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Accumulation Features of Hydroxylated-PCBs (OH-PCBs) in the Blood of Pigs Collected from a Dumping Site for Municipal Wastes in India

Hazuki MIZUKAWA[1], Kei NOMIYAMA[1], Tatsuya KUNISUE[2], Michio X. WATANABE[1], Annamalai SUBRAMANIAN[1], Shin TAKAHASHI[1] and Shinsuke TANABE[1]

[1]*Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan*
[2]*Wadsworth Center, NYS Department of Health, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509, U.S.A.*

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Abstract—Concentrations of hydroxylated polychlorinated biphenyls (OH-PCBs) and PCBs were analyzed in the blood of pigs collected from a dumping site (DS) for municipal wastes in India. OH-PCB concentrations in the blood of pigs from DS were significantly higher than those from control site (CS), although no significant difference was found in PCB concentrations. Predominant isomers were CB153, CB180 and CB187 for PCBs, and 4OH-CB107, 4OH-CB162 and 4OH-CB146 for OH-PCBs. These results were different from accumulation patterns reported previously in other mammals and birds. It could be due to species-specific metabolic capacity by phase I cytochrome P450 (CYP) enzymes and/or phase II conjugation enzymes and binding affinity to TTR in different animals. Concentrations of penta- through hepta-OH-PCB congeners in piglet were higher than those in the mother, indicating that these congeners were transferred to the piglet through the placenta. Mean OH-PCBs/PCBs ratio in the blood of pigs was comparable to those in the blood of carnivorous animals, but higher than in human blood samples. Moreover OH-PCBs/PCBs ratios of pigs from DS were higher than those from CS. These observations imply that the hepatic metabolism in pigs from DS was accelerated by the CYP enzymes induced by higher exposure to PCBs and dioxins.

Keywords: hydroxylated-PCBs (OH-PCBs), pig, India, thyroid hormone

INTRODUCTION

Polychlorinated biphenyls (PCBs) are reported to cause variety of toxic effects (Safe, 1994). Especially, the neurotoxicity and thyroid hormone (TH) disturbance are considered to be caused by the hydroxylated PCB metabolites (OH-PCBs). The OH-PCBs are formed by oxidative metabolism of PCBs by cytochrome P450 monooxygenases (CYP) in the liver after PCBs were ingested into the body. The introduction of hydroxyl groups in the PCB molecules increases their polarities

and facilitates excretion from the body. If the hydroxyl group is in the *para*-position of a biphenyl structure and has adjacent chlorine atoms, the structure resembles thyroxin (T4). OH-PCBs and T4 are known to competitively bind with transthyretin (TTR) which is a TH transport protein, and thus TH homeostasis is possibly disrupted by OH-PCBs. The OH-PCBs have been detected in the blood, liver and brain of various marine and terrestrial mammals and birds (Kunisue *et al*., 2007; Gebbink *et al*., 2008; Jaspers *et al*., 2008; Kunisue and Tanabe, 2009). These reports clearly indicate that the capacity to metabolize PCBs the resulting OH-PCB congener patterns differ widely among animal species. Particularly, OH-PCBs levels were found to be higher than parent PCBs in the blood of some terrestrial mammals (Kunisue and Tanabe, 2009); the toxicological risk of OH-PCBs to terrestrial mammals might be high. However few studies have analyzed OH-PCBs in terrestrial mammals, and accumulation features of these compounds are not well known.

Huge amounts of industrial wastes are dumped in open dumping areas in India, leading to contamination by PCBs, dioxins and related compounds (DRCs). At the open waste DS, pigs feed on garbage probably with a high accumulation of PCBs and other contaminants; as a result CYPs drug-metabolizing enzymes are induced in the liver resulting in the production of OH-PCBs. Moreover, we presume that domestic swine might be a potential source of contaminants for near by residents who consume the meat as food.

The present study elucidates the contamination status and accumulation characteristics of PCBs and the metabolites, OH-PCB by analyzing the blood samples of pigs collected from DS and CS in India.

MATERIALS AND METHOD

Sample

The blood samples (n = 22) of pigs (*Sus scrofa domesticus*) were collected from the dumping site at Chennai city (DS) and Chidambaram (CS) in India during December 2002. The sample set consisted of fourteen individuals (five males and nine females) in DS and eight individuals (four males and four females) in CS. Whole blood samples were used for chemical analysis of PCBs and OH-PCBs. These samples were stored in Environmental Specimen Bank (*es*-BANK) for Global Monitoring of Ehime University, Japan. The whole blood samples were kept at –25°C until chemical analysis.

Chemical analysis

Whole blood samples (10 g) were homogenized with 2-propanol and 50% methyl *t*-butyl ether (MTBE)/hexane. The extracts were washed with 5% NaCl in hexane-washed water. $^{13}C_{12}$-labeled OH-PCBs (4OH-T_3CB29, 4′OH-T_4CB61, 4OH-T_4CB79, 4′OH-P_5CB120, 4OH-P_5CB107, 4′OH-H_6CB159, 4OH-H_6CB146, 4′OH-H_7CB172, 4OH-H_7CB187) and PCBs (CB28, 52, 95, 101, 105, 118, 138, 153, 156, 157, 167, 170, 178, 180, 189, 194, 202, 206, 208, 209) were spiked as

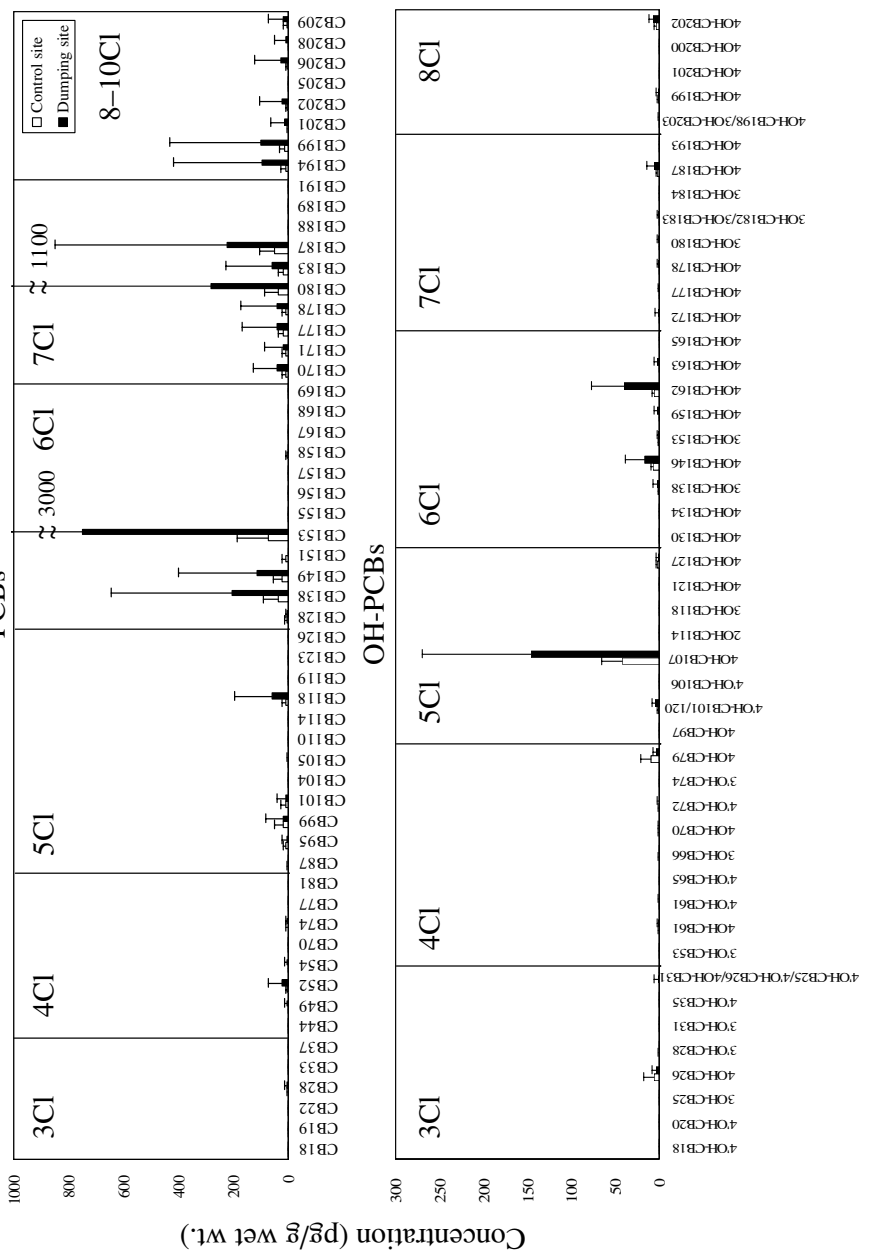


Fig. 1. Concentrations of PCB and OH-PCB isomers (pg/wet wt.) in the blood of pigs collected from India.

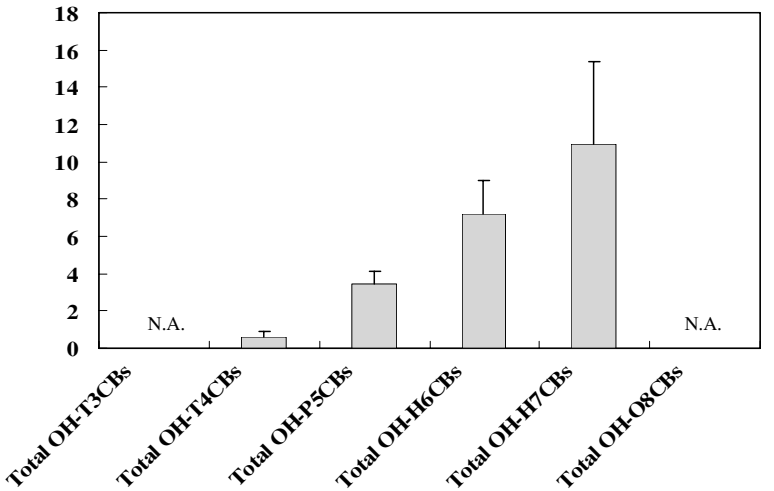


Fig. 2. Concentration ratios of OH-PCB congeners between piglets and mother. N.A.: No analysis was conducted because the concentration was below detection limit in mother.

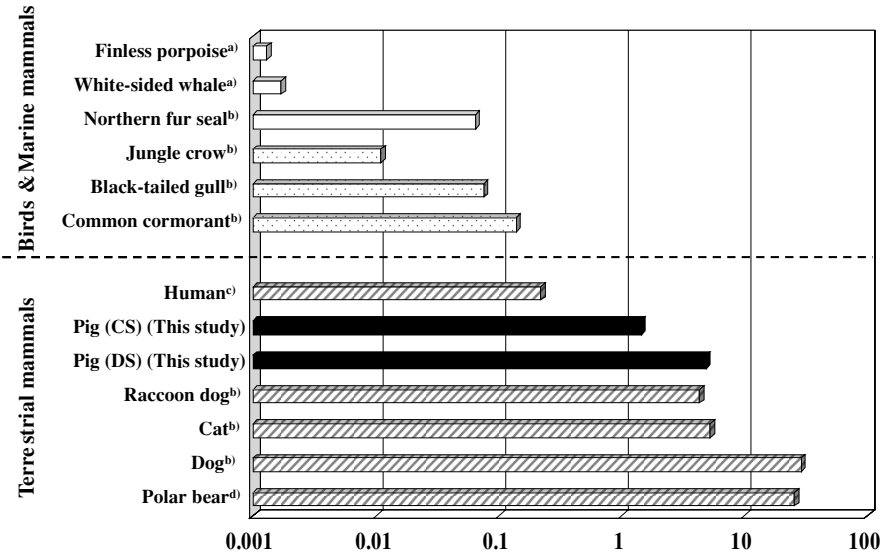
internal standards (IS). 1M KOH in 50% ethanol/water was added and shaken to partition. The fat in the organic phase containing PCBs was removed by gel permeation chromatography (GPC), and the extract was passed through activated silica-gel packed in a glass column. $^{13}C_{12}$-labeled PCBs (CB15, 70) were spiked as syringe spikes for PCBs analysis. The KOH solution phase containing OH-PCBs was passed through a column packed with inactivated silica-gel (5% H_2O deactivated) and concentrated. Then concentrated solution was derivatized overnight by using trimethylsilyldiazomethane. The fat in the derivatized solution was removed by GPC and passed through activated silica-gel packed in a column and concentrated nearly to dryness. $^{13}C_{12}$-labeled PCBs (CB77, 157) were spiked as syringe spikes for OH-PCBs.

Identification and quantification of PCBs and OH-PCBs were made using a gas chromatograph (GC: 6890 series, Agilent) coupled with high resolution mass spectrometer (HRMS: JMS-800D, JEOL) at a resolution of 10,000. GC/HRMS was equipped with a capillary column (DB-5MS for OH-PCBs and DB-1MS for PCBs, J&W Scientific) and operated in electron impact and selected ion monitoring mode (EI-SIM).

RESULTS AND DISCUSSION

Contamination status and accumulation patterns of PCBs and OH-PCBs

Mean concentrations of Σ_{62}PCBs in the blood of pig from CS and DS ranged from below the limit of quantification (LOQ) to 1,300 pg g^{-1} wet wt. and LOQ to



a) Nomiyama *et al*., 2009, b) Kunisue and Tanabe, 2009, c) Nomiyama *et al*., 2010, d) Gebbink *et al*., 2008

Fig. 3. Concentration ratios of OH-PCBs to PCBs in the blood of pigs from India and in human and wildlife blood.

23,000 pg g^{-1} wet wt., respectively. Among detected PCB isomers, CB153 was the highest, followed by CB180, CB187 and CB138 (Fig. 1). It was showed no difference of congener profiles between CS and DS. In this study, 24 OH-PCB isomers were identified in the blood of pigs. Mean concentrations of $\sum_{52}$OH-PCBs in the blood from CS and DS ranged from 34 to 200 pg g^{-1} wet wt. and 58 to 630 pg g^{-1} wet wt., respectively. In identified OH-PCB isomers, 4OH-CB107 concentration was the highest in the blood of pigs collected from both sites, followed by 4OH-CB162, 4OH-CB146 and 4OH-CB79 (Fig. 1). The OH-PCBs identified in pigs blood generally had a *para*-substituted OH-group with chlorine atoms on the adjacent carbons, and in the non-phenolic ring the chlorine atoms were present in *meta*- and *para*-positions. These OH-PCBs structures have high binding affinity to TTR in the blood because of their structural similarity to T4 (Lans *et al*., 1993; Brouwer *et al*., 1998; Cheek *et al*., 1999). OH-PCB levels in the blood from DS were significantly higher than those of CS ($p < 0.05$), although no significant difference of PCB concentrations was found between DS and CS. This result suggests that pigs from DS might have been exposed not only PCBs but also to dioxins and polycyclic aromatic hydrocarbon compounds (PAHs), hence had accelerated induction of drug-metabolizing enzymes (e.g. CYPs). The accumulation profiles of OH-PCB isomers observed in the blood of pigs was different from other mammals, birds and human (Kunisue *et al*., 2007; Gebbink *et al*., 2008; Jaspers *et al*., 2008; Kunisue and Tanabe, 2009; Nomiyama *et al*.,

2010a, b). It could be due to species-specific metabolic capacity by phase I CYP enzymes and/or phase II conjugation enzymes and binding affinity to TTR in different animals. Particularly, 4OH-CB107 distinctly remained in pigs' blood from India, suggesting that, this metabolite is difficult to excrete because of its high binding affinity to TTR.

Maternal transfer of OH-PCBs

Comparison of three piglets and their mother pig from DS showed that both PCBs and OH-PCBs levels in piglets were higher than in mother pig, suggesting that these compounds were transferred by cord blood from the mother pig to piglets in addition to metabolize of PCBs within piglets. When concentration ratios of OH-PCB homologues were estimated between piglets and mother pig (piglets/mother), hepta-chlorinated OH-PCBs showed the highest ratio (Fig. 2). This may be due to the easier transfer of higher-chlorinated OH-PCBs from mother to fetus through placenta and/or phase II conjugation enzymes in piglets may have low metabolic capacity. The higher piglet/mother concentration ratios were found for 4OH-CB107, 4OH-CB162 and 4OH-CB187. It is considered that these OH-PCB isomers could be easily bound to TTR cord blood. Especially, it is reported that increased levels of 4OH-CB107 significantly decreased fetal plasma T4 and FT4 levels during the gestation days (Meerts *et al.*, 2002), high transfer of OH-PCBs via placenta to piglet may have the adverse effects.

OH-PCBs/PCBs concentration ratios

When concentration ratio of OH-PCBs to PCBs was calculated, the ratio observed in the DS was largely higher than those of CS ($p > 0.05$) (Fig. 3). This result implies that the hepatic metabolism in pigs from DS was accelerated by the CYP enzymes as a result of induction by higher levels of dioxins and perfluorooctanesulfonate (PFOS) and also due to PAHs exposure (Watanabe *et al.*, 2010). The OH-PCBs/PCBs ratios observed in this study were similar to those of other terrestrial mammals (except for human) (Kunisue and Tanabe, 2009), but 1–3 orders of magnitude higher than those in human (Nomiyama *et al.*, 2010a), marine mammal (Nomiyama *et al.*, 2010b) and avian species (Kunisue and Tanabe, 2009), but lower than polar bear (Gebbink *et al.*, 2008) (Fig. 3). This suggests that pigs have high metabolic capacity by induction of drug-metabolizing enzymes (e.g. CYPs) similar to some carnivorous species such as cat, dog and raccoon dog.

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H. Mizukawa (e-mail: Hazuki.Mizukawa@gmail.com)