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Hydroxylated Polychlorinated Biphenyls (OH-PCBs): Recent Advances in Wildlife Contamination Study

Masahide Kawano*, Jun Hasegawa, Takeshi Enomoto, Hisao Onishi, Yu Nishio, Muneaki Matsuda and Tadaaki Wakimoto

Department of Environment Conservation, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan

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The exposure of wildlife and human population to environmental hazardous contaminants has been of global concern for over several decades. More recently, focus has been directed towards potential toxicological effects such as the endocrine disrupting function of xenobiotics. The hydroxylated metabolites of PCBs (OH-PCBs) should be pointed out as these compounds. OH-PCBs have emerged as important classes of environmental contaminants in wildlife and humans because of their ability to bind with the thyroxin transport protein, transthyretin (TTR), and their interaction with thyroid hormone receptors. However, data on their occurrence in wildlife and their behavior in the matrices of environment are limited. Topics include the formation of OH-PCBs, their physicochemical properties (octanol-water partition coefficient, Kow), analytical procedures and contamination status in wildlife. The guidance for improving the study of OH-PCB contamination is also briefly mentioned.

1. Introduction

Large quantities of polychlorinated biphenyl (PCB) chemicals have been produced globally and dispersed all over the world, and almost all the organisms worldwide are contaminated by these chemicals.[1,2] PCBs are a type of persistent organic pollutant (POP). At present, the production and usage of PCBs have been banned and controlled by an international treaty, the so-called Stockholm Convention, due to their persistency in the environment and their toxic effects on organisms including humans.[3] Although PCBs are

*E-mail: mkawano@agr.ehime-u.ac.jp

still causing environmental problems for humans and wildlife, a relatively new class of compounds containing a halogen group and at least one hydroxyl group have attracted a great deal of scientific interest in the field of environmental contamination research. Such compounds are mainly the metabolites and products of POPs. The analysis of POPs in environmental samples has so far been focused on detecting parent compounds because of their persistence and toxicities; however, recent research has been mainly focused on their metabolites and their toxicological effects, especially the degradation products of PCBs. The hydroxylated (OH-) metabolites of PCBs have emerged as an important class of environmental contaminants in humans and wildlife.[4,5]

The occurrence of OH-PCBs was first found in the feces of Baltic guillemot (*Uria algae*) and grey seal (*Halichoerus grypus*) species in the mid-1970s,[6] the dawn of the environmental chemical pollution age. This was an important discovery, which occurred about ten years after parent PCB contamination was found in wildlife. The hydroxylated metabolites of PCBs are believed to be rapidly excreted from the body and/or are degraded to water-soluble conjugated metabolites. However, more recently OH-PCBs have attracted a great deal of scientific attention because of the findings that certain OH-PCBs can bind to the thyroxine transport protein transthyretin (TTR), and that they interact with thyroid and estrogen hormone receptors.[7–9] In the present review, we focus on recent advances in the understanding of the physicochemical properties, octanol-water partition coefficient (log Kow), analytical methods and occurrence of OH-PCBs in the environment and wildlife.

2. Formation of OH-PCBs in Organisms

The introduction of the OH group into the aromatic ring of the parent PCB congener can occur either by direct insertion in the ring or by arene epoxide formation and subsequent epoxide hyrolase-mediated ring opening with or without an intermolecular 1,2-shift (NIH-shift) of H and Cl atoms.[4,10] The formation of an intermediate arene oxide is catalyzed by cytochrome P450s (CYPs), for example, CYP1A, CYP2B, probably CYP2C, and CYP3A enzymes.[4] OH-PCBs are susceptible to further metabolism via a conjugation reaction with glucuronic acid or sulfate, which increases the water solubility of the compound and facilitates its excretion.[4] The formation of 4OH-PCB187 metabolites is shown in Fig. 1.

3. Physicochemical Properties

Understanding the physicochemical properties of OH-PCBs is important to elucidate their accumulation in water and their transfer to fish in the aquatic environment. Although OH-PCBs have been detected in the blood samples of some organisms this may be due to the degradation of parent PCBs to OH-PCBs by microorganisms in water, sediment and soil or those in higher organisms such as birds and mammals. A large number of low chlorinated OH-PCBs have been detected to date in water and sediment samples.[11,12]

The log Kow data of OH-PCBs are insufficient because of the lack of commercially available standards for many of them. Tampal *et al.*[13] reported the log Kow of OH-PCB congeners with one to seven chlorine atoms. The results indicate that the higher the number of chlorine atoms substituted in the biphenyl ring, the higher the log Kow value. The log Kow values of heptachlorinated OH-PCBs are around six,[13] which is roughly one order of

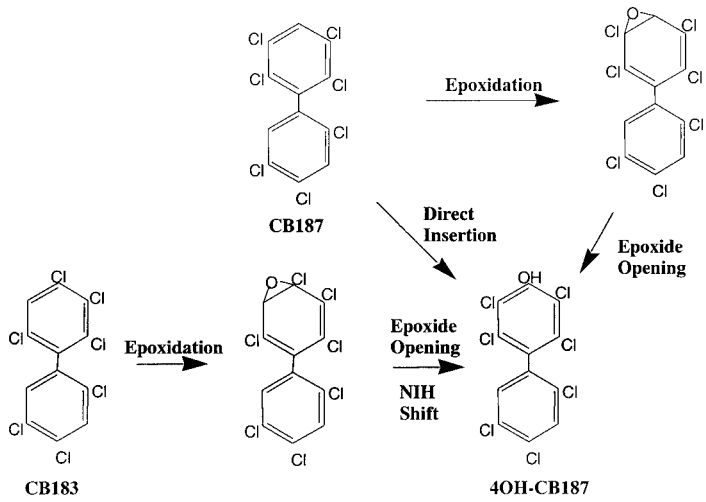

Fig. 1. Metabolic formation of 4OH-CB187 from CB187 and CB183.

magnitude lower than the values of the respective parent PCBs.[13,14] Although the log Kow value is one order lower than that of the corresponding parent PCBs, OH-PCBs also have a potential to accumulate in water and be transferred to aquatic organisms to a certain extent. Consequently, higher trophic organisms, such as waterfowl and other fish-eating organisms, accumulate OH-PCBs in two possible ways. The first is through food such as fish contaminated with OH-PCBs, and the second is through the metabolism of the parent PCBs by their metabolic enzymes such as CYPs that produce the corresponding OH-PCBs as metabolites.

4. Analytical Procedure

OH-PCB metabolites were found specifically in blood of organisms due to their relatively high water solubilities; consequently, blood is normally used as a target biological sample for evaluating the level of contamination by OH-PCBs.[4,5,15] Moreover, in order to understand the processes involved in the formation and excretion of OH-PCBs, estimating the levels of the parent PCBs is also important. Accordingly, simultaneous analytical methods for detecting OH-PCBs and parent PCBs are necessary and indispensable.

The extraction of OH-PCBs and PCBs from the blood samples generally entails a simple liquid-liquid partition of whole blood, plasma, or serum with organic solvents after adjustment of the pH of the blood samples. This analytical procedure was used by Jansson *et al.*[6] in their earlier work. Since then, Bergman *et al.*[15] and Hovander *et al.*[16] have basically used this analytical method with a slight modification for the analysis of a part of relatively highly chlorinated OH-PCB congeners. Therefore, this method is not for the analysis of almost all OH-PCB congener residues in organisms as the target compounds. In short, in the present study, blood sample was diluted with water and a polar organic solvent. The diluted sample was acidified and then extracted with organic solvents such as hexane/methyl *tert*-butyl ether (MTBE). The combined organic phase was concentrated and lipid

content was determined gravimetrically from an aliquot of the sample. The remaining extract was then redissolved in hexane and then partitioned into neutral (Extraction 1) and phenolic substances (Extraction 2) using aqueous potassium hydroxide. Extraction 1 was transferred to a sulfuric acid-impregnated silica gel column for the elimination of lipid residues. The first eluate with hexane was collected for parent PCBs analysis. The phenolic compounds such as OH-PCBs in Extraction 2 were methylated using diazomethane. Lipid residues in Extract 2 were removed by hexane/concentrated sulfuric acid partitioning. The hexane phase was transferred to a sulfuric acid-impregnated silica gel column and methylated phenolic PCBs were eluted with dichloromethane. A gas chromatograph (GC) equipped with an electron capture detector (ECD) was used for qualitative and quantitative analysis. High resolution (HR)GC-low resolution (LR) mass spectrometry (MS) quantification was performed for only one pooled sample.[15] Liquid-liquid partitioning strategies take advantage of the polarity and acidic character of OH-PCBs for separation from nonpolar substances such as parent PCBs and other xenobiotics.

We have recently developed a conventional method for the simultaneous analyses of OH-PCBs and parent PCBs by high resolution (HR)GC coupled with HR (resolution number 10,000 or more) MS.[17,18] HRGC/HRMS is useful for congener specific and highly sensitive analyses and is certified and widely used for PCDD/Fs analysis. The method was used as follows: isopropyl alcohol was added and then acidified by adding hydrochloric acid to the blood sample. OH-PCBs and parent PCBs were extracted using a mixture of dichloromethane and hexane (50%, v/v). The lipids were removed by shaking the combined extract with concentrated sulfuric acid. The acid degrades lipids and other interfering molecules, leaving behind the relatively stable OH-PCBs as well as PCBs. Silica gel (5% water w/w, deactivated) was used to separate parent PCBs as the first eluate (Fraction 1) using heaxne, from OH-PCBs as the second eluate (Fraction 2) using dichloromethane/hexane. Heat-activated aluminum oxide was used for a further cleanup of Fraction 1 with dichloromethane/hexane as the mobile phase. Prior to the cleanup of Fraction 2 with silica gel impregnated with sulfuric acid (44%, w/w), the concentrated solution of the fraction was treated with an excess of diazomethane for the methylation of OH-PCBs. Finally, OH-PCBs in the solutions were quantified using HRGC/HRMS.

The cleanup technique using alkaline digestion after diazomethane derivatization developed by Okumura[19] has a good efficiency for the elimination of interfering substances. This procedure was used for sediment analysis by Sakiyama *et al.*[12]

Instrumental analysis by HRGC/HRMS provides highly reliable data on concentrations (trace levels) as well as congener-specific analysis; consequently, we propose that HRGC/HRMS analysis can be used widely for OH-PCBs analysis.

5. Occurrence in Environment

The first study of OH-PCBs in wildlife was published in the mid-1970s by Jansson *et al.*,[6] which examined the presence of OH-PCBs in the droppings of seals and seabirds. The presence of these compounds has been determined in wildlife such as fish, marine mammals and birds, and also in humans. Only a limited number of OH-PCB congeners and species of wildlife have been investigated so far. OH-PCB congeners identified to date in the biota

are briefly summarized in Table 1.

More than 60 congeners of OH-PCBs have been detected in fish blood.[11] They could only be identified quantitatively because of the lack of commercially available standards. Consequently, many constituents detected in the biota could not be identified because of the same reason. As shown in Table 1, there are many OH-PCB congeners in fish plasma.[11,20,21] The number of OH-PCB congeners detected in bird blood is limited such that the number is not as high as the number detected in fish plasma. It is possible to safely say that the majority of OH-PCBs in birds are formed from the limited number of PCBs that persistently remain.

It seems that the OH-PCB congener profiles determined in biological samples were different among species, such as fish, birds, marine mammals and humans. In this context, it is important to consider the relationships between OH-PCBs and parent PCBs. The total concentrations of the OH-PCBs in fish of the Great Lakes were relatively low compared with ∑PCBs (<0.01%).[20] OH-PCB residue concentrations in marine mammals such as whales were also relatively low.[22, 23] On the other hand, the ratios, ∑OH-PCBs/∑PCBs, in terrestrial mammals and birds are relatively high, indicating their high metabolic capacity for the formation of OH-PCBs and retention in blood. Such possibilities in the species-specific formation of OH-PCB congeners in the biota have been estimated.[4] Many studies have already shown that 4OH-CB187 is one of the major congeners in biotic organisms. For example, 4OH-CB187 is the major congener in birds[24–26] as well as in polar bears.[27,28] Furthermore, 4OH-CB146 is the dominant congener in seabirds collected from the North Pacific Ocean.[24] The 4OH-PCB146 and 4OH-PCB187 congeners may possess relatively strong affinities for thyroid hormone transport proteins (TTRs), as they mimic the natural substrate T4.[4,7,29] Sandala *et al.*[28] found that the total concentration of OH-PCBs in the blood samples of polar bears was higher than that of any other contaminant examined in their studies (including PCBs, CHLs, DDTs, chlorobenzenes, HCHs, Mirex, dieldrin and octachlorostyrene).

OH-PCBs were also detected in water[11] and sediment samples.[12] A large number of OH-PCB congeners were identified in the sediment samples from Osaka Bay, Japan (Table 2). Additionally, four unidentified hydroxylated trichlorobiphenyls (OH-TriCBs), one unidentified OH-TetraCB and nine OH-PentaCBs were found in the same samples. Poorly chlorinated OH-PCBs are the predominant contaminants of the sediment samples. This may be because low chlorinated OH-PCBs are formed easily from the parent PCBs by microorganisms in the sediment environment.[30] Consequently, some of them dissolve in water and accumulate in aquatic organisms such as fish depending on their congener-specific physicochemical properties such as a log Kow value.

6. Perspectives of Study on Environmental Contamination by OH-PCBs

A large number of organohalogen compounds have been known to accumulate in lipid-rich tissues and organs due to their lipophilic properties and persistency. The majority of the compounds identified in the environmental samples are neutral. On the other hand, the phenolic halogenated compounds such as OH-PCBs are water-soluble; therefore, it was believed that they do not accumulate in the biota. Consequently, less concern was shown

Table 1
Concentration (ng/g wet weight) of hydroxylated biphenyls in wildlife.

Species		Location	No of Congeners	4OH-CB107	4'OH-CB130	3'OH-CB138	4OH-CB146	3OH-CB153	4OH-CB172	4OH-CB187	ΣOH-PCB	ΣPCB	Reference
		Year	Detected	Ave. Range	Ave. Range	Ave. Range	Ave. Range	Ave. Range	Ave. Range	Ave. Range	Ave. Range	Ave. Range	Author (Ref. No.)
Fish													
Brown bullhead	Plasma (*n*=N.A.)	South Ontario 2002	>60	N.A.[*1] N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	Darling *et al.* 2004 (11)
Lake trout	Plasma (*n*=5)	Lake Ontario 2000–2001	11	0.0049 0.0017–0.008	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	0.1045[*2] 0.0371–0.16	N.A. N.A.	511 37–1,246	Campbell *et al.* 2003 (20)
Large-mouth bass	Plasma (*n*=3)	Detroit River 2001	N.A.	0.31 0.19–0.51	0.18 <0.001–0.48	2.94 <0.001–8.76	N.A. N.A.	N.A. N.A.	N.A. N.A.	5.62 0.34–16.19	11.33 1.4–30.6	163.68 118.6–210.6	Li *et al.* 2003(21)
Mammal													
Grey seal	Blood coagulate (*n*=5)	Baltic Sea 1992	13	1,500 90–1,700	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	30,000 7,900–83,000	Bergman *et al.* 1994 (15)
Ringed seal	Plasma (*n*=5)	Kuujjuaq, Canada 1997	N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	0.081 N.A.	27.1 N.A.	Sandau *et al.* 2000 (27)
Polar bear	Whole blood (*n*=19)	East Greenland 1999–2001	23[*3]	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	182.3 93.5–382.1	46.1 12.6–204.2	Sandala *et al.* 2004 (28)
Polar bear	Plasma (*n*=30)	Rasolute Bay, Canada 1997	37	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A.[*4]	92.6 26.4–576	46.9 16.1–161	Sandau *et al.* 2000 (27)
Bowhead whale	Plasma (*n*=20)	Barrow, Alaska 1997–2000	2	N.A. N.A.	0.94 0.32–3.29	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	0.39 0.31–2.39	1.52 0.53–3.8	2.78 1.09–9.37	Hoekstra *et al.* 2003 (22)
Killer whale	Whole blood (*n*=1)	Vancouver, Canada N.A.	>5	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.	2.041 N.A.	12.5 N.A.	Bennett *et al.* 2002 (23)

Species		Location Year	No of Congeners Detected	4OH-CB107 Ave. Indiv. data	4'OH-CB130 Ave. Indiv. data	3'OH-CB138 Ave. Indiv.data	4OH-CB146 Ave. Indiv. data	3OH-CB153 Ave. Indiv. Data	4OH-CB172 Ave. Indiv. Data	4OH-CB187 Ave. Indiv. Data	ΣOH-PCB Ave. Range	ΣPCB Ave. Range	Reference Author (Ref. No.)
Bird													
Osprey	egg	Svelvik., Os ;	13	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	Berger
	(*n*=2)	Hedmark, Norwey		N.A.	N.A.	N.A.	0.016	N.A.	N.A.	0.057	N.A.	N.A.	*et al.*
		1994, 2000											2004 (26)
Golden	egg	Os ; Hedmark,	4	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	Berger
eagle	(*n*=2)	Selbu, Norwey		N.A.			N.A.	0.012	N.A.	0.022	N.A.	N.A.	*et al.*
		1998, 2000											2004 (26)
White-	egg	Vikna, Lødingen,	16	N.A.	N.A.	N.A.	0.022	N.A.	N.A.	0.081	N.A.	N.A.	Berger
tailed	(*n*=2)	Norwey		N.A.	N.A.	N.A.	0.018, 0.026	N.A.	N.A.	0.061, 0.100	N.A.	N.A.	*et al.*
sea eagle		1996, 2000											2004 (26)
Peregrine	egg	Telemark,	30	0.0185	0.039	0.051	0.247	0.063	0.062	0.371	N.A.	N.A.	Berger
falcon	(*n*=2)	Nærøy		0.016, 0.021	0.026, 0.052	0.033, 0.0 68	0.171, 0.322	0.036, 0.090	0.045, 0.079	0.353, 0.388	N.A.	N.A.	*et al.*
		1995, 2000											2004 (26)

Species		Location Year	No. of Congeners Detected	4OH-CB107 Ave. Range	4'OH-CB130 Ave. Range	3'OH-CB138 Ave. Range	4OH-CB146 Ave. Range	3OH-CB153 Ave. Range	4OH-CB172 Ave. Range	4OH-CB187 Ave. Range	ΣOH-PCB Ave. Range	ΣPCB Ave. Range	Reference Author (Ref. No.)
Bird													
Laysan	Plasma	Midway	7	N.A.	N.A.	N.A.	N.A.[*5]	N.A.	N.A.	N.A.[*5]	11.5	18.3	Klasson-
albatross	(*n*=5)	Atoll		N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	5.9–18.5	13.7–22.3	Wehler
		1993–1994											1998 (24)
Black-	Plasma	Midway	6	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	27.1	83.8	Klasson-
footed	(*n*=5)	Atoll		N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	20–33	42–160	Wehler
albatross		1993–1994											1998 (24)
White-	Whole	Swedish	26[*6]	N.A.	N.A.	N.A.	0.89	N.A.	N.A.	2.8	N.A.	95	Olsson
tailed	blood	Baltic coast		N.A.	N.A.	N.A.	0.74–1.1	N.A.	N.A.	2.3–3.4	N.A.	83–110[*7]	*et al.*
sea eagle	(*n*=15)	1996–1998											2000 (25)

[*1]: Data not available
[*2]: Most abundant OH-PCB congener measured
[*3]: Twenty-one OH-PCB congeners and two dihydroxylated congeners. 4'OH-CB120 and 4OH-CB187 were major congeners, which on average contributed approximately 30% to the total OH-PCBs
[*4]: Major OH-PCB congener
[*5]: 4OH-CB146 and 4OH-CB187 accounted for greater mass, 70 to 90% of the total OH-PCBs
[*6]: 24 OH-PCB congeners and two dihydroxylated congeners
[*7]: 95% confidence interval for 15 samples

Table 2
OH-PCBs identified in sediment samples from Osaka Bay, Japan (Sakiyama *et al.*, 2004).

OH-Tri CB	OH-Tetra CB	OH-Penta CB
5'-OH-CB18	3'-OH-CB42	5-OH-CB110
5'-OH-CB17	3 -OH-CB68	
3'-OH-CB18	4'-OH-CB49	
3'-OH-CB17	2'-OH-CB79	
2'-OH-CB35	4'-OH-CB68	
4'-OH-CB25	4'-OH-CB44	
5'-OH-CB33	4'-OH-CB72	
4 -OH-CB39	4 -OH-CB72	
3'-OH-CB33		
6'-OH-CB26		
4'-OH-CB18		
3 -OH-CB28		
5 -OH-CB25		
4 -OH-CB26		
5 -OH-CB28		
4 -OH-CB31		
4'-OH-CB26		
4'-OH-CB33		
4'-OH-CB20		
4'-OH-CB35		

for them from the viewpoint of environmental contamination.[4,5] However, a growing number of studies have shown that OH-PCBs are retained in blood of wildlife and humans. Moreover, it has been suggested that they possess endocrine-disrupting activity; in particular, they have been shown to interfere with the transport of thyroid hormones by competitive inhibition of TTR binding; their chemical structures are similar to the thyroid hormone thyroxine (T4).[4,7,29] OH-PCBs and other phenolic halogenated compounds are apparently different from other persistent organic compounds in their neutral behavior as well as in their environmental behavior and toxicological effects on organisms. In particular, phenolic halogenated compounds combine easily with compounds produced in glands and organs and cause toxicological effects. Furthermore, as they have halogen atoms in their biphenyl rings, they may be persistent as other organohalogen compounds. It has been found that poorly chlorinated OH-PCBs are predominant in the aquatic environment, particularly in sediments[12]; although highly chlorinated OH-PCBs may be predominant in higher trophic organisms such as birds and humans. From the viewpoint of environmental chemistry and the limited data so far available, environmental matrices (water, air, soil and sediment) as well as wildlife should be analyzed.

Some published literatures show that OH-PCB exposure at the fetal development stage of organisms can cause serious effects on brain development.[29,31] This is caused by the disruption of hormonal systems that control brain development. On the basis of these findings, we suggest that it is essential to investigate in detail the toxicological and physiological effects, and behavioral fate of these compounds in wildlife and humans.

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