Published in final edited form as:

Ecotoxicology. 2016 September ; 25(7): 1438–1444. doi:10.1007/s10646-016-1693-z.

Toxicity of hydroxylated polychlorinated biphenyls (HO-PCBs) using the bioluminescent assay Microtox®

Renu Bhalla, Rouzbeh Tehrani, and Benoit Van Aken*

Department of Civil and Environmental Engineering, Temple University, 1947 N. 12th Street, Philadelphia, PA 19122, USA

Abstract

Hydroxylated polychlorinated biphenyls (HO-PCBs) are toxic contaminants which are produced in the environment by biological or abiotic oxidation of PCBs. The toxicity of a suite of 23 mono-hydroxylated derivatives of PCBs and 12 parent PCBs was determined using the bacterial bioluminescent assay Microtox®. All HO-PCBs tested exhibited higher toxicity than the corresponding parent PCB, with effect concentration 50% (EC_{50}) ranging from 0.07 to 133 mg L⁻¹. The highest toxicities were recorded with 4-hydroxylated derivatives of di-chlorinated biphenyls (EC_{50} = 0.07 to 0.36 mg L⁻¹) and 2-hydroxylated derivatives of tri-chlorinated biphenyls carrying a chlorine substituent on the phenolic ring (EC_{50} = 0.34 to 0.48 mg L⁻¹). The toxicity of HO-PCBs generally decreased when the degree of chlorination increased. Consistently with this observation, a significant positive correlation was measured between toxicity (measured by EC_{50}) and octanol-water partition coefficient (pK_{ow}) for the HO-PCBs under study (Pearson's correlation coefficient, $r = 0.74$), which may be explained by the lower solubility and bioavailability generally associated with higher hydrophobicity. This study is the first one which assessed the toxicity of a suite of PCBs and HO-PCBs using the bioluminescent assay Microtox®, showing an inverse correlation between toxicity and hydrophobicity.

Keywords

Microtox®; bioluminescent assay; hydroxylated polychlorinated biphenyl–HO-PCB; structure-activity relationship–SAR; toxicity

Introduction

Polychlorinated biphenyls (PCBs) represent a class of 209 congeners made of a biphenyl core to which 1 to 10 chlorine atoms are attached (Borja et al. 2005). PCBs exhibit high chemical and physical stability and high dielectric constant, making them useful in a variety of industrial applications, including lubricants, dielectric fluids, and plasticizers. Because of their toxicity and persistence in the environment, the production of PCBs was banned in most countries by the late seventies (Field and Sierra-Alvarez 2008). It is usually estimated

*Corresponding author: bvanaken@temple.edu.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

that over 1.5 million tons of PCBs were produced worldwide and they are today detected in virtually every compartment of the ecosystem, including air, water, soil, sediments, and living organisms (Pieper and Seege 2008). PCBs have been reported to exert detrimental effects on wildlife and humans, including immunotoxicity, neurotoxicity, developmental toxicity, reproductive toxicity, and carcinogenicity (Takeuchi et al. 2011; Grimm et al. 2015).

Hydroxylated derivatives of PCBs (HO-PCBs) are produced in the environment by biological and abiotic oxidation of PCBs (Tehrani and Van Aken, 2014). The first step of the metabolism of PCBs by higher organisms often involves the transient formation of hydroxylated (HO-) derivatives (Montano et al., 2013). PCBs in mammalian cells are thought to be oxidized into HO-metabolites by the cytochrome P-450 monooxygenase system (Letcher et al. 2000). HO-PCBs have been detected in Great Lake fish and they were shown to be formed in PCB-exposed rainbow trout (Buckman et al. 2006). Likewise, higher plants and wood-decaying fungi were shown to transform lesser-chlorinated PCBs into various mono- and di-HO metabolites (Chroma et al. 2003; Sietmann et al. 2006; Van Aken et al. 2010). Hydroxylation of PCBs constitutes the first step of a detoxification sequence, which involves typically activation (phase I), transferase-mediated conjugation (phase II), and excretion or sequestration (phase III) (Letcher et al. 2000). Besides biological processes, it has been suggested that HO-PCBs can be formed in the atmosphere through abiotic gas-phase reaction with hydroxyl radicals ($\cdot\text{OH}$) (Anderson and Hites 1996; Mandalakis et al. 2003). As reaction products from PCBs, HO-PCBs are widely dispersed in the environment and they have been detected in a variety of samples, including air, water, sediments, and animal tissues (Flanagan and May 1993; Kawano et al. 2005; Buckman et al. 2006; Ueno et al. 2007; Marek et al. 2013; Awad et al. 2016). HO-PCBs have raised environmental concerns because of their toxicity for higher organisms (Montano et al. 2013; Grimm et al. 2015). In fact, the toxicity of many halogenated contaminants – including PCBs – on the endocrine and neural systems has been attributed to the formation of HO-metabolites (Montano et al. 2013).

Although the toxicity of HO-PCBs toward higher organisms is well documented, their effects on bacteria have received little attention – even though bacteria are the major organisms responsible for natural attenuation of PCBs. A few publications have reported the toxic effects of HO-PCBs on bacteria (Camara et al. 2004; Parnell et al. 2006; Geng et al. 2012), however these studies focused more on PCB biodegradation and considered only a limited number of HO-derivatives. The objective of the present study is to determine and compare the toxic effect of a suite of mono-HO PCB congeners using the microbial bioluminescent assay Microtox[®].

Methods

Chemicals

Microtox[®] reagents and consumables were purchased from Azur Environmental (Fairfax, CA). PCB 2 and its 4-HO derivative (3-chlorobiphenyl-CB and 4-HO-3-CB), PCB 3 and its 2'-, 3'-, and 4'-HO derivatives (4-CB, 2'-HO-4-CB, 3'-HO-4-CB, and 4'-HO-4-CB), 4-HO derivative of PCB 8 (4-HO-2,4'-dichlorobiphenyl-DCB), PCB 12 and its 4'-HO derivative

(3,4-DCB and 4'-HO-3,4-DCB), 2'- and 4'-HO derivatives of PCB 35 (2'-HO-3,4,5'-trichlorobiphenyl-TCB and 4'-HO-3,3',4-TCB), PCB 36 and its 2'- and 4'-HO derivatives (3,3',5-TCB, 2'-HO-3,5,5'-TCB, and 4'-HO-3,3',5-TCB), PCB 39 and its 4-HO derivative (3,4',5-TCB and 4-HO-3,4',5-TCB), 4'-HO derivative of PCB 68 (4'-HO-2,3',4,5'-tetrachlorobiphenyl-TeCB), and 4'-HO derivative of PCB 79 (4'-HO-3,3',4,5'-TeCB) were custom-synthesized by the Superfund Research Program Synthesis Core at the University of Iowa (Iowa City, IA). The HO-PCBs were synthesized in purity of 99% or higher by coupling a chlorinated boronic acid with a chlorinated bromoanisole followed by demethylation with boron bromide (BBr₃) as described elsewhere (Lehmle and Robertson 2001). 3-HO derivative of PCB 3 (3-HO-4-CB), PCB 8 (2,4'-DCB), PCB 9 and its 2'-, 3'-, and 4'-HO derivatives (2,5-DCB, 2'-HO-2,5-DCB, 3'-HO-2,5-DCB, and 4'-HO-2,5-DCB), PCB 30 and its 2'-, 3'-, and 4'-HO derivatives (2,4,6-TCB, 2'-HO-2,4,6-TCB, 3'-HO-2,4,6-TCB, and 4'-HO-2,4,6-TCB), PCB 35 (3,3',4-TCB), PCB 61 and its 2'-, 3'-, and 4'-HO derivatives (2,3,4,5-TeCB, 2'-HO-2,3,4,5-TeCB, 3'-HO-2,3,4,5-TeCB, and 4'-HO-2,3,4,5-TeCB), PCB 68 (2,3',4,5'-TeCB), and PCB 79 (3,3',4,5'-TeCB) were purchased from Accustandard (New Haven, CT) in purity of 99% or higher.

Microtox[®] assay

The toxicity of the parent PCBs and their HO-derivatives was determined using the standard Microtox[®] Basic Toxicity test following the manufacturer recommendations (Johnson 2005). Bioluminescence levels were recorded on a Microtox[®] Model 500 Analyzer using original Microtox[®] solutions and reagents (Azur Environmental).

PCBs and HO-PCBs were first dissolved in GC-grade acetone (5,000-mg L⁻¹) and then homogeneously dispersed in aqueous Microtox[®] Diluent Solution (2% NaCl) to a final concentration of 50 mg L⁻¹. A phenol solution (50-mg L⁻¹) was used for quality control of the assay. Microtox[®] Diluent Solution containing 1% (v/v) acetone was used to detect the potential toxic effect of the solvent. When the toxicity of PCB derivatives was too high for recording reliable data, additional dilutions were prepared to final concentrations of 2.5 or 5.0 mg L⁻¹.

The lyophilized luminescent bacterium, *Vibrio fischeri* (Microtox[®] Reagent), kept at -80°C was revitalized in 1 mL of Microtox[®] Reconstitution Solution (0.01% NaCl). The cell suspension was immediately transferred into a glass cuvette that was placed in the reagent well of the Microtox[®] Analyzer at 5°C. Two mL of two-fold serial dilutions of PCBs and HO-PCBs were prepared in Microtox[®] Diluent Solution to which 10 µL of reconstituted Reagent was added. The cuvettes were incubated at 15°C and bioluminescence was recorded at time 0 and after 5 and 15 min.

Data analyses

Data analysis was performed using the MicrotoxOmni[®] Data Reduction software, which uses a *p*-order inhibition model (Johnson 2005). The assay endpoint was the effective concentration 50% (EC₅₀), which is defined as the concentration of toxicant that results in 50% of bioluminescence reduction by reference to the non-exposed control. EC₅₀ values and confidence intervals at 95% were calculated from the model parameters – after linearization

– using Microsoft Excel (Redmond, WA). The fitting of experimental data to the model was expressed by the coefficient of determination, R^2 .

The pK_{ow} of PCBs and HO-PCBs were estimated using the SPARC (Automated Reasoning in Chemistry) on-line calculator (Hilal et al. 2004). EC_{50} values recorded after 5 min were plotted against pK_{ow} and linear regressions, Pearson's correlation coefficients (r), and associated p -values were computed using Prism 5.04 (Graphpad, La Jolla, CA).

Results and discussion

We obtained 23 high purity mono-HO PCBs and their 12 parent compounds, including mono- to tetra-chlorinated congeners, from academic (University of Iowa) and commercial sources (Accustandard). The toxicity of all congeners was measured using the microbial bioluminescent assay Microtox[®]. Exposure of the luminescent marine bacteria, *V. fischeri*, to a toxic substance results in a decrease of light emission, which is recorded to determine the toxicity. The Microtox[®] assay is widely accepted as a standard toxicity testing method (ASTM 2009) and has been used to assess the environmental toxicity of various contaminants, including metals, pesticides, aromatic hydrocarbons, explosives, and PCBs (Chu et al. 1997; Kemble et al. 2000; Ingersoll et al. 2002).

Results of the Microtox[®] assay – EC_{50} with 95% confidence intervals recorded after 5 and 15 min and R^2 of the dose-response curves – are presented in Table 1. The EC_{50} values of the standard phenol solution (50 mg L^{-1}) fell within the range prescribed by the manufacturer (13 to 26 mg L^{-1}) and no toxicity was detected with the acetone controls.

Because PCBs can be present at high concentration in the environment (e.g., $28,000 \text{ mg kg}^{-1}$ was detected in sediments of the Hudson River), a concentration of 50 mg L^{-1} was used in this study (Sondossi et al. 1991; Camara et al. 2004). Because higher-chlorinated congeners are not water-soluble at this level, a homogenous dispersion was prepared by dissolution in acetone prior to addition to the aqueous phase, according to guidelines for testing low-solubility toxicants (Rufli et al. 1998; OECD/OCDE 2006).

Based on the Microtox[®] readings, most parent PCBs did not exhibit a detectable toxicity at the concentration of 50 mg L^{-1} (i.e., EC_{50} non determined–ND). Only three lesser-chlorinated parent PCBs showed a recordable toxicity at 50 mg L^{-1} : 3-CB, 2,4'-DCB, and 2,5-DCB with 5-min $EC_{50} = 13.1$, 2.64, and 14.5 mg L^{-1} , respectively (Table 1). Hormesis (i.e., metabolic stimulation by exposure to low concentrations of toxic chemicals) was frequently observed with the parent PCBs, resulting in higher light emission in exposed samples as compared with the non-exposed controls (Shen et al. 2009).

All mono-HO PCBs under study exhibited a recordable toxicity after 5 and 15 min of exposure at the concentration tested (ranging from 2.5 to 50 mg L^{-1}) (Table 1). Recorded 5-min EC_{50} values of mono-HO derivatives ranged from 0.07 mg L^{-1} (4-HO-2,4'-DCB) to 133 mg L^{-1} (4'-HO-2,3,4,5-TeCB). The data fitted generally well the toxicity model, with coefficients of determination, $R^2 = 0.9$ for 36 analyses (83%) and 0.8 for 51 analyses (98%) (Table 1). The highest toxicities were recorded with 4-HO derivatives of di-chlorinated biphenyls (2,4'-, 2,5-, and 3,4-DCB) and 2-HO derivatives of tri-chlorinated

biphenyls carrying a chlorine atom on the phenolic ring (3,3',4- and 3,3',5-TCB), with 5-min EC₅₀ ranging from 0.07 to 0.48 mg L⁻¹ (Table 1).

Our results showed that the toxicity of HO-PCBs generally decreased with the degree of chlorination. The EC₅₀ of HO-PCBs were then plotted against the estimated pK_{ow} – a measurement of the hydrophobicity, which is directly related to the degree of chlorination. A significant correlation was observed between EC₅₀ and pK_{ow} when considering the 23 HO-congeners under study (Pearson's correlation coefficient, $r = 0.74$, $p = < 0.0001$; Figure 1A). Stronger correlations were detected within 3-HO ($r = 0.99$, $p = 0.0007$; Figure 1C) and 4-HO congeners ($r = 0.97$, $p < 0.0001$; Figure 1D), when they were considered separately. No significant correlation was detected with 2-HO congeners (Figure 1B). An inverse relationship between toxicity and degree chlorination of PCBs (i.e., positive correlation between EC₅₀ and pK_{ow}) has been reported in prior publications and was primary linked to the lower bioavailability associated with hydrophobic molecules (Camara et al. 2004). An alternative explanation for the observed correlation between toxicity and hydrophobicity could be related to the reactivity of PCBs and HO-PCBs, which generally decreases when the degree of chlorination increases. Even though the metabolism of PCBs and their derivatives is generally considered a detoxification mechanism, HO-PCBs can be further hydroxylated, generating toxic reactive derivatives. *In vitro* studies have shown that HO-PCBs, through the formation of quinones and reactive oxygen species (ROS), could damage DNA and form adduct with proteins, lipids, and nucleic acids (Grimm et al. 2015). These mechanisms could also explain to toxicity of reactive lower-chlorinated HO-PCBs toward bacterial cells.

Consistently with our findings, a few publications have reported that hydroxylation of PCBs results in higher toxicity for bacteria. Based on the oxygen uptake rate, Sondossi et al. (1991) recorded a high toxicity of three mono-HO PCBs (4-HO-2-CB, 4-HO-3-CB, and 4-HO-5-CB) at the concentration of 0.5 and 3.0 mM (~100 and ~600 mg L⁻¹) toward the PCB degrader *Comamonas testosteroni* B-356. The HO-PCBs were found to be significantly more toxic than the HO-biphenyls and chlorobiphenyls tested. Camara et al. (2004) studied the toxicity of PCBs using a recombinant *E. coli* expressing subsets of biphenyl dioxygenase genes (*bph*). Based on cell viability, the authors observed a high toxicity of di-HO derivatives originating from the metabolism of parents PCBs. In accordance with our observations, a lower toxicity was detected when the degree of chlorination increased from mono- to tri-chlorinated biphenyls. Parnell et al. (2006) exposed the PCB degrader, *Burkholderia xenovorans* LB400, to the commercial mixture, Aroclor 1242 (500 mg L⁻¹), and observed that induction of the biphenyl pathway resulted in overexpression of several detoxification genes, suggesting that the toxicity associated with PCBs was partly due to the formation of PCB metabolites. More recently, Geng et al. (2012) reported the inhibitory effect of four *ortho*-HO PCBs (with 2, 3, 4, and 5 chlorine atoms) on *Escherichia coli*, although none of the parent PCBs tested exhibited a recordable effect. The authors reported that HO-PCBs were actively pumped out of the cell by the AcrAB-TolC drug efflux system, which is known to be involved in cell resistance to hydrophobic pollutants. Besides PCBs, hydroxylation of other aromatic compounds has been reported to increase their toxicity, which is assumed to be related to the higher solubility of HO-derivatives as compared with the parent compounds (Camara et al. 2004; Geng et al. 2012).

A further examination of the structure-activity relationship (SAR) between the HO-PCBs reveals some remarkable patterns. First, higher-chlorinated HO-congeners – tri- and tetra-chlorinated – with the HO substituent in position 2 consistently exhibited a higher toxicity than the corresponding 3-HO and 4-HO substituted ones (Table 1). Moreover, tri-chlorinated congeners with the HO substituent in position 2 and a chlorine atom in position 5 – on the phenolic ring – showed a much higher toxicity (5-min EC_{50} = 0.34 and 0.48 mg L⁻¹ for 2'-HO-3,4,5'- and 2'-HO-3,5,5'-TCB, respectively) than all other higher-chlorinated HO-PCBs (5-min EC_{50} ranging from 1.05 to 133 mg L⁻¹). These observations may be explained by the steric hindrance around the HO group, which has been suggested to lower the activity of conjugative enzymes, therefore preventing detoxification of the molecule (Tampal et al. 2002). Among lower-chlorinated HO-congeners – mono- and di-chlorinated –, the highest toxicity was observed with the 4-HO substituted compounds (5-min EC_{50} = 0.92 mg L⁻¹), with the exception of 4'-HO-4-CB (5-min EC_{50} = 4.79 mg L⁻¹). A similar observation was reported by Sondossi et al. (1991) who recorded higher toxicity – based on *E. coli* respiration rate – of 4-HO-2-CB and 4-HO-3-CB as compared with 2-HO-5-CB. Furthermore, our results also show that lower-chlorinated 2-HO congeners exhibited lower toxicity (5-min EC_{50} = 6.75 mg L⁻¹) than the corresponding 3-HO and 4-HO congeners (5-min EC_{50} = 4.79 mg L⁻¹), which may be explained by the higher reactivity of the latter ones, potentially resulting in damaging oxidative species. Studying the mutagenic activity of HO-derivatives of 4-CB in a rat hepatocyte model, Espandiari et al. (2004) observed that only 3'-HO- and 4'-HO-4-CB – but not 2'-HO-4-CB – were active, likely through further hydroxylation/oxidation to reactive compounds (e.g., quinones) susceptible to induce oxidative stress and DNA damage. An alternative explanation could be the higher conjugation rate of 2-HO congeners as compared with 3-HO- and 4-HO substituted ones. Investigating the implication of uridine diphosphate glucuronosyl transferase (UGT) in the conversion of HO-PCBs, Tampal et al. (2002), reported a higher rate of conjugation rate of 2'-HO-4-CB as compared with 3'-HO- and 4'-HO-4-CB, therefore potentially leading to a lower toxicity.

Our results also showed that the toxicity of lesser-chlorinated PCBs and HO-PCBs decreased (i.e., increase of EC_{50}) from 5 to 15 min of exposure – observed for all mono-chlorinated and most (5 out of 7) di-chlorinated compounds –, although the toxicity of higher-chlorinated HO-PCBs increased from 5 to 15 min – observed with all tetra-chlorinated and most (5 out of 8) tri-chlorinated compounds (Table 1). A decrease of toxicity with the exposure time has been reported elsewhere and may be explained by an increasing bioluminescence in response to toxic stress (Antonelli et al. 2009) or defense mechanisms, such as efflux pumps (Geng et al. 2012). Consistently with our observation, Camara et al. (2004) reported that exposure of *E. coli* cells for one, six, and 24 h resulted in a decrease of cell viability which was more significant with higher- chlorinated congeners (i.e., tri-chlorinated vs. mono- and di-chlorinated). The increasing toxicity with the time which was observed with higher-chlorinated congeners may be due to a slower penetration of the compounds into the cells, leading to an increase of intracellular concentration.

The toxicity of HO-PCBs – as other halogenated phenolic compounds – for higher organisms is generally attributed to their endocrine disrupting activity, targeting both the estrogen and thyroid systems, and their neurotoxic potential (Montano et al. 2013; Grimm et

al. 2015). On the other hand, little information is available about the toxicity mechanisms of HO-PCBs for bacteria, making difficult to explain further the observed SAR.

Using a suite of 23 HO-congeners, the present study shows for the first time a significant negative correlation between the toxicity and hydrophobicity of HO-PCBs, which can be explained by the lower bioavailability and reactivity of higher-chlorinated congeners. Our results may have important environmental implications because bacteria are the major actors of the biodegradation of PCBs in the environment under both aerobic and anaerobic conditions (Borja et al. 2005; Pieper and Seeger 2008). The higher toxicity of HO-PCBs could inhibit bacterial activity and partially explain the recalcitrance of certain PCB congeners to biodegradation (Camara et al. 2004).

Acknowledgments

This work was funded by the Iowa Superfund Basic Research Program, National Institute of Environmental Health Sciences, Grant P42ES013661. The authors want to thank Hans-Joachim Lehmler (University of Iowa) for providing most PCBs and HO-PCBs used in this study.

References

- American Society for Testing and Materials–ASTM. Standard Test Method for Assessing the Microbial Detoxification of Chemically Contaminated Water and Soil Using a Toxicity Test with a Luminescent Marine Bacterium. ASTM International; West Conshohocken, PA: 2009. Method D5660–96
- Anderson PN, Hites RA. HO radical reactions: The major removal pathway for polychlorinated biphenyls from the atmosphere. *Environ Sci Technol.* 1996; 30:1756–1763.
- Antonelli M, Mezzanotte V, Panouilleres M. Assessment of peracetic acid disinfected effluents by microbiotests. *Environ Sci Technol.* 2009; 43:6579–6584. [PubMed: 19764220]
- Awad A, Martinez A, Marek R, Hornbuckle K. Occurrence and distribution of two hydroxylated polychlorinated biphenyl congeners in Chicago air. *Environ Sci Technol Lett.* 2016; 3:47–51.
- Borja J, Taleon DM, Aurensenia J, Gallardo S. Polychlorinated biphenyls and their biodegradation. *Process Biochem.* 2005; 40:1999–2013.
- Buckman A, Wong C, Chow E, Brown S, Solomon K, Fisk A. Biotransformation of polychlorinated biphenyls (PCBs) and bioformation of hydroxylated PCBs in fish. *Aquat Toxicol.* 2006; 78:176–185. [PubMed: 16621064]
- Camara B, Herrera C, Gonzalez M, Couve E, Hofer B, Seeger M. From PCBs to highly toxic metabolites by the biphenyl pathway. *Environ Microbiol.* 2004; 6:842–850. [PubMed: 15250886]
- Chroma L, Moeder M, Kucerova P, Macek T, Mackova M. Plant enzymes in metabolism of polychlorinated biphenyls. *Fresenius Environ Bull.* 2003; 12:291–295.
- Chu S, He Y, Xu X. Determination of acute toxicity of polychlorinated biphenyls to *Photobacterium phosphoreum*. *Bull Environ Contamin Toxicol.* 1997; 58:263–267.
- Espandiar P, Glauert HP, Lehmler H-J, Lee EY, Srinivasan C, Robertson LW. Initiating activity of 4-chlorobiphenyl metabolites in the resistant hepatocyte model. *Toxicol Sci.* 2004; 79:41–46. [PubMed: 14976334]
- Field JA, Sierra-Alvarez R. Microbial transformation and degradation of polychlorinated biphenyls. *Environ Pollut.* 2008; 155:1–12. [PubMed: 18035460]
- Flanagan WP, May RJ. Metabolite detection as evidence for naturally occurring aerobic PCB biodegradation in Hudson River sediments. *Environ Sci Technol.* 1993; 27:2207–2212.
- Geng S, Fang J, Turner KB, Daunert S, Wei Y. Accumulation and efflux of polychlorinated biphenyls in *Escherichia coli*. *Anal Bioanal Chem.* 2012; 403:2403–2409. [PubMed: 22349406]

- Grimm F, Hu D, Kania-Korwel I, Lehmler H, Ludewig G, Hornbuckle K, Duffel M, Bergman A, Robertson L. Metabolism and metabolites of polychlorinated biphenyls. *Crit Rev Toxicol*. 2015; 45:245–272. [PubMed: 25629923]
- Hilal SH, Karickhoff SW, Carreira LA. Prediction of the solubility, activity coefficient and liquid/liquid partition coefficient of organic compounds. *QSAR Combinat Sci*. 2004; 23:709–720.
- Ingersoll CG, MacDonald DD, Brumbaugh WG, Johnson BT, Kemble NE, Kunz JL, May TW, Wang N, Smith JR, Sparks DW, Ireland DS. Toxicity assessment of sediments from the Grand Calumet River and Indiana Harbor Canal in northwestern Indiana, USA. *Arch Environ Contam Toxicol*. 2002; 43:156–167. [PubMed: 12115041]
- Johnson, BT. Microtox® toxicity test. In: Blaise, C.; Ferard, JF., editors. *Small-scale Freshwater Environmental Toxicity Test Methods*. Kluwer Academic; Dordrecht, The Netherlands: 2005. p. 1-39.
- Kawano M, Hasegawa J, Enomoto T, Onishi H, Nishio Y, Matsuda M, Wakimoto T. Hydroxylated polychlorinated biphenyls (HO-PCBs): Recent advances in wildlife contamination study. *Environ Sci*. 2005; 12:315–24. [PubMed: 16609671]
- Kemble NE, Hardesty DG, Ingersoll CG, Johnson BT, Dwyer FJ, MacDonald DD. An evaluation of the toxicity of contaminated sediments from Waukegan Harbor, Illinois, following remediation. *Arch Environ Contam Toxicol*. 2000; 39:452–461.
- Lehmler HJ, Robertson LW. Synthesis of hydroxylated PCB metabolites with the Suzuki-coupling. *Chemosphere*. 2001; 45:1119–1127. [PubMed: 11695625]
- Letcher, RJ.; Klasson-Wehler, E.; Bergman, A. Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. In: Hutzinger, O.; Paasivirta, J., editors. *Earth and Environmental Science*, vol. 3, *Anthropogenic Compounds*. Springer; Berlin, Germany: 2000. p. 315-359.
- Mandalakis M, Berresheim H, Stephanou E. Direct evidence for destruction of polychlorobiphenyls by HO radicals in the subtropical troposphere. *Environ Sci Technol*. 2003; 37:542–547. [PubMed: 12630470]
- Marek R, Martinez A, Hornbuckle K. Discovery of hydroxylated polychlorinated biphenyls (HO-PCBs) in sediment from a Lake Michigan waterway and original commercial Aroclors. *Environ Sci Technol*. 2013; 47:8204–8210. [PubMed: 23862721]
- Montano M, Gutleb A, Murk A. Persistent toxic burdens of halogenated phenolic compounds in humans and wildlife. *Environ Sci Technol*. 2013; 47:6071–6081. [PubMed: 23635024]
- Organisation for Economic Co-operation and Development–OECD/OCDE. OECD guidelines for the testing of chemicals. Vol. 221. OECD; Paris, France: 2006 Mar 23. *Lemna* sp. growth inhibition test.
- Parnell JJ, Park J, Denev V, Tsoi T, Hashsham S, Quensen J, Tiedje JA. Coping with polychlorinated biphenyl (PCB) toxicity: Physiological and genome-wide responses of *Burkholderia xenovorans* LB400 to PCB-mediated stress. *Appl Environ Microbiol*. 2006; 72:6607–6614. [PubMed: 17021212]
- Pieper DH, Seeger M. Bacterial metabolism of polychlorinated biphenyls. *J Mol Microbiol Biotechnol*. 2008; 15:121–138. [PubMed: 18685266]
- Rufli H, Fisk PR, Girling AE, King JMH, Lange R, Lejeune X, Stelter N, Stevens C, Suteau P, Tapp J, Thus J, Versteeg DJ, Niessen HJ. Aquatic toxicity testing of sparingly soluble, volatile, and unstable substances and interpretation and use of data. *Ecotoxicol Environ Saf*. 1998; 39:72–77. [PubMed: 9515079]
- Shen KL, Shen CF, Lu Y, Tang XJ, Zhang CK, Chen XC, Shi JY, Lin Q, Chen YX. Hormesis response of marine and freshwater luminescent bacteria to metal exposure. *Biol Res*. 2009; 42:183–187. [PubMed: 19746263]
- Sietmann R, Gesell M, Hammer E, Schauer F. Oxidative ring cleavage of low chlorinated biphenyl derivatives by fungi leads to the formation of chlorinated lactone derivatives. *Chemosphere*. 2006; 64:672–685. [PubMed: 16352329]
- Sondossi M, Sylvestre M, Ahmad D, Masse R. Metabolism of hydroxybiphenyl and chloro-hydroxybiphenyl by biphenyl/chlorobiphenyl degrading *Pseudomonas testosteroni*, strain B-356. *J Ind Microbiol*. 1991; 7:77–88.

- Takeuchi S, Shiraishi F, Kitamura S, Kuroki H, Jin K, Kojima H. Characterization of steroid hormone receptor activities in 100 hydroxylated polychlorinated biphenyls, including congeners identified in humans. *Toxicology*. 2011; 289:112–121. [PubMed: 21843587]
- Tampal N, Lehmler H-J, Espandiari P, Malmberg T, Robertson LW. Glucuronidation of hydroxylated polychlorinated biphenyls (PCBs). *Chem Res Toxicol*. 2002; 15:1259–1266. [PubMed: 12387623]
- Tehrani R, Van Aken B. Hydroxylated polychlorinated biphenyls in the environment: Sources, fate, and toxicities. *Environ Sci Pollut Res*. 2014; 21:6334–6345.
- Ueno D, Darling C, Alaei M, Campbell L, Pacepavicius G, Teixeira C, Muir D. Detection of hydroxylated polychlorinated biphenyls (HO-PCBs) in the abiotic environment: Surface water and precipitation from Ontario, Canada. *Environ Sci Technol*. 2007; 41:1841–1848. [PubMed: 17410773]
- Van Aken B, Correa PA, Schnoor JL. Phytoremediation of polychlorinated biphenyls: New trends and promises. *Environ Sci Technol*. 2010; 44:2767–2776. [PubMed: 20384372]

Fig. 1.

The effect concentration 50% (EC_{50} ; mg L⁻¹) was plotted as a function of the octanol-water partition coefficient (K_{ow}) for different groups of mono-HO congeners. The regression lines (solid line), the confidence intervals at 95% (dashed curves), the regression line equations, and the coefficients of determination (R^2) are presented. The error bars represent the standard deviations of EC_{50} . **Panel A** shows the plots of all 23 mono-HO congeners under study. **Panel B** shows the plots of the 2-HO congeners. **Panel C** shows the plots of the 3-HO congeners. **Panel D** shows the plots of the 4-HO congeners.

Toxicity of PCBs and HO-PCBs determined using the Microtox[®] assay. The effect concentration 50% (EC₅₀) after 5 min and 15 min (confidence intervals at 95%), coefficients of determination (R²), and octanol-water partition coefficients (K_{ow}) are presented

Table 1

PCBs and HO-PCBs	Abbreviation	EC50 (CI 95%) (mg L ⁻¹)		R ²	K _{ow}	
		5 min	15 min		5 min	15 min
3-Chlorobiphenyl	3-CB	13.1 (7.5–22.7)	45.7 (8.1–257)	0.95	0.81	4.59
4-Hydroxy-3-	4-HO-3-CB	0.92 (0.75–1.14)	1.15 (0.92–1.44)	0.99	1.00	4.49
4-Chlorobiphenyl	4-CB	ND ^a	ND			4.60
2'-Hydroxy-4-	2'-HO-4-CB	7.30 (4.19–12.7)	9.39 (3.44–25.7)	0.98	0.90	4.28
3'-Hydroxy-4-	3'-HO-4-CB	3.41 (2.44–4.77)	4.34 (2.43–7.75)	1.00	0.99	4.49
4'-Hydroxy-4-	4'-HO-4-CB	4.79 (4.08–5.62)	6.94 (5.36–8.98)	1.00	1.00	4.50
3-Hydroxy-4-	3-HO-4-CB	6.34 (2.17–18.5)	5.69 (3.01–10.8)	0.93	0.98	4.38
2,4'-Dichlorobiphenyl	2,4'-DCB	2.64 (1.15–6.06)	16.1 (6.77–38.5)	0.98	0.87	5.11
4-Hydroxy-2,4'-	4-HO-2,4'-DCB	0.07 (0.06–0.08)	0.09 (0.05–0.16)	1.00	0.99	5.11
2,5-Dichlorobiphenyl	2,5-CDB	14.5 (5.57–38.0)	28.5 (11.5–70.5)	0.85	0.90	5.20
2'-Hydroxy-2,5-	2'-HO-2,5-DCB	6.75 (3.81–12.0)	6.44 (3.05–13.6)	0.98	0.96	4.88
3'-Hydroxy-2,5-	3'-HO-2,5-DCB	3.01 (0.40–22.4)	2.92 (2.45–3.49)	0.90	1.00	5.11
4'-Hydroxy-2,5-	4'-HO-2,5-DCB	0.18 (0.10–0.30)	0.25 (0.10–0.63)	0.99	0.96	5.11
3,4-Dichlorobiphenyl	3,4-DCB	ND	ND			5.19
4'-Hydroxy-3,4-	4'-HO-3,4-DCB	0.36 (0.18–0.72)	0.41 (0.04–4.25)	0.98	0.82	5.10
2,4,6-Trichlorobiphenyl	2,4,6-TCB	ND	ND			5.97
2'-Hydroxy-2,4,6-	2'-HO-2,4,6-TCB	4.82 (0.81–28.8)	5.46 (1.02–29.2)	0.86	0.86	5.58
3'-Hydroxy-2,4,6-	3'-HO-2,4,6-TCB	9.09 (5.82–14.2)	9.77 (3.62–26.3)	0.98	0.89	5.87
4'-Hydroxy-2,4,6-	4'-HO-2,4,6-TCB	21.6 (16.6–28.0)	19.2 (10.2–36.3)	0.99	0.93	5.88

PCBs and HO-PCBs	Abbreviation	EC50 (CI 95%) (mg L ⁻¹)		R ²		K _{ow}
		5 min	15 min	5 min	15 min	
3,3',4'-Trichlorobiphenyl	3,3',4'-TCB	ND	ND			5.66
2'-Hydroxy-3,4,5'-	2'-HO-3,4,5'-TCB	0.34 (0.31–0.37)	0.41 (0.04–4.38)	1.00	0.61	5.45
4'-Hydroxy-3,3',4'-	4'-HO-3,3',4'-TCB	11.2 (8.94–14.1)	8.57 (6.35–11.6)	0.99	0.99	5.48
3,3',5'-Trichlorobiphenyl	3,3',5'-TCB	ND	ND			5.65
2'-Hydroxy-3,5,5'-	2'-HO-3,5,5'-TCB	0.48 (0.31–0.72)	0.35 (0.25–0.48)	0.98	0.99	5.44
4'-Hydroxy-3,3',5'-	4'-HO-3,3',5'-TCB	1.20 (0.89–1.62)	0.94 (0.59–1.52)	0.99	0.98	5.45
3,4',5'-Trichlorobiphenyl	3,4',5'-TCB	ND	ND			6.65
4-Hydroxy-3,4',5'-	4-HO-3,4',5'-TCB	2.57 (1.62–4.10)	1.29 (0.71–2.34)	0.97	0.95	5.46
2,3,4,5'-Tetrachlorobiphenyl	2,3,4,5'-TeCB	ND	ND			6.55
2'-Hydroxy-2,3,4,5'-	2'-HO-2,3,4,5'-TeCB	1.05 (1.00–1.10)	0.80 (0.38–1.68)	1.00	0.90	6.24
3'-Hydroxy-2,3,4,5'-	3'-HO-2,3,4,5'-TeCB	33.3 (11.2–98.6)	18.3 (13.8–24.2)	0.88	0.99	6.46
4'-Hydroxy-2,3,4,5'-	4'-HO-2,3,4,5'-TeCB	133 (75.8–234)	73.2 (23.0–233)	0.99	0.95	6.46
2,3',4,5'-Tetrachlorobiphenyl	2,3',4,5'-TeCB	ND	ND			6.29
4'-Hydroxy-2,3',4,5'-	4'-HO-2,3',4,5'-TeCB	31.0 (19.9–48.4)	10.3 (6.97–15.3)	0.98	0.98	6.11
3,3',4,5'-Tetrachlorobiphenyl	3,3',4,5'-TeCB	ND	ND			6.26
4'-Hydroxy-3,3',4,5'-	4'-HO-3,3',4,5'-TeCB	60.6 (29.6–124)	12.5 (8.59–18.2)	0.97	0.98	6.09

^aND: Not determined.