolite profile observed in incubations without NAC at NAC concentrations > 1 mM due to the higher levels of 4,5-136 (Table 1).

Effect of glutathione and NAC on the atropisomeric enrichment of PCB 136 and its metabolites

The direction and extent of the atropisomeric enrichment of PCB 136 and its two HO-PCB metabolites was determined using atropselective gas chromatography to determine if glutathione and NAC co-treatment affected the atropselectivity of PCB 136 metabolism. (–)-PCB 136 was enriched in all incubations, with EF values in the range of 0.43 to 0.47 (Figure 2). Moreover, both HO-PCB metabolites displayed considerable atropisomeric enrichment. The second eluting atropisomer of 5-136 (E_2 -5-136) and the first eluting atropisomer of 4-136 (E_1 -4-136) were enriched in all incubations. Co-treatment with glutathione or NAC (0 to 10 mM) did not affect the direction or extent of the atropisomeric enrichment of PCB 136 or its two HO-PCB metabolites.

Discussion

The microsomal metabolism observed in the present study is consistent with earlier *in vitro* studies using recombinant CYP2B1 enzyme (Lu et al. 2013), rat liver microsomes (Wu et al. 2011) and precision-cut rat liver tissue slices (Wu et al. 2013b) from rats pretreated with phenobarbital, an inducer of hepatic CYP2B enzymes. Briefly, 5-136 was the major metabolite formed by CYP2B enzymes in these *in vitro* systems, whereas 4-136 and 4,5-136 were only minor metabolites. Both 5-136 and 4-136 were further metabolized by CYP2B enzymes to 4,5-136, a catechol metabolite of PCB 136 (Waller et al. 1999). While 5-136 was the major metabolite in this and other studies (Wu et al. 2013b, Wu et al. 2011), it is important to emphasize that the HO-PCB metabolite profile depended on the P450 isoform composition (Wu et al. 2011) of the respective *in vitro* model and, in addition, was highly species dependent (Ohta et al. 2005, Sundström & Jansson 1975, Wu et al. 2014). Moreover, metabolite profiles can significantly differ from *in vivo* studies due to biotransformation and transport processes that are not present in *in vitro* assays. Growing experimental evidence suggests that these OH-PCB metabolites themselves are developmental neurotoxicants (Kodavanti et al. 2003, Niknam et al. 2013, Pessah et al. 2006).

The presence of physiologically relevant concentrations of glutathione significantly increased the levels of 4,5-136, with 4,5-136 representing over 10 % of the Σ HO-PCBs at 10 mM glutathione. A similar effect on 4,5-136 levels was observed in experiments using NAC. Several animal studies demonstrate that PCB catechol metabolites, such as 3',4'-dihydroxy-4-chlorobiphenyl and 4,5-136, are efficiently eliminated, most likely as the corresponding sulfate or glucuronide conjugates (Birnbaum 1983, Dhakal et al. 2014, Haraguchi et al. 2004, Wu et al. 2015). It is therefore possible that reduced hepatic glutathione stores are associated with a decreased formation and elimination of 4,5-136 conjugates, and additional studies are needed to investigate whether reduced glutathione levels, e.g. due to a disease (Pastore et al. 2003), alter the phase II metabolism of PCB 136 and other PCB congeners *in vivo*.

Neither the extent of PCB 136 metabolism nor the levels of 5-136 and 4-136 in the microsomal incubations were affected by the presence of glutathione or NAC. Therefore, it seems unlikely that the increase in 4,5-136 levels observed in our study was due to an antioxidant-mediated increase in PCB and/or HO-PCB metabolism rates because to the best of our knowledge there is no evidence that the redox environment regulates the activity of P450 enzymes involved in PCB metabolism. However, this possibility cannot be completely dismissed because rat CYP2B enzymes involved in the metabolism of PCB 136 (Lu et al. 2013, Warner et al. 2009) contain several cysteine residues. Moreover, several studies have shown that human CYP3A4 activity is increased in the presence of glutathione, but not other thiol containing antioxidants (Gillam et al. 1993, Kim & Kim 1998) due to an allosteric effect of glutathione on CYP3A4 (Davydov et al. 2008).

Since PCB 136 and structurally related PCBs (e.g., PCB 84) enantioselectively affect cellular targets implicated in PCB developmental neurotoxicity (Lehmler et al. 2005, Pessah et al. 2009, Yang et al. 2014), the enantiomeric fractions of PCB 136 and its OH-PCB metabolites were measured for each microsomal incubation. The goal was to determine if the presence of varying concentrations of thiol antioxidants alters the enantioselectivity of the P450 enzyme-mediated oxidation of PCB 136. We observed considerable atropisomeric enrichment of (–)-PCB 136, E₂-5-136 and E₁-4-136, which is consistent with our earlier microsomal metabolism studies using rat liver microsomes (Wu et al. 2011). Moreover, the enrichment of (–)-PCB 136 was in agreement with an *in vivo* disposition study in rats (Kania-Korwel et al. 2008). However, the EF values of PCB 136 and its HO-PCB metabolites in the microsomal incubations were similar across all glutathione and NAC concentrations investigated. Therefore, it seems unlikely that differences in the glutathione levels in metabolically active tissues [e.g., due to co-exposures to xenobiotics, including certain PCB congeners (Lai et al. 2010), or diseases associated with a reduced levels of glutathione (Pastore et al. 2003)] modulate the levels of neurotoxic PCB and/or OH-PCB enantiomers in target tissues and, thus, affect PCB developmental neurotoxicity *in vivo*.

Reactive arene oxide intermediates have been implicated in the covalent binding of PCB 136 metabolites to microsomal protein in a study with pooled human liver microsomes (Schnellmann et al. 1983). However, the trapping of arene oxide intermediates by glutathione or NAC is expected to affect levels of mono- and not di-hydroxylated PCB 136 metabolites, which was not observed in the present study. Moreover, the chemical reaction

of an arene oxides derived from PCB 52 (2,2',5,5'-tetrachlorobiphenyl) with thiol oxidants appeared to be relatively slow (Preston et al. 1984) and likely did not occur to a significant extent during the short incubation time used in the present study. Alternatively, a couple of studies demonstrated that reactive quinone metabolites are responsible for the binding of PCBs (Hesse et al. 1978) and hexachlorobenzene (van Ommen et al. 1986) to microsomal proteins. In both studies, antioxidants prevented the binding of the quinone intermediates to microsomal proteins by reducing the quinone back to a hydroquinone metabolite or by forming covalent adducts. Similarly, it is possible that thiol antioxidants alter the balance of PCB catechol vs. PCB quinone metabolites by preventing the oxidation of 4,5-136 to the corresponding PCB quinone or by reducing the PCB quinones back to 4,5-136. However, further studies of the chemistry of *ortho* PCB quinones, such as the *ortho* quinone formed from 4,5-136, are needed to confirm this hypothesis because conflicting evidence suggests that thiol antioxidants, such as glutathione, do not act as a reducing agent in the presence of chlorinated *para* quinones, but forms covalent adducts (Song et al. 2013, Song et al. 2009, van Ommen et al. 1986).

Conclusions

The present study demonstrates that thiol antioxidants do not alter the stereoselectivity of the metabolism of PCB 136. However, the antioxidant-dependent increase in the levels of the catechol metabolite 4,5-136 suggests that, similar to other chlorinated aromatic compounds, a reactive PCB 136 quinone may be formed in microsomal preparations, and that the balance of catechol vs. quinone metabolite may depend of the cellular glutathione status. This observation may have important toxicological implications because PCB quinones play in important role in the toxicity of PCBs. Further studies are therefore needed to elucidate how thiol antioxidants alter PCB metabolite profiles in microsomal incubations and if hepatic antioxidant levels also affect PCB metabolite profiles and toxicity *in vivo*.

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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 Poll Res*. 2013:1–12.
- 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]
- Davydov DR, Davydova NY, Tsalkova TN, Halpert JR. Effect of glutathione on homo- and heterotropic cooperativity in cytochrome P450 3A4. *Arch Biochem Biophys*. 2008; 471:134–145. [PubMed: 18206979]

- Dhakal K, He X, Lehmler HJ, Teesch LM, Duffel MW, Robertson LW. Identification of sulfated metabolites of 4-chlorobiphenyl (PCB3) in the serum and urine of male rats. *Chem Res Toxicol*. 2012; 25:2796–804. [PubMed: 23137097]
- Dhakal K, Uwimana E, Adamcakova-Dodd A, Thorne PS, Lehmler HJ, Robertson LW. Disposition of phenolic and sulfated metabolites after inhalation exposure to 4-chlorobiphenyl (PCB3) in female rats. *Chem Res Toxicol*. 2014
- 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; 91:475–483. [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; 40:233–245. [PubMed: 6805966]
- Gillam EMJ, Baba T, Kim BR, Ohmori S, Guengerich FP. Expression of modified human cytochrome-P450 3a4 in *Escherichia coli* and purification and reconstitution of the enzyme. *Arch Biochem Biophys*. 1993; 305:123–131. [PubMed: 8342945]
- Grimm FA, Hu D, Kania-Korwel I, Lehmler H-J, Ludewig G, Hornbuckle KC, Duffel MW, Bergman A, Robertson LW. Metabolism and metabolites of polychlorinated biphenyls. *Crit Rev Toxicol*, Early Online. 2015;1–28.10.3109/10408444.2014.999365
- 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]
- Hesse S, Mezger M, Wolff T. Activation of [C-14] chlorobiphenyls to protein-binding metabolites by rat-liver microsomes. *Chem Biol Interact*. 1978; 20:355–365. [PubMed: 418886]
- Hu D, Hornbuckle KC. Inadvertent polychlorinated biphenyls in commercial paint pigments. *Environ Sci Technol*. 2010; 44:2822–2827. [PubMed: 19957996]
- 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, Vyas S, Song Y, Lehmler HJ. Gas chromatographic separation of methoxylated polychlorinated biphenyl atropisomers. *J Chromatogr A*. 2008; 1207:146–154. [PubMed: 18760792]
- Kania-Korwel I, Barnhart CD, Stamou M, Truong KM, El-Komy MH, Lein PJ, Veng-Pedersen P, Lehmler HJ. 2,2',3,3',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. Toxicokinetics of chiral polychlorinated biphenyls across different species—a review. *Environ Sci Pollut Res Int*. 2015a10.1007/s11356-015-4383-0
- Kania-Korwel I, Lehmler HJ. Chiral polychlorinated biphenyls: absorption, metabolism and excretion—a review. *Environ Sci Pollut Res Int*. 2015b10.1007/s11356-015-4150-2
- Kim BR, Kim DH. Influence of glutathione on the catalytic activity of reconstituted cytochrome P450 3A4. *Biochem Biophys Res Commun*. 1998; 242:209–212. [PubMed: 9439637]
- Kodavanti PR, Ward TR, Derr-Yellin EC, McKinney JD, Tilson HA. Increased [³H]phorbol ester binding in rat cerebellar granule cells and inhibition of ⁴⁵Ca²⁺ buffering in rat cerebellum by hydroxylated polychlorinated biphenyls. *Neurotoxicology*. 2003; 24:187–198. [PubMed: 12606291]
- 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]
- Lai I, Chai Y, Simmons D, Luthe G, Coleman MC, Spitz DH, WM, Ludewig G, Robertson LW. Acute toxicity of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) in male Sprague-Dawley rats: effects on

- hepatic oxidative stress, glutathione and metals status. *Environ Int.* 2010; 36:918–923. [PubMed: 19969354]
- Lehmler H-J, 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.* 2010; 44:2757–2766. [PubMed: 20384371]
- Lehmler HJ, Robertson LW, Garrison AW, Kodavanti PRS. Effects of PCB 84 enantiomers on [^3H] phorbol ester binding in rat cerebellar granule cells and $^{45}\text{Ca}^{2+}$ -uptake in rat cerebellum. *Toxicol Lett.* 2005; 156:391–400. [PubMed: 15763638]
- Liu Y, Apak TI, Lehmler HJ, Robertson LW, Duffel MW. Hydroxylated polychlorinated biphenyls are substrates and inhibitors of human hydroxysteroid sulfotransferase SULT2A1. *Chem Res Toxicol.* 2006; 19:1420–1425. [PubMed: 17112228]
- Lu Z, Kania-Korwel I, Lehmler H-J, Wong CS. Stereoselective formation of mono- and di-hydroxylated polychlorinated biphenyls by rat cytochrome P450 2B1. *Environ Sci Technol.* 2013; 47:12184–12192. [PubMed: 24060104]
- Mariussen E, Fonnum F. Neurochemical targets and behavioral effects of organohalogen compounds: an update. *Crit Rev Toxicol.* 2006; 36:253–89. [PubMed: 16686424]
- Megson D, O'Sullivan G, Comber S, Worsfold PJ, Lohan MC, Edwards MR, Shields WJ, Sandau CD, Patterson DGJ. Elucidating the structural properties that influence the persistence of PCBs in humans using the National Health and Nutrition Examination Survey (NHANES) dataset. *Sci Total Environ.* 2013; 461–462C:99–107.
- Mitchell MM, Woods R, Chi LH, Schmidt RJ, Pessah IN, Kostyniak PJ, LaSalle JM. Levels of select PCB and PBDE congeners in human postmortem brain reveal possible environmental involvement in 15q11-q13 duplication autism spectrum disorder. *Environ Mol Mutagen.* 2012; 53:589–598. [PubMed: 22930557]
- Niknam Y, Feng W, Cherednichenko G, Dong Y, Joshi SN, Vyas SM, Lehmler H-J, Pessah IN. Structure-activity relationship of select meta- and para-hydroxylated non-dioxin-like polychlorinated biphenyls: From single RyR1 channels to muscle dysfunction. *Toxicol Sci.* 2013; 136:500–513. [PubMed: 24014653]
- 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; 35:319–30. [PubMed: 16019954]
- Pastore A, Federici G, Bertini E, Piemonte F. Analysis of glutathione: implication in redox and detoxification. *Clinica Chimica Acta.* 2003; 333:19–39.
- 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- Ca^{2+} 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]
- 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]
- Preston BD, Miller JA, Miller EC. Reactions of 2,2',5,5'-tetrachlorobiphenyl 3,4-oxide with methionine, cysteine and glutathione in relation to the formation of methylthio-metabolites of 2,2',5,5'-tetrachlorobiphenyl in the rat and mouse. *Chemico-Biological Interactions.* 1984; 50:289–312. [PubMed: 6430573]
- Sacco JC, James MO. Glucuronidation in the polar bear (*Ursus maritimus*). *Mar Environ Res.* 2004; 58:475–479. [PubMed: 15178068]
- Sacco JC, James MO. Sulfonation of environmental chemicals and their metabolites in the polar bear (*Ursus maritimus*). *Drug Metab Dispos.* 2005; 33:1341–1348. [PubMed: 15951448]
- Schecter A, Colacino J, Haffner D, Patel K, Opel M, Papke O, Birnbaum L. Perfluorinated compounds, polychlorinated biphenyls, and organochlorine pesticide contamination in composite

- food samples from Dallas, Texas, USA. *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]
- Slim R, Toborek M, Robertson LW, Lehmler HJ, Hennig B. Cellular glutathione status modulates polychlorinated biphenyl-induced stress response and apoptosis in vascular endothelial cells. *Toxicology and Applied Pharmacology.* 2000; 166:36–42. [PubMed: 10873716]
- Song EQ, Ma XY, Tian XG, Liu J, Liu LC, Dong H, Song Y. The effect of the structure of polychlorinated biphenyls on their hydroxylation, oxidation, and glutathionyl conjugation reactions. *Biomed Environ Sci.* 2013; 26:138–147. [PubMed: 23336137]
- Song Y, Wagner BA, Witmer JR, Lehmler H-J, Buettner GR. Nonenzymatic displacement of chlorine and formation of free radicals upon the reaction of glutathione with PCB quinones. *Proc Natl Acad Sci USA.* 2009; 106:9725–9730. [PubMed: 19497881]
- Srinivasan A, Lehmler HJ, Robertson LW, Ludewig G. Production of DNA strand breaks in Vitro and reactive oxygen species in Vitro and in HL-60 cells by PCB metabolites. *Toxicological Sciences.* 2001; 60:92–102. [PubMed: 11222876]
- Sundström G, Jansson B. The metabolism of 2,2',3,5',6-pentachlorobiphenyl in rats, mice and quails. *Chemosphere.* 1975; 4:361–370.
- Tampal N, Lehmler H-J, Espandiani P, Malmberg T, Robertson LW. Glucuronidation of hydroxylated Polychlorinated Biphenyls (PCBs). *Chem Res Toxicol.* 2002; 15:1259–1266. [PubMed: 12387623]
- 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.
- van Ommen B, Adang AEP, Brader L, Posthumus MA, Muller F, van Bladeren PJ. The microsomal metabolism of hexachlorobenzene - origin of the covalent binding to protein. *Biochem Pharmacol.* 1986; 35:3233–3238. [PubMed: 3768018]
- 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]
- Wu X, Pramanik A, Duffel MW, Hrycak EG, Bandiera SM, Lehmler HJ, Kania-Korwel I. 2,2',3,3',6,6'-Hexachlorobiphenyl (PCB 136) is enantioselectively metabolized to hydroxylated metabolites by rat liver microsomes. *Chem Res Toxicol.* 2011; 24:2249–2257. [PubMed: 22026639]
- Wu X, Duffel MW, 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 K, Duffel M, Lein PJ, Lehmler H-J. 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 HJ. 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]
- Wu X, Barnhart C, Lein PJ, Lehmler HJ. Hepatic metabolism affects the atropselective disposition of 2,2',3,3',6,6'-hexachlorobiphenyl (PCB 136) in mice. *Environ Sci Technol.* 2015; 49:616–625. [PubMed: 25420130]
- 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; 138:379–392. [PubMed: 24385416]

Zhu Y, Mapuskar KA, Marek RF, Xu W, Lehmler HJ, Robertson LW, Hornbuckle KC, Spitz DR, Aykin-Burns N. A new player in environmentally induced oxidative stress: polychlorinated biphenyl congener, 3,3'-dichlorobiphenyl (PCB11). *Toxicol Sci.* 2013; 136:39–50. [PubMed: 23997111]

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Figure 1.

The effect of thiol antioxidants on the microsomal metabolism of PCB 136. (A) Simplified metabolism scheme showing the metabolism of PCB 136 to mono- and di-hydroxylated metabolites and the effect of increasing concentrations of (B) glutathione or (C) *N*-acetyl-cysteine (NAC) on the relative levels of PCBs 136 and its metabolites in microsomal incubations with racemic PCB 136 (5 μ M). All results are expressed as percent change with respect to incubations without glutathione or NAC. The data represent the mean $\pm$ standard deviation for three different microsomal incubations. * $p < 0.05$, significantly different from incubations without glutathione or NAC as determined using Student's *t*-test.

Figure 2.

Glutathione or N-acetyl-cysteine (NAC) had not effect on enantioselective oxidation of PCB 136, with an enantiomeric enrichment of the (A) first eluting atropisomer of PCB 136, (B) second eluting atropisomer of 5-136, and (C) the first eluting atropisomer of 4-136. The dotted line indicates the EF value of the racemic standard. The data represent the mean $\pm$ standard deviation for three different microsomal incubations.

TABLE 1

Changes in the metabolite profiles of HO-PCB 136 metabolites in rat liver microsomal incubations containing different concentrations (0 to 10 mM) of glutathione or *N*-Acetyl-cysteine (NAC).

Antioxidant		HO-PCB 136 metabolite profile			
Name	mM	5-136 (percent of Σ HO-PCB [§])	4-136 (percent of Σ HO-PCB [§])	4,5-136 (percent of Σ HO-PCB [§])	Σ HO-PCB [§] as percent of PCB 136
Glutathione	0	96±1	1.4±0.1	3.1±1.4	62±4
	0.01	95±3	1.3±0.1	4.0±2.8	65±1
	0.1	93±1*	1.3±0.1	6.0±0.9*	65±1
	1	91±1*	1.3±0.0	8.1±0.9*	65±1
	5	89±1*	1.3±0.0	9.6±0.7*	65±0.3
	10	88±0.4*	1.3±0.0	11±0.4*	64±1
NAC	0	96±1	1.4±0.1	3.1±1.4	62±4
	0.01	94±3	1.3±0.0	4.6±3.0	65±1
	0.1	95±1	1.3±0.1	3.5±1.1	64±2
	1	93±3	1.2±0.0	5.7±3.0	63±3
	5	91±0.3*	1.2±0.0*	8.2±0.3*	66±1
	10	87±0.2*	1.2±0.0*	12±0.2*	67±1

[§] Σ HO-PCBs indicated the total amount of detected HO-PCB 136 including 3–150 (below limit of detection), 5-136, 4-136 and 4,5-136 in the incubation system.

* p < 0.05, significantly different from incubations without glutathione or NAC as determined with Student's t-test.