PCBs AND HYDROXYLATED PCB METABOLITES IN THE TISSUES OF WILD BIRDS AND FISH PREY FROM JAPAN

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Abstract
The residue profile of polychlorinated biphenyls (PCBs) and hydroxylated polychlorinated biphenyls (HO-PCBs) in the blood, liver and brain tissues of 3 wild bird species (grey heron *Ardea cinerea*, black kite *Milvus migrans*, great cormorant *Phalacrocorax carbo*) collected from Japan were investigated. In all species, the residue levels of PCBs were in the order of liver > brain > blood, whereas the levels of HO-PCBs were in the order of blood > liver > brain. The concentration ratios of ΣHO-PCBs/ΣPCBs showed species- and tissue-specific. The predominant HO-PCB congeners were consistently 4-HO-CB187 and 4-HO-CB146 in grey herons and black kites, and 3-HO-CB153 in addition to those two congeners in great cormorants. To evaluate the dietary uptake of HO-PCBs for fish-eating birds, the concentrations of HO-PCBs in the whole body of several fish species which are prey of great cormorants were also determined. Although HO-PCBs were detected in all fish samples, ΣHO-PCBs levels were 3-4 orders of magnitude lower than ΣPCBs. Therefore, it seems that HO-PCBs exposure to predatory birds were exclusively dependent on their own biotransformation of PCBs rather than dietary uptake.

Introduction
It is well known that polychlorinated biphenyls (PCBs) are persistent organic pollutants and metabolized to hydroxylated polychlorinated biphenyls (HO-PCBs) by cytochrome P450 (CYP) enzyme-mediated oxidation in organisms. A large number of investigations for the levels of HO-PCBs in blood compartment (mainly plasma) of human and wildlife have been reported as described in recent two reviews [1, 2]. It is speculated that the residue profile of HO-PCBs is vary among species due to some factors such as the differences in metabolic capacity and affinity of binding protein. Because the omnivorous and piscivorous avian species which are the top predator of food chain tend to bioaccumulate PCBs, the high exposure of HO-PCBs by metabolism of PCBs is quite possible. It has been reported the levels of HO-PCBs in the blood plasma or egg in wild birds, for example, albatrosses [3], gulls [4-6] and eagles [7, 8], but the study for other tissue is very limited [6]. The aim of this study is to determine the concentrations of PCBs and HO-PCBs in the tissues such as blood, liver and brain of three different avian species, and compare the residue profile among species and tissues. Additionally, dietary uptake of HO-PCBs from their fish prey was investigated.

Materials and Methods
6 grey herons (*Ardea cinerea*) and 6 black kites (*Milvus migrans*) were collected from Matsuyama, Ehime, Japan in 2003-2004. 4 great cormorants (*Phalacrocorax carbo*) and 5 species of freshwater fish (largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, ayu *Plecoglossus altivelis*, pale chub *Zacco platypus*, three-lips *Opsariichthys uncirostris*) were collected from Lake Biwa, Shiga, Japan in 2005. Whole blood, liver and brain tissues of birds and whole body homogenates (pooled from 2-4 individuals) of fish were stored at –20 °C until analysis.

The samples (2-5 g of bird tissues, 16-37 g of pooled fish) were homogenized in 2-propanol and denaturized with HCl. ^{13}C-labeled PCBs (mono to decachlorinated 10 congeners) and ^{13}C-labeled HO-PCBs (penta to heptachlorinated 4 congeners) were spiked as surrogate standards and extracted three times with dichloromethane/hexane (1:1). The extracts were treated with concentrated sulfuric acid to remove lipids, and then fractionated using deactivated silica gel column (3 g; 5% H_2O, w/w). PCBs were eluted with hexane, followed by HO-PCBs were eluted with dichloromethane/hexane (1:3). PCBs fraction was further cleaned-up by

activated alumina column. HO-PCBs fraction was derivatized to the methoxylated analogues (MeO-PCBs) using diazomethane, and purified using sulfuric acid-silica gel column (2 g; 44% H_2SO_4, w/w). Each fractions were concentrated and spiked ^{13}C-labeled PCB138 as injection standard. Identification and quantification of PCBs and MeO-PCBs were carried out using HRGC (6890 series, Agilent technologies) / HRMS (JMS-800D, JEOL) at resolution of R>10,000 (10% valley). The gas chromatographic separation of PCB and MeO-PCB congeners was performed using HT8-PCB column (60m × 0.25mm i.d, SGE Analytical Science) and DB-5MS column (30m × 0.25mm i.d, 0.25μm film thickness, Agilent Technologies), respectively. Tri to octachlorinated congeners of PCBs and seven HO-PCB congeners, that is 4-HO-CB107 (4-HO-2,3,3',4',5-pentaCB), 4'-HO-CB130 (4'-HO-2,2',3,3',4,5'-hexaCB), 3'-HO-CB138 (3'-HO-2,2',3,4,4',5'-hexaCB), 3-HO-CB153 (3-HO-2,2',4,4',5,5'-hexaCB), 4-HO-CB146 (4-HO-2,2',3,4',5,5'-hexaCB), 4'-HO-CB172 (4'-HO-2,2',3,3',4,5,5'-heptaCB), 4-HO-CB187 (4-HO-2,2',3,4',5,5',6-heptaCB) were determined. The recoveries of both ^{13}C-labeled PCBs and HO-PCBs were consistently in the range of 80-110%.

Results and Discussion

Residue profile of PCBs and HO-PCBs in the tissues of wild birds

As shown in Figure1, despite relatively similar levels of ΣPCBs among species, those of ΣHO-PCBs quite varied. The residue levels of ΣPCBs were the order of liver > brain > blood in all species, whereas those of ΣHO-PCBs were blood > liver > brain. The several congeners of HO-PCBs which structurally resemble to thyroxine selectively retain in the blood owing to the strong affinity to the thyroxine transport protein such as transthyretin (TTR) [9, 10]. In all species, the levels of ΣHO-PCBs in liver were about half of blood, which corresponding to the case of Norwegian Arctic glaucous gulls [6]. The levels of ΣHO-PCBs in brain were relatively lower than liver. It was suggested that the blood-brain barrier could not completely protective for HO-PCBs as previously observed in polar bears [11] and some species of cetaceans [12].

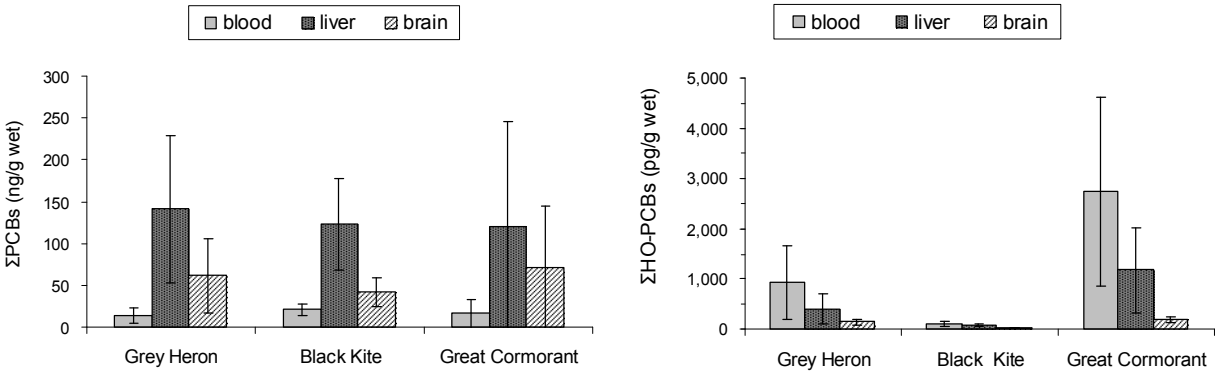

Figure 1. Arithmetic mean concentrations of ΣPCBs and ΣHO-PCBs in the blood, liver and brain of grey herons, black kites and great cormorants. Error bars are standard deviations.

The predominant congeners of HO-PCBs were consistently 4-HO-CB187 and 4-HO-CB146 in grey herons and black kites, and 3-HO-CB153 in addition to those two congeners in great cormorants (Figure 2). 4-HO-CB187 and 4-HO-CB146 are major HO-PCB congeners in most other avian species [3-8]. In addition, many unidentified HO-PCB congeners which matched the theoretical isotopic ratio of two selected monitor ions according to authentic standards were found in all samples. ΣHO-PCBs/ΣPCBs ratios of blood were higher than those of liver and brain in all species. ΣHO-PCBs/ΣPCBs ratios of black kites were apparently lower in all tissues compared to grey herons and great cormorants. It is speculated that the variation of HO-PCB congener patterns and ΣHO-PCBs/ΣPCBs ratios among species are mainly due to the differences of exposure levels and metabolic capacity for PCBs, and/or affinity of binding protein and conjugate ability for HO-PCBs.

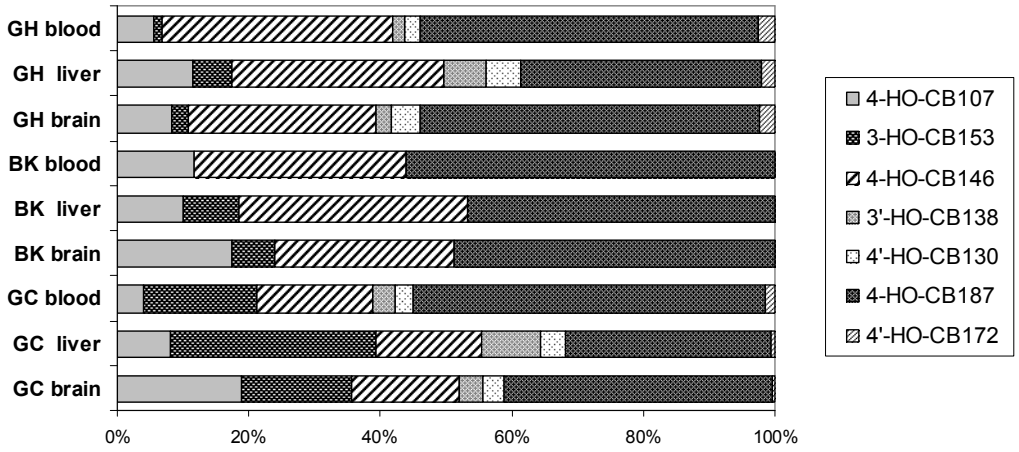

Figure 2. Mean compositions of HO-PCB congeners in the blood, liver and brain of grey herons (GH), black kites (BK) and great cormorants (GC).

Dietary uptake of HO-PCBs in fish-eating birds

Recently, HO-PCBs were found in the blood plasma of some freshwater fish species from Great Lakes [13, 14] and rainbow trout exposed to Aroclor mixtures [15]. In the present study, the whole body of freshwater fish which are main prey of great cormorants were analyzed to evaluate dietary uptake of HO-PCBs. The results show that 4-HO-CB107, 4-HO-CB146 and 4-HO-CB187 were detected in largemouth bass and bluegill, and 3-HO-CB153 in addition to those three congeners in ayu, pale chub and three-lips. The levels of ΣHO-PCBs were 3-4 orders of magnitude lower than ΣPCBs in these fish samples (Figure 3).

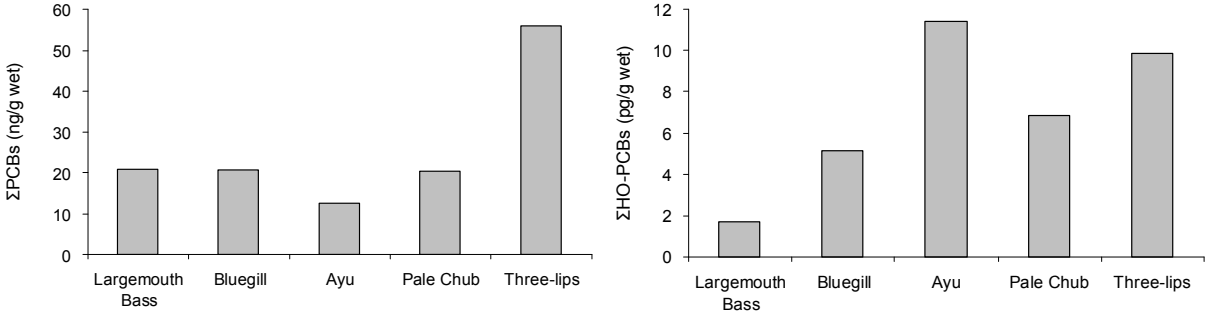

Figure 3. Concentrations of ΣPCBs and ΣHO-PCBs in the whole body of fish prey.

Although the biological half-lives of HO-PCBs in avian species are not clear, it has been reported that those of 4-HO-CB107 and 4-HO-CB187 in rats were 3.8 and 15 days, respectively, which are relatively shorter than the most parent congeners [16]. These results suggest that HO-PCBs exposure to predatory birds is thought to be exclusively dependent on their own biotransformation of PCBs rather than dietary uptake.

Acknowledgements
We would like to thank Dr. A. Sudo, Eaglet Office Inc. and Dr. K. Kameda, Lake Biwa museum for sample collection in Lake Biwa.

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