

# HYDROXYLATED PCBs AND PCBs; THEIR DETECTION IN WATER SAMPLES AND SUSPECTED SOURCES

Satoshi Nanba, Yuma Shiki, Masahide Kawano, Muneaki Matsuda, Masatoshi Morita

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

## 【Introduction】

Hydroxylated PCBs (HO-PCBs) are known to occur as metabolites of PCBs in organisms. Some isomers of HO-PCBs can compete with thyroxine for binding sites on transthyretin (TTR), because of similar structure to the thyroid hormones<sup>(1)</sup>. As the result they may disrupt thyroxine hormonal action. Other sources of HO-PCBs in aquatic environment may be by reaction of PCBs with OH radicals<sup>(2)</sup> and microbial PCB oxidation<sup>(3)</sup>. HO-PCBs have been detected in snow, rain, and surface water samples in Ontario Canada, and higher concentration HO-PCBs was found in water sample collected near sewage treatment plant (STP) than other area<sup>(4)</sup>. It was indicated that STP is one of the source of HO-PCBs into the aquatic environment<sup>(4)</sup>. In other study, high concentrations of HO-PCBs were detected in feces of Great Cormorant (*Phalacrocorax carbo*)<sup>(5)</sup>, indicating that HO-PCBs are spread through wildlife feces to the environment.

Information of HO-PCBs in the environment is still limited, and it seemed important to elucidate the sources, concentrations and distribution of HO-PCBs in the environment is an urgent problem. In this study, HO-PCBs and PCBs were determined in surface water of Lake Biwa to understand general environmental pollution and in paper mill effluent to identify the industrial sources of HO-PCBs.

## 【Materials and methods】

### • Sample collection

**Lake Biwa** The surface water samples of Lake Biwa, Shiga, Japan, were collected at three locations (Northern point, Middle point and Southern point) in Lake Biwa on December 3 to 7, 2007 (Fig.1). Northern point was relatively less polluted by industrial activities compared with other areas of Lake Biwa. Middle point was near the colony of the common cormorant. It was thought that the water near there was influenced by HO-PCBs in feces of Great Cormorants. Southern point was located in densely populated region with developed industries. <sup>13</sup>C<sub>12</sub>-labeled HO-PCB standards {4'-HO-CB12(di), 4'-HO-CB 29(tri), 4'-HO-CB61 (tetra), 4'-HO-CB120 (penta), 4'-HO-CB159 (hexa), 4'-HO-CB187 (hepta)} and <sup>13</sup>C<sub>12</sub>-labeled PCBs (CB3, CB15, CB28, CB52, CB118, CB153, CB180, CB194) were spiked onto approximate 100L of the surface water samples of Lake Biwa. The surface water samples of Lake Biwa were passed through SP850 resin column without filtration.

**Paper mill effluent** The effluent water of paper producing from wood chip, and the effluent water of recycled paper production were collected along river mouth of Kinsei-river, Kawano-e Sikokutyuuou, Ehime, Japan, on January 25, 2008. Aforementioned <sup>13</sup>C<sub>12</sub>-labeled HO-PCB standards and <sup>13</sup>C<sub>12</sub>-labeled PCB standards were spiked onto each effluent sample (20L). These effluent samples were filtrated on 0.6µm glass fiber filter (GFF) and passed through SP850 resin column.

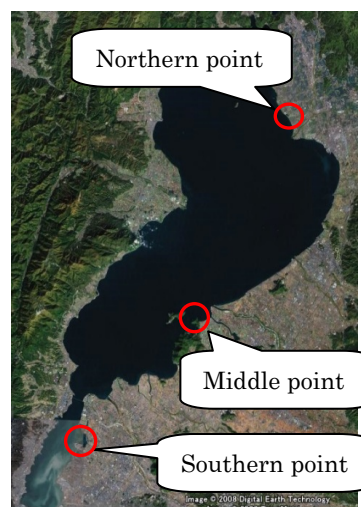


Fig.1 Three sampling locations in Lake Biwa

• **Extraction and clean up procedures**

HO-PCBs and PCBs collected on SP850 resin and GFF were extracted with acetone using Soxhlet apparatus. The eluate (100mL) was added with 400mL of 5% NaCl solution (less than pH2) and 50mL hexane, and the solution was mixed. HO-PCBs and PCBs was recovered in hexane layer and subjected to further clean-up procedure. The recovered hexane layer was concentrated and was passed through silica-gel column (3g; 5% water, w/w). PCBs were recovered with elution of 60mL hexane and were 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 on silica-gel column (1g; 5% water, w/w). Methylated HO-PCBs were recovered with elution of 50mL dichloromethane/hexane (10% v/v) and concentrated under nitrogen gas flow for GC-MS analysis. The <sup>13</sup>C<sub>12</sub>-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” {(4HO-CB2 (mono), 4HO-CB14 (di) }. 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  $\Sigma$ HO-PCBs.

**【Result and discussion】**

• **Lake Biwa**

Concentrations and homolog patterns of  $\Sigma$ HO-PCBs and  $\Sigma$ PCBs in Lake Biwa samples are shown in Figs.2 and 3. The concentrations of  $\Sigma$ HO-PCBs were high to low in the order of the southern point, the central point, and the northern point (below the detection limits) (Fig.2). The major components of HO-PCBs in middle point were mono- and dichlorinated homologs. The higher chlorinated HO-PCBs homologs (tri- to penta-) are predominated at the Southern point than the middle point.

The concentration of  $\Sigma$ PCBs were in the order of the southern point > central point > northern point (Fig.3). This tendency is similar to that of  $\Sigma$ HO-PCBs. In addition, the southern point is predominated by higher chlorinated PCBs homologs (tetra- to hexa-) as compared to other points (di- to tetra). The tendency of homolog pattern in the southern point predominated higher chlorinated compounds than other point was common with PCBs and HO-PCBs.

South part of Lake Biwa was located in densely populated region with industries such as capacitor production factory. A relatively high input of PCBs might occur in the past, and it is for this reason that southern point was higher concentration of  $\Sigma$ HO-PCBs and  $\Sigma$ PCBs than other area.

In Lake Biwa samples, a similarity was observed concentration and the homolog pattern between HO-PCBs and PCBs. HO-PCBs in Lake Biwa samples seems to come mostly from PCBs.

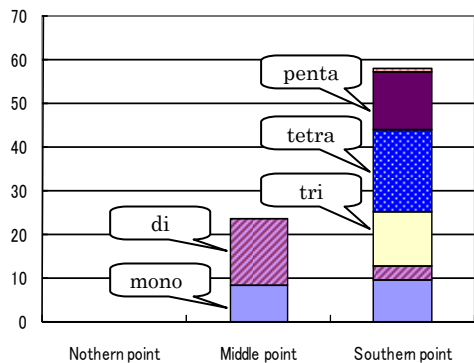


Fig.2 Concentrations (pg/L) and homolog pattern of  $\Sigma$ HO-PCBs in Lake Biwa samples.

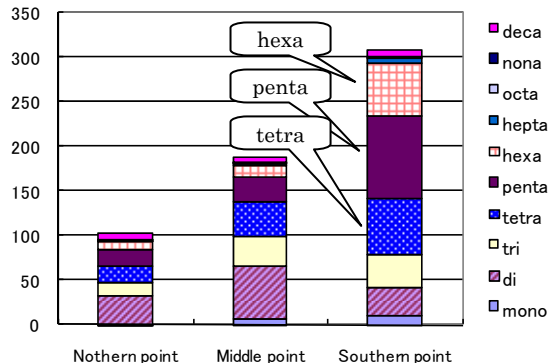


Fig.3 Concentrations (pg/L) and homolog pattern of  $\Sigma$ PCBs in Lake Biwa samples.

• **Paper mill effluents**

Concentrations and homolog patterns of  $\Sigma$ HO-PCBs and  $\Sigma$ PCBs in paper mill effluent samples are shown in Figs.4 and 5.  $\Sigma$ HO-PCBs determined in the filtrate of recycled paper production was the highest concentration (1300pg/L) and it was higher than  $\Sigma$ PCBs concentration (860pg/L) in the same sample. It may be presumed that HO-PCBs in the effluent of recycled paper production were formed in a different mechanism from hydroxylation of PCBs. In producing recycled paper process, the bleaching with chlorine is still done in part and there is a possibility that HO-PCBs are formed by chlorination of phenylphenol during chlorine bleaching process. To confirm this hypothesis, the experiment was done to react ortho-, meta-, para-phenylphenol and chlorine bleaching agent in water and isomer patterns were compared.

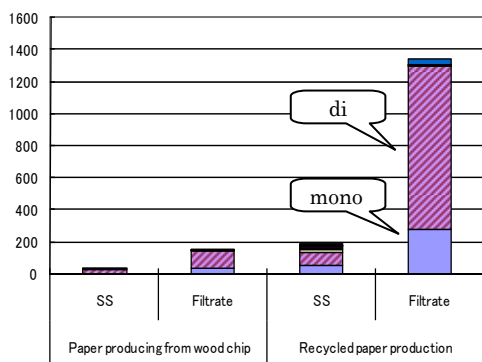


Fig.4 Concentrations (pg/L) and homolog patterns of  $\Sigma$ HO-PCBs in paper mill effluent samples  
 ※SS ; suspended substance

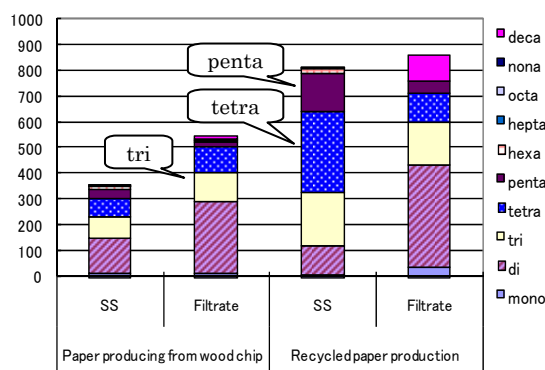


Fig.5 Concentrations (pg/L) and homolog patterns of  $\Sigma$ PCBs in paper mill effluent samples  
 ※SS ; suspended substance

• **The model experiment for formation of chlorinated phenylphenol**

Ortho-, meta-, para-phenylphenol (each 1 $\mu$ mol) were added to 100mL of dilute chlorine bleaching agent solution (0.6% NaClO, pH=8). The solution was mixed and left overnight. After that, 50mL of hexane was added to extract chlorinated phenylphenol. The recovered hexane layer was methylated with trimethylsilyl diazomethane and concentrated under nitrogen gas flow for GC/MS analysis. GC/MS analysis and identified HO-PCBs were done same way as previous determination.

Mass fragmentograms of HO-PCBs (mono- and dichlorinated homologs) in the filtrate of the effluent of recycled paper production and chlorinated samples of ortho-, meta-, para-phenylphenol showed in Figs.6 and 7. 4-HO-CB2, 4-HO-CB14 and four unidentified HO-PCBs congener A, B, C, D peaks in the three of chlorinated phenylphenol samples matched well with retention time the peaks in the effluent of recycled paper production. The result suggests that HO-PCBs can be formed by chlorination of phenylphenol in the producing recycled paper process.

In the result of previous study by Onodera et al, mainly 2-chloro-4-phenylphenol (4-HO-CB1) and 2,6-dichloro-4-phenylphenol (4-HO-CB10) were identified as chlorinated para-phenylphenol isomers by the reaction of phenylphenol and hypochlorite in water (pH=4~10)<sup>(6)</sup>, it was different from that identified in our experiment {3-chloro-4-phenylphenol (4-HO-CB2), 3, 5-chloro-4-phenylphenol (4-HO-CB14)}. The reason of this distinction may be the difference of the experiment conditions such as the reaction time and the concentration ratio of phenylphenol and hypochlorous acid etc.

It had been thought previously that HO-PCBs were formed biologically by metabolizing PCBs within organisms, microbial PCB oxidation and reaction of PCBs with OH radicals. However, chlorination of phenylphenol might be another source of HO-PCBs.

The effluent of paper making  
from used paper (filtrate)

Chlorination of  
ortho-phenylphenol

Chlorination of  
meta-phenylphenol

Chlorination of  
para-phenylphenol

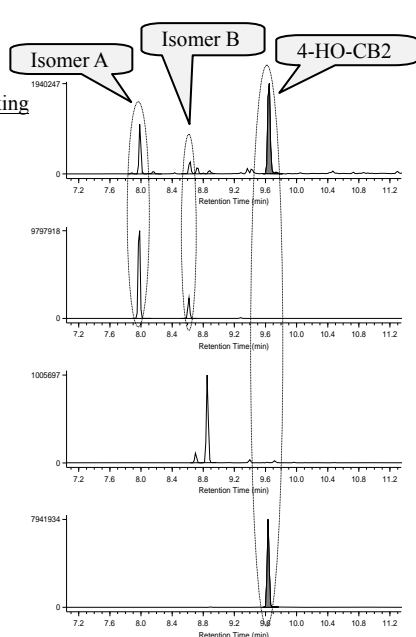


Fig.6 Mass fragmentograms of HO-monoCBs

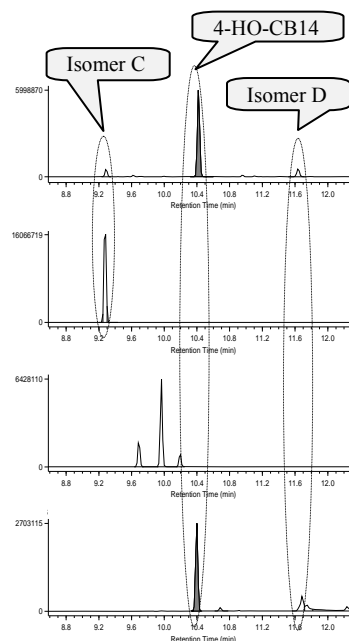


Fig.7 Mass fragmentograms of HO-diCBs

### 【Reference】

- (1) Brouwer A, Morse D.C. Lans M.C. Schuur A.G. Murk A.J. Klasson-Wehler E. Bergman A. Visser T.J. *Toxicol. Ind. Health* 1998, 14, 59-84.
- (2) David L, Sedlak and Andera W, Andren *Elsevier Science Ltd* 1994, 28-5, 1207-1215
- (3) Flanagan W.P, May R.J. *Environ. Sci. Technol.* 1993, 27, 2207-2212
- (4) Ueno D, Darling C, Alaei M, Campbell L, Pacepavicius G, Teixeira C, Muir D. *Environ Sci Technol.* 2007; 41: 1841-8
- (5) Shiki Y, Hasegawa J, Matsuda M, Kawano M, Sudou A, Morita M *17<sup>th</sup> Symposium on Environmental Chemistry Program and Abstracts* P69
- (6) Onodera S, Iino N, Matsuda M, Ishikura S *Journal of Chromatography*, 1983, 265, 201-213