

# ACCUMULATION OF CHLORINATED POLYCYCLIC AROMATIC HYDROCARBONS IN FISH AND SEDIMENT FROM TOKYO BAY, JAPAN

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## Introduction:

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDF), and mono-ortho polychlorinated biphenyl (PCB) congener are chemically stable and recognized as persistent hydrophobic organic chemicals, which could be biomagnified via food chain. Therefore, they are accumulated in organic-rich matrices such as aquatic sediment and biota<sup>1</sup>.

Chlorinated polycyclic aromatic hydrocarbons (CIPAHs) are compounds that contain chlorine replaced one to several hydrogen atoms. They are produced by the chlorination of PAHs, which is likely to increase their toxicities and persistences in the environment and CIPAHs are structurally similar to PCDD, PCDF, and PCBs. Ohura et al. (2009) reported that some CIPAHs have more aryl hydrocarbon receptor (AhR) activities than their parent PAHs<sup>2</sup>. CIPAHs elicit dioxin-like activities comparable to PCB. The toxicity and persistence of CIPAHs increases with the number of halogen atoms substituted<sup>2,3</sup>.

CIPAHs have been widely detected in various environments including atmosphere in Tokyo, Japan<sup>4</sup> and sediment cores collected from Tokyo Bay, Japan<sup>5</sup>. However, the information on the concentration levels of CIPAHs in biota has been limited. In addition, the bioavailability of CIPAHs in the sediment for fishes is not available, especially biota sediment accumulation factors (BSAFs).

In this study, we determined the concentrations of CIPAHs in sediments and fishes collected from Tokyo bay, Japan. The effects of type of PAHs and CIPAHs on their BSAFs were investigated.

## Materials and methods:

### Sample collection

Sediment and fish samples were collected from Tokyo Bay, Japan. Sediment samples were collected from nine stations ([35°35.00' N, 139°54.43' E], [35°35.00' N, 139°58.00' E], [35°35.00' N, 140°01.17' E], [35°31.56' N, 139°58.00' E], [35°32.18' N, 140°01.17' E], [35°29.36' N, 139°49.32' E], [35°29.36' N, 139°54.43' E]) Fish samples includes conger-eel (*Conger myriaster*), sea bass (*Lateolabrax japonicus*), and marbled sole (*Limanda yokohamae*). All sediment and fish samples were stored under a cool and dark place.

### Extraction and clean-up procedures for fish and sediment samples

Fish samples were brayed with anhydrous sodium sulfate. Then, PAHs and CIPAHs in fish and sediment were extracted by the Soxhlet extraction method with 250 mL of dichloromethane after spiking recovery standards. The fish extract was purified and fractionated by using an activated carbon column connected with a 2% KOH silica gel column. The sediment extract was purified and fractionated by using an activated carbon column connected with a silica gel column. After washing the connected column with 10% dichloromethane/hexane, the silica gel column and 2% KOH silica gel column was removed, and the activated carbon column was reversed. Then, the target compounds (PAHs and CIPAHs) were eluted from the activated carbon column with 120 mL of toluene. The toluene fraction was spiked with internal standards and concentrated to 100 µL for the analysis by a gas chromatograph with high resolution mass spectrometer (HRGC-HRMS).

### HRGC-HRMS analysis

The concentrations of PAHs and CIPAHs in the samples were determined by the HRGC-HRMS. Gas chromatographic separation was accompanied by a 60-m BPX-DXN fused silica capillary column (0.25 mm internal diameter). Two microliters of the aliquot were injected in splitless mode at 280°C. The temperature of the column oven was kept at 130°C for 1 min, raised with rate of 5°C min<sup>-1</sup> to 250°C, raised with 10°C min<sup>-1</sup> to 320°C, and held for 18 min. The HRMS was operated in an electron-impact selected ion monitoring (SIM) mode at resolution  $R > 10,000$  (10% valley). Peaks were identified by comparison of the retention times of samples to those of standards if the signal-to-noise ( $S/N$ ) ratio was  $> 3$  and were quantified if target/qualifier ion ratio were within 15% of the theoretical values.

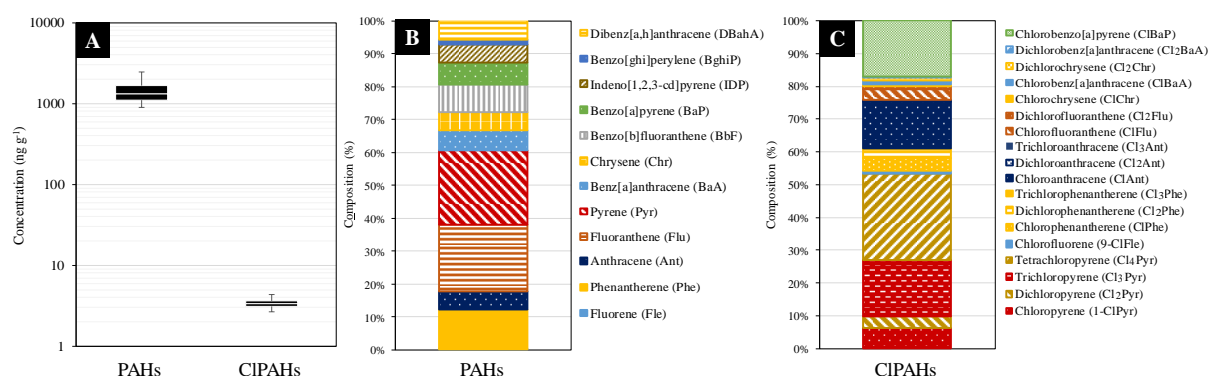
## Results and discussion:

### *Concentration and composition of PAHs and CIPAHs in sediment from Tokyo Bay*

Figure 1A shows the total concentration of PAHs and CIPAHs in sediment collected from Tokyo Bay. The PAHs concentration were 900–2400 ng g<sup>-1</sup>. The concentrations of CIPAHs were 2.6–4.3 ng g<sup>-1</sup>. The PAH concentrations were 210–670 times higher than those of CIPAHs. Horii et al. (2009) showed that the concentrations of CIPAHs except Cl<sub>3</sub>Pyr and Cl<sub>4</sub>Pyr in Tokyo Bay were 36–1200 pg g<sup>-1</sup>. In our study, the concentrations of CIPAHs except Cl<sub>3</sub>Pyr and Cl<sub>4</sub>Pyr were 1600–2400 pg g<sup>-1</sup>, which were comparable to the previous research<sup>5</sup>. The PAH and CIPAH concentrations in these samples had no large difference. In this study, the sampling was only conducted in northern part of Tokyo Bay. These sampling sites are located in inside of Tokyo Bay. These results suggested that the circulation was unlikely to occur in interior from Tokyo Bay. Yamashita et al. (2000) showed that PCDD and PCDF concentrations were about 10–30 and 2–5 ng g<sup>-1</sup>, respectively<sup>6</sup>. The concentrations of CIPAHs in Tokyo Bay were comparable to those of PCDD/PCDF.

We found that a number of CIPAH isomers and highly chlorinated CIPAHs were observed in the sediment collected from Tokyo bay. Figure 1B shows the composition of PAHs. Three to six rings of PAHs were detected in the sediments. Fluoranthene (Flu) and pyrene (Pyr) were detected at high concentration. Figure 1C shows the composition of CIPAHs. Cl<sub>n</sub>Pyr accounted for 55% of total CIPAHs. In particular, high concentrations of Cl<sub>3</sub>Pyr and Cl<sub>4</sub>Pyr were detected in the sediments compared to the concentrations of ClPyr and Cl<sub>2</sub>Pyr. Comparing with ClPyr and Cl<sub>2</sub>Pyr, Cl<sub>3</sub>Pyr and Cl<sub>4</sub>Pyr have a larger molecular size, which leads to increase the environmental persistence and bio-accumulating properties. There could be unknown emission sources of Cl<sub>3</sub>Pyr and Cl<sub>4</sub>Pyr in Tokyo Bay. These results suggested that highly chlorinated PAHs were present in the aquatic environment and CIPAHs could pose a potential as persistent organic pollutants.

A lot of unidentified isomers of CIPAHs was observed from the sediment samples. Peaks of isomers whose analytical standards are not available were detected based on the isotope patterns of molecular ions [M, M+2] by GC-HRMS analyses. For example, ClPhe/Anthracene (Ant), dichlorinated phenanthrene (Cl<sub>2</sub>Phe)/Ant, Cl<sub>3</sub>Phe/Ant have unidentified isomers, which suggests that the total concentration of CIPAHs could be underestimated.



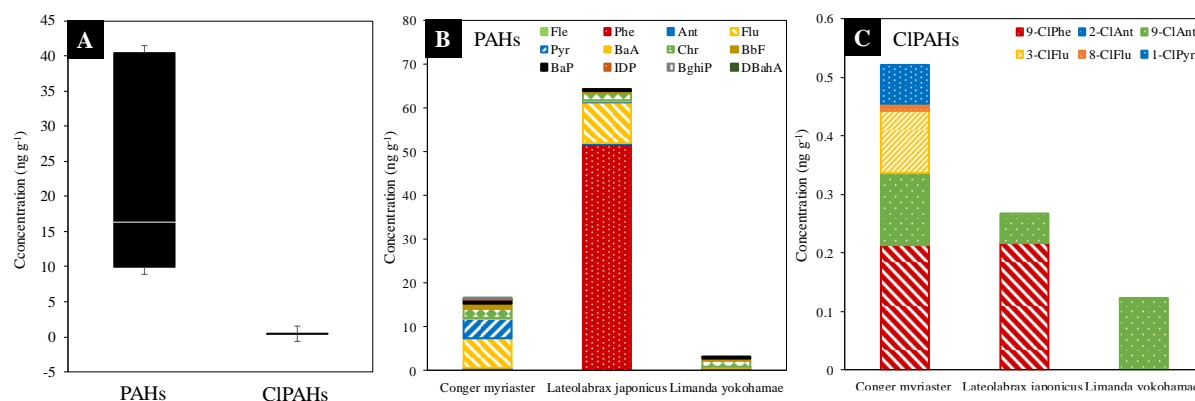
**Figure 1: Total Concentrations of PAHs and CIPAHs (A) and Compositions of PAHs (B) and CIPAHs (C) in Sediments Collected from Tokyo Bay, Japan**

### *Concentration and composition of CIPAHs in Fish in Tokyo Bay*

Figure 2A shows the concentrations of PAHs and CIPAHs in fish samples collected from Tokyo Bay.

*Lateolabrax japonicus* is a wild and higher animal in aquatic environments. *Conger myriaster* and *Limanda yokohamae* occur in the bottom of the bay. The concentrations of PAHs and CIPAHs in the fish samples were 3.2–64 ng g<sup>-1</sup> and 0.12–0.52 ng g<sup>-1</sup>. The concentrations of PAHs in *Conger myriaster* and *Limanda yokohamae* were 32 and 26 times higher than those of CIPAHs, respectively. The concentrations of PAHs in *Lateolabrax japonicus* are 240 times higher than those of CIPAHs. The concentration ranges were dramatically varied by the samples. The concentrations of CIPAHs were 10–100 times higher than those of dioxins. Phe, Flu, and Pyr were at high concentrations in these samples. These PAHs were 3–4 rings. 5 rings PAHs (benzo[*b*]fluoranthene [BbF] and Benzo[*a*]pyrene [BaP]) were also detected from all the samples. 6-rings PAHs (indeno[1,2,3-*cd*]pyrene [IDP], benzo[*ghi*]perylene [BghiP], and dibenz[*a,h*]anthracene [DBahA]) were detected at low concentration. These results suggested that smaller molecular PAHs were likely to occur in fishes compared to larger molecular PAHs.

9-CIPhe, 2-ClAnt, 9-ClAnt, 3-ClFlu, 8-ClFlu, and 1-ClPyr were detected from the fish samples. These CIPAHs were 3–4 rings. Despite highly chlorinated PAHs were at high concentration in the sediment, they were not detected from the fish samples.



**Figure 2: Total Concentrations of PAHs and CIPAHs (A) and Compositions of PAHs (B) and CIPAHs (C) in Fishes Collected from Tokyo Bay, Japan**

#### *Biota sediment accumulation factors (BSAFs) of CIPAHs in fish in Tokyo Bay*

Biota sediment accumulation factors (BSAFs) (concentration in biological samples [ $\text{ng g-lipids}^{-1}$ ] / concentration in sediment [ $\text{ng g}^{-1}$ ]) were calculated for the samples in Tokyo Bay (Figure 3A). The BSAFs of PAHs were 0.0016–0.37 (Phe), 0.0040 (Ant), 0.00014–0.035 (Flu), 0.0001–0.014 (Pyr), 0.0025–0.0043 (BaA), 0.017–0.028 (chrysene [Chr]), 0.0041–0.0090 (BbF), and 0.0048–0.011 (BaP). The BSAF values of 9-CIPhe were 2.0 (*Lateolabrax japonicus*) and 1.9 (*Conger myriaster*). The BSAF values of 9-ClAnt were 1.3 (*Lateolabrax japonicus*), 3.3 (*Conger myriaster*), and 3.3 (*Limanda yokohamae*). The BSAF values of 3-ClFlu, 8-ClFlu, and 9-ClFlu were 1.2 (*Conger myriaster*), 0.7 (*Conger myriaster*), and 0.3 (*Conger myriaster*), respectively. Previous researches showed that BSAFs of PAHs for tilapia were  $< 0.3^7$ . BSAFs of PAHs in this study were comparable to the previous researches. Some studies showed that the accumulation of PAHs is largely affected by compounds and species<sup>8</sup>. In Fishes, water is the predominant source of exposure to organic compounds with  $\text{Log } K_{ow} (< 5)$ , while sediment particles can contribute substantially to bioaccumulation for those with high  $\text{Log } K_{ow} (> 5)^9$ . The values of  $\text{Log } K_{ow}$  of PAHs were 4.6 (Phe), 4.5 (Ant), 5.2 (Flu), 5.2 (Pyr), 5.8 (BaA), 5.8 (Chr), 6.5 (BbF), and 6.23 (BaP). Aqueous and dietary routes were important for PAH uptake to fishes.

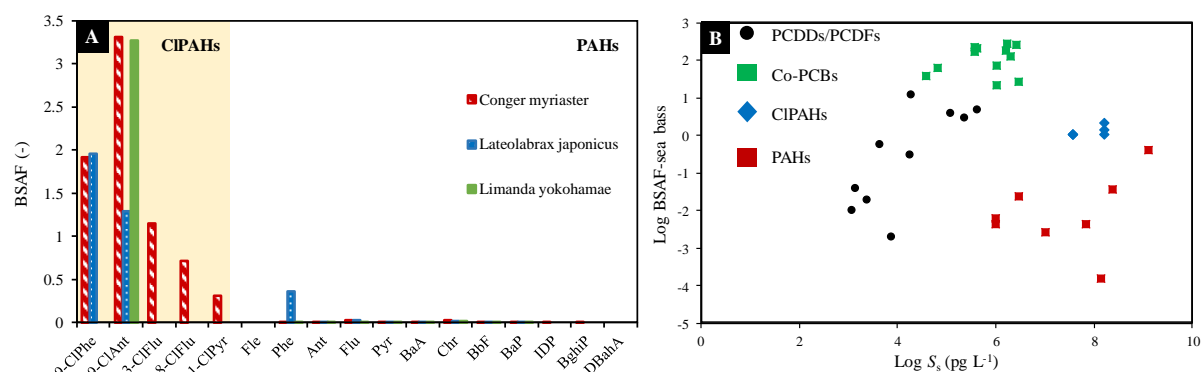
Previous study showed that among PAHs with  $\text{Log } K_{ow}$  less than 5, no metabolism was documented for naphthalene, Phe, and Ant in some fishes<sup>10</sup>. BSAFs of CIPhe and ClAnt were higher than those of the other CIPAHs. This suggested that metabolism is important for bioaccumulation for CIPAHs.

BSAFs of smaller molecular CIPAHs were higher than those of larger molecular CIPAHs. This indicated that high molecular weight CIPAHs were more difficult to be bioconcentrated. Ni et al. (2013) have proposed that lower molecular weight PAHs had higher detection rates than those of higher molecular weight PAHs<sup>11</sup>. This trend was agree in the concentration of PAHs and CIPAHs in fishes.

BSAF of 9-CIPhe (*Lateolabrax japonicus*) was 5 times higher than that of Phe. BSAF of 9-ClAnt (*Lateolabrax japonicus*) was 300 times higher than that of Ant. In some fish species (*Lateolabrax japonicus*, *Conger myriaster*, and *Limanda yokohamae*), BSAFs of CIPAHs were much higher than those of PAHs. These results suggested that CIPAHs were more easily bioconcentrated in fishes than PAHs. In other words, the substitution of proton with chlorine molecule in PAHs could increase bio-accumulating property.

#### *Comparison among PAHs, CIPAHs, PCDDs/PCDF, and Co-PCB*

Figure 3B shows the relationship between BSAFs and water solubilities ( $\text{Log } S_s$ ) of PAHs, CIPAHs, PCDDs/PCDF, and Co-PCB. The values of BSAFs were following order: Co-PCB  $>$  PCDD/PCDFs  $>$  CIPAHs  $>$  PAHs. CIPAHs were located between dioxins (PCDD/PCDFs and Co-PCB) and PAHs in the figure. PCDD/PCDFs and Co-PCB have a correlation. PAHs have higher water solubilities compared to dioxins. Although the water solubilities of CIPAHs and PAHs are comparable, BSAFs of them were largely different. In addition, highly chlorinated PAHs were not detected from the fish samples. Previous studies reported that BSAFs of PCDD/PCDFs for crabs were decreased with increases in the degree of chlorination<sup>12</sup>.



**Figure 3: Biota Sediment Accumulation Factors (BSAF) of PAHs and CIPAHs in Fishes in Tokyo Bay (A), Relationship Between Water Solubility ( $\log S_s$ ) and BSAFs for Sea Bass (B)**

### Conclusions:

CIPAHs were detected in fish and sediment samples collected from Tokyo Bay, Japan. BSAFs of CIPAHs were higher than those of PAHs. These results suggested that CIPAHs were more easily bio-concentrated in fished than PAHs. Highly chlorinated PAHs were detected from the sediments, but not from the fish samples. These findings suggest that research on the metabolism of highly chlorinated CIPAHs is desired.

### Acknowledgements:

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