HYDROXYLATED POLYCHLORINATED BIPHENYLS IN GREAT CORMORANT (*PHALACROCORAX CARBO*)

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Abstract

In order to understand, detailed contamination status of hydroxylated polychlorinated biphenyls (HO-PCBs) in human and wildlife, we have developed the analytical methods for lower-chlorinated HO-PCBs in addition to higher-chlorinated ones together with PCB, and applied to the determination in the tissues and organs of great cormorant (*Phalacrocorax carbo*).HO-PCBs and PCBs were detected in all cormorant samples analyzed. It was found that significantly high concentrations of total HO-PCBs were present in the blood samples, and the highest concentration was found in the gallbladder. The residue pattern of HO-PCB congeners in great cormorant was different between blood and gallbladder, probably reflecting metabolic pathway of PCBs and HO-PCBs.

Introduction

The pollution of PCBs has been spread globally even to the polar region, and elevated concentrations of PCBs have been found in various trophic organisms. Accumulated PCBs in organism are slowly metabolized and HO-PCBs are ones of the major metabolic products of PCBs. It has been reported that certain HO-PCBs are present in human blood, and wildlife such as marine mammals[1, 2] and as well as other environmental samples such as river water, rain and snow so far[3].

In recent years, toxicity of HO-PCB has been attracted attention: adverse effect to cerebral nerve development[4], estrogenic hormonal activity[5], possible disturbance of a thyroid hormone action and inhibition of the sulfation and glucuronidation of 3-hydroxy-benzo (a) pyrene[6]. Previous analytical studies have been performed mainly for higher (penta-, hexa- and hepta-) chlorinated HO-PCBs, but little is known for lower chlorinated (di-, tri- and tetra-) HO-PCBs. HO-PCBs are complex mixtures of congeners, total number of congeners being 837, has the different toxicity by congeners.

The purpose of this study was to develop an analytical method for lower chlorinated HO-PCBs in addition to higher chlorinated ones, and to understand the behavior of HO-PCBs in organisms as well as in the environment. It seems important to analyze HO-PCBs and PCBs simultaneously, because HO-PCBs originate from PCBs. In previous reports, HO-PCBs and PCBs analysis was to use liquid-liquid extraction under an acidic or alkalic conditions[1-3]. However some problems may be suggested became the following clean-up steps are complex and some interfering matrices could not be eliminated efficiently. Therefore we examined separation of HO-PCBs and PCBs by using silica-gel (5% water, w/w) which has previously been used for the analysis of higher chlorinated HO-PCBs in human samples[7]. The method was applied to the determination of the compounds in the tissues and organs of great cormorant.

Materials and Methods

The great cormorants were collected in Lake Biwa, Shiga, Japan in July, 2006. These samples (n=3) were stored at -20℃ until analysis. Tissues and organs, including blood, liver, brain, gall bladder, kidney and muscle were analyzed.

The sample(3~5g) was homogenized and acidified with addition of hydrochloric acid(1mL), and then $^{13}C_{12}$-labeled HO-PCB standards {4'-HO-CB 29(tri), 4'-HO-CB61 (tetra), 4'-HO-CB120 (penta), 4'-HO-CB159 (hexa), 4-HO-CB187 (hepta)} and $^{13}C_{12}$-labeled PCBs (CB3, CB15, CB28, CB52, CB118, CB153, CB180, CB194) were spiked onto sample. HO-PCBs and PCBs were extracted in three times with acetonitrile (20mL) under acidic condition.

Hexane was added to acetonitrile extract for partition. Less polar constituents dissolved in the acetonitrile extract was distributed in hexane layer (10ml). After collecting hexane layer, acetonitrile (saturated with hexane)

was again added, and the same procedure was repeated three times. The acetonitrile layer was collected. Then a partition of 400mL of 5% NaCl solution (less than pH2) and 50ml hexane was added, and HO-PCBs and PCBs was recovered in hexane layer and subjected to further clean-up procedure.

The hexane layer was concentrated and then was passed through silica-gel column (3g; 5% water, w/w). PCBs were recovered with elution of 60mL hexane and the fraction was concentrated under nitrogen gas flow for GC-MS analysis. HO-PCBs were recovered with elution of 100mL dichloromethane/hexane (30% v/v). HO-PCBs in the latter fraction were methylated with trimethylsilyl diazomethane. After the derivatization, the solution was chromatographed with double layer column containing deactivated (5% water, w/w) silica-gel (2g; higher layer) and activated Florisil column (5g; lower layer). Methylated HO-PCBs were recovered with elution of 50mL dichloromethane/hexane (20% v/v) and concentrated under nitrogen gas flow for GC-MS analysis. The $^{13}C_{12}$-labeled standards (CB138) were spiked onto samples as syringe spike. HO-PCBs and PCBs were analyzed using GC (6890 series, Agilent, USA) / MS (JMS-800D, JEOL, Japan) at high resolution of 10,000. A mass spectrometer was operated in selected-ion monitoring (SIM) mode.

HO-PCBs were quantified using MeO-PCB standards derivatized by the same procedure as samples. The peaks that matched the retention times and isotopic ratios of primary and secondary ions of those compounds in the standard solutions were quantified as "identified HO-PCBs"{(4'HO-CB72 (tri), 4'HO-CB26 (tetra), 4HO-CB107 (penta), 3HO-CB153 (hexa), 4HO-CB146 (hexa), 3'HO-CB138 (hexa), 4'HO-CB130 (hexa), 4HO-CB187 (hepta), 4'HO-CB172 (hepta)}. The peaks that have different retention times from authentic standards but have the same isotopic ratios of primary and secondary ions were considered as unidentified HO-PCB congeners. The sum of identified HO-PCBs and unidentified HO-PCBs was referred to total HO-PCBs.

Recoveries of the $^{13}C_{12}$- HO-PCBs and $^{13}C_{12}$- PCBs congeners were within 60-120% and within 70-90%, respectively, after the whole procedures.

Results and Discussion

HO-PCBs and PCBs were detected in all samples analyzed. Total HO-PCBs and PCBs measured in tissues and organs of cormorants are shown in Fig.1. The highest concentration was determined in the gallbladder samples and the second in blood samples, 30 times and 10 times being higher than the other tissues and organs, respectively. In these samples, the concentrations of HO-PCBs were found 4 and 9 times higher than the concentrations of PCBs, respectively. On the contrary, concentrations of PCBs were higher than the concentrations of HO-PCBs in the other organs.

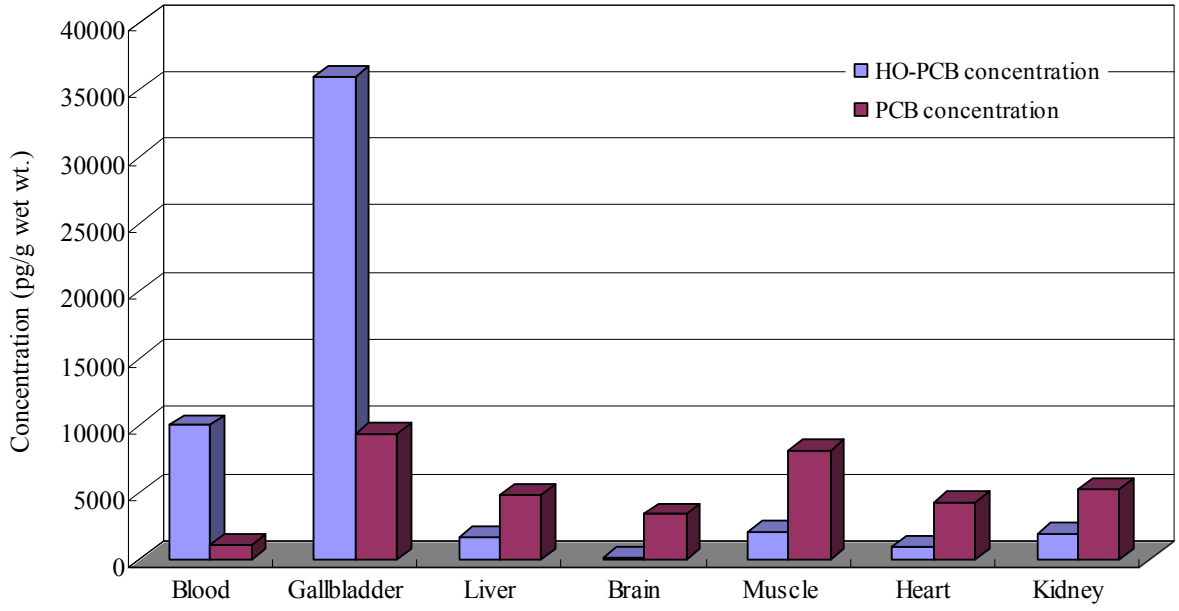

Fig. 1 Analytical results of total HO-PCBs and PCBs concentrations in great cormorant.

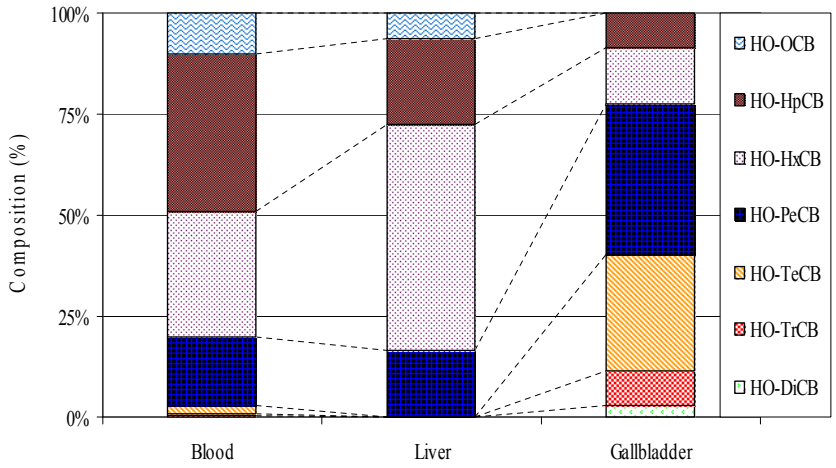

Fig. 2 Composition of HO-PCBs determined in blood, liver and gallbladder of great cormorant.

Composition of HO-PCBs determined in blood, liver and gallbladder are shown in Fig.2. It is interesting that the composition patterns of HO-PCB congeners are completely different between blood and gallbladder samples. In the blood samples, higher chlorinated HO-PCBs (pentachlorinated or more chlorinated) occupied it by almost 96% of total HO-PCBs. However in the gallbladder, tri-, tetra-, and penta- chlorinated HO-PCBs accounted for around 80%.

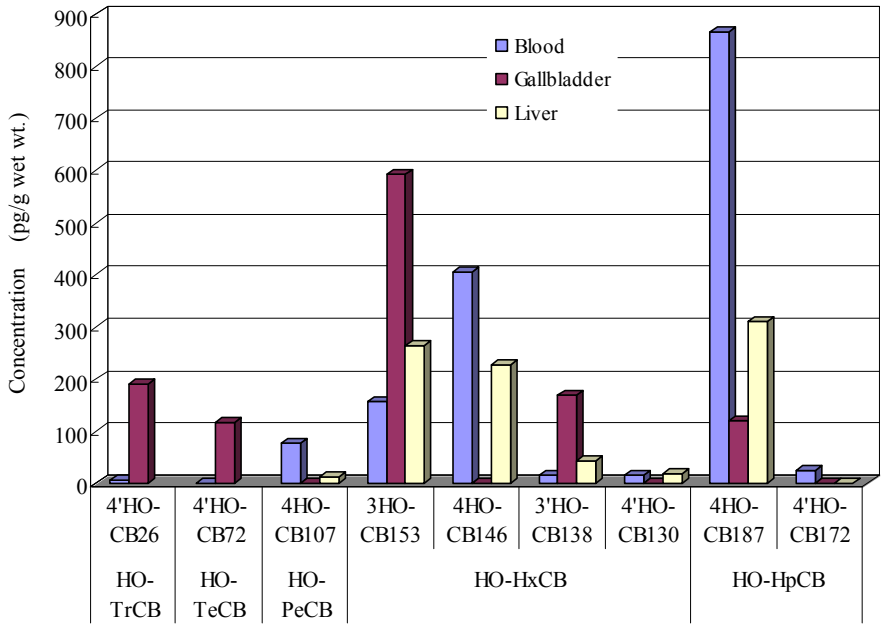

Fig. 3 HO-PCB congeners profiles in blood, liver and gallbladder.

The congeners that are identified in blood, in gallbladder, and in liver of great cormorant are illustrated in Fig.3. The figure indicates that relatively higher chlorinated and para-hydroxylated PCBs are predominant in blood, indicating the presence of protein to bind HO-PCBs in the blood of great cormorant. One may imagine the affinity with thyroxine binding protein such as transthyretin[8]. The structural similarity to thyroxine was suggested as an explanation for HO-PCB retention in organisms. The protein bound HO-PCBs in blood might be transported to brain and other critical organs.

On the other hand, in the gallbladder, it seems that the concentrations of the congeners of lower chlorinated HO-PCBs or para- and meta-hydroxylated PCBs are comparatively high. These results seem in similar pattern with the previous reports in the chick embryo experiment[9]. This residue pattern of the compounds may be reflected by metabolic pathway of PCBs and HO-PCBs.

One explanation is like that; PCBs are metabolized in liver, and the product HO-PCBs is excreted into bile, the HO-PCB congeners as PCBs metabolite being appeared in high concentration in gallbladder. A part of HO-PCBs excreted into bile was reabsorbed in gastrointestinal tract and appears in blood, which has high affinity with blood protein being remaining at higher concentration.

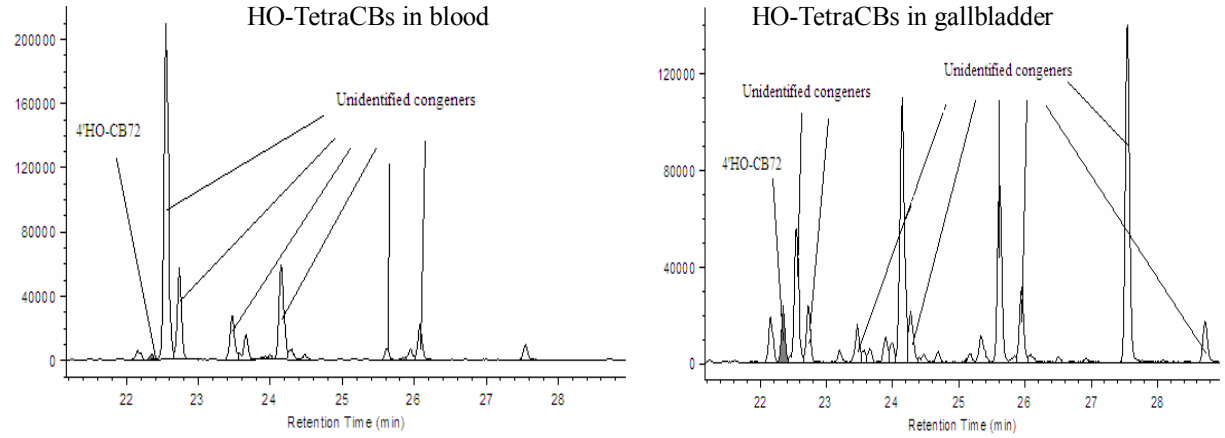

Fig. 4 Mass fragmentograms of OH-TetraCBs determined in blood and gallbladder samples of great cormorant.

Many unidentified congeners and isomers were found in all samples analyzed. Even in blood and gallbladder samples more than 11 and 17 peaks appears, respectively (Fig.4). Ratios of unidentified HO-PCBs/total HO-PCBs were 84% and 95%, respectively, in blood and gallbladder. It seems to be important to understand the chemical structures and behavior of unidentified congeners in order to understand toxicological and environmental meaning of HO-PCBs.

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