Speciation of Extractable Organhalogens According to Molecular Size

in Various Environmental Matrices

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Introduction
Studies on extractable organohalogens (EOX, X = Cl, Br) have indicated that there are a number of unidentified organochlorine/bromine compounds (OHCs) in the environment[1,2]. Some of these unidentified compounds may have bioaccumulation potential and toxicity similar to those of persistent organic pollutants (POPs). However, there are also large numbers of naturally produced OHCs. Few studies have conducted the speciation of EOX and investigated the mass balance of identified/unidentified OHCs in the separated fraction.

In general, the molecular size of POP-like OHCs are relatively small. Thus, the low molecular size fraction of EOX is an important indicator of the potential for POP-like OHCs. By investigating the mass balance of identified/unidentified OHCs in the low molecular size fraction of EOX, we can evaluate the potential for POP-like unidentified OHCs.

To clarify the important sinks, sources, and dynamics of POP-like unidentified OHCs, we need to compare various environmental matrices using the same extraction method. And we used different solvents to extract OHCs with a wide range of polarity.

In this study, we conducted gel permeation chromatography (GPC) fractionation of extracts and separated EOX into two fractions, low molecular size EOX (EOX-L) and high molecular size EOX (EOX-H), and also evaluated how much of the identified Cl, Br (Cl and Br derived from some identified compounds) was attributable to EOX-L. We compared these results (both EOX-L and identified Cl, Br) in various environmental samples and considered which environments have high potential for POP-like unidentified OHCs.

Materials and methods
Samples: Natural forest soils were collected from a forest on Mt. Yatsugatake (Yatsugatake Experimental Forest of Tsukuba University, Nagano Prefecture) and a forest located in the Tango Peninsula, Kyoto Prefecture, in the summer of 2016. Soils from the O, A, and B layers (depth: ~5, 5–10, 25–40 cm respectively) were collected at each site. Soil standard samples were acquired from the Japan Society for Analytical Chemistry (JSAC) standard reference material (SRM) 0422 (surface soil) and 0421 (depth: 3–10 cm). Both samples were collected at a forest near a municipal solid waste incinerator (MSWI). Sediment and finless porpoise blubber samples were utilized for the intercalibration study[4,5]. House dust was acquired from the NIST SRM 2585. Urban particulate matter (PM) was taken from the NIST SRM 1648a. MSWI bottom ash and MSWI fly ash were taken from the JSAC SRMs 0512 and 0511, respectively. Concentrations of some individual OHCs in samples, not including natural forest soils, were given as certified, reference, or reported values.

Extraction, fractionation, and analysis: Samples (5 g for soils and sediment, 2 g for house dust, ash, and blubber, and 0.5 g for urban PM) were extracted by ultrasonic extraction. Samples were subsequently extracted by three different solvents: aceton 10 mL, acetone/hexane (1:1) 10 mL and toluene 10 mL. Each fraction was combined and reduced to 10 mL by rotary evaporation. Then, a 5 mL aliquot (remaining 5 mL was kept for other studies) was washed to remove inorganic Cl and Br by partitioning crude extracts between 5% Na_2SO_4 solution and mixture of methyl *tert*-butyl ether/hexane (1:1, v/v). Organic phase was reduced to 10 mL by rotary evaporation and applied for the fractionation by GPC. Extracts were passed through a glass column (2 cm ID × 50 cm) packed with 50 g biobeads S-X3. The column was then eluted with a mixture of cyclohexane/ethyl acetate (1:1). The first 120 mL of eluate was considered as the high molecular size fraction, and the second 120 mL was considered as the low molecular size fraction. This fractionation point was determined by a standard solution containing corn oil and mixture of di- to deca-brominated diphenyl ethers. Each fraction was concentrated to 10 mL, and 2 mL was used for neutron activation analysis. The sample was placed into a

polyethylene (PE) bag and dried under normal temperature and pressure. Then, the PE bag was sealed and placed into another PE bag, which was also sealed. Samples were irradiated for 5 min with a thermal neutron flux of $2.0-2.4 \times 10^{13}$ cm^{-2} $\cdot$ S^{-1} at Kyoto University Reactor (KUR). The radioactivities of ^{38}Cl ($t_{1/2}$ = 37.18 min, E_γ = 1642, 2168 keV) and ^{80}Br ($t_{1/2}$ = 17.6 min, E_γ = 616 keV) were measured for 60 s using a Ge semiconductor detector.

Results and discussion

EOX-L+EOX-H in this study and EOX in previous studies: The sum of EOX-L and EOX-H (EOX-L+H) can be considered as EOX (total length of white and black bars in Figure 1A, B). The concentration of EOCl-L+H observed in the sediment in this study was 2-fold greater than the EOCl reported for Finnish lake sediments (3.8 µg/g, dry weight[6]) and 115-fold smaller than the concentration reported for sediment collected in the vicinity of a former chloralkali plant (in the range of 655−822 µg/g, dry weight[1]). A considerable concentration
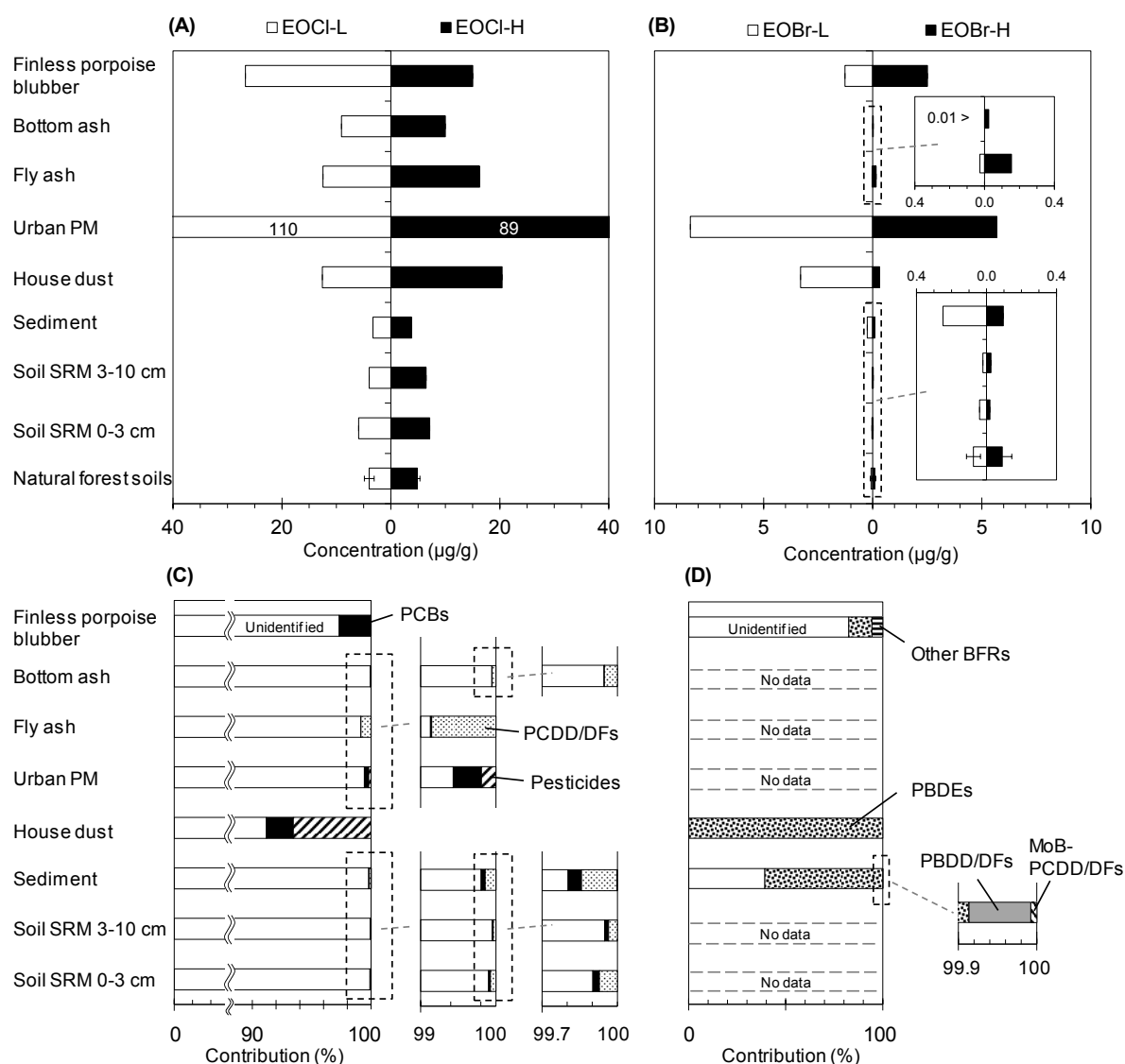

Figure 1. (A) Concentrations of EOCl-L (white bars) and EOCl-H (black bars). **(B)** Concentrations of EOBr-L (white bars) and EOBr-H (black bars). **(C)** Contributions of identified Cl from each individual compound in EOCl-L. **(D)** Contributions of identified Br from each individual compound in EOBr-L.

of EOBr-L+H was observed, whereas it was not detected in the sediment collected near a former chloralkali facility[1]. This indicates that Osaka Bay, the location where the sediment of this study was collected, was not particularly contaminated by wastes from chloralkali processes, but may have been contaminated by synthetic brominated compounds from industries. It is also possible that more natural OHCs are produced in Osaka Bay, as reported in a previous study on natural dioxins in the Seto Inland Sea (near Osaka Bay)[7]. The concentrations of EOCl-L+H (42 µg/g, wet weight) and EOBr-L+H (3.8 µg/g, wet weight) in finless porpoise blubber obtained in this study were similar to those reported for harbor porpoise from the Baltic sea (EOCl: 22–48 µg/g, wet weight; EOBr: 0.57–2.2 µg/g, wet weight)[8]. The concentrations of EOCl-L+H and EOBr-L+H observed in fly ash in this study were 29 µg/g and 0.2 µg/g, respectively. These are comparable to those of a previous report (EOCl: 4.98–24.17; EOBr: 0.21–2.53)[9]. The concentrations observed in bottom ash (EOCl-L+H: 19 µg/g; EOBr-L+H: 0.02 µg/g) were also similar to the literature values (EOCl: 2.75–17.73 µg/g; EOBr: not detected–0.21 µg/g)[9].

Ratio of EOX-L to EOX-H (EOX-L/H): The concentration of EOCl-L/H was 0.6–1.0, and that of EOBr-L/H was 0.3–10. This indicates that the molecular size distribution patterns of organobromine compounds ranged more widely than those of organochlorine compounds, according to the environments in which they existed. As many naturally produced OHCs are high molecular size compounds[10] and synthetic POP-like OHCs are generally low molecular size compounds, EOX-L/H can serve as a good indicator of the extent to which each sample is affected by human activity. EOBr-L/H was high in sediment, house dust, and urban PM, indicating that these samples were more affected by synthetic POP-like organobromine compounds.

Contributions of individual OHCs to EOX-L: The contributions of individual OHCs were calculated from the certified, reference, or reported concentrations of some individual compounds (Figure 1C, D). In forest soil, 12 polychlorinated biphenyl (PCB) congeners and polychlorinated dibenzo-*p*-dioxins/furans (PCDD/DFs) contributed less than 0.1% to EOCl-L. This is a relatively smaller contribution than for other samples, indicating that the impact of synthetic OHCs is not as high compared to that of naturally produced OHCs. Nevertheless, because both the identified Cl and EOCl-L were higher in surface soil than in undersoil, some unidentified OHCs from MSWI may have accumulated in the surface soil. In sediment, the 12 PCB congeners and PCDD/DFs contributed 0.2% to EOCl-L. Kannan et al. reported that organochlorine pesticides (dichlorodiphenyltrichloroethanes [DDTs] + hexachlorocyclohexane isomers [HCHs] + chlordane compounds [CHLs] + hexachlorocyclohexane isomers [HCB]), PCBs, PCDDs/DFs, and polychlorinated naphthalenes (PCNs) accounted for 48% of EOCl[1]. The difference is due to their high concentration of PCBs; they reported a concentration of ΣPCBs of 375 µg/g, which is 16,000 times larger than the concentration of the sediment in this study.[1] Without an extremely high contamination, more than 90% of the total is expected to be accounted for by unidentified natural compounds. Keppler et al. reported that halide ions can be alkylated during the oxidation of organic matter and that low molecular size halocarbons are produced naturally in soils and sediments.[11] On the other hand, 8 polybrominated diphenyl ether (PBDE) congeners, PBDD/DFs, and Monobrominated-PCDD/DFs contributed 61% to EOBr-L. This suggests that high concentrations of PBDEs cause higher concentrations of EOBr-L in sediment. In urban PM, 58 PCB congeners and 34 chlorinated pesticides contributed 0.57% to EOCl-L, although the concentration of ΣPCBs (717 µg/g) was even higher than that of contaminated sediment collected near a former chloralkali facility. This suggests the presence of unidentified organochlorine compounds that significantly contribute to the EOCl-L in urban PM. Xu et al. reported that known organochlorine compounds (HCH, DDT, DDE, DDD, heptachlor, chlordane, and PCBs) account for 0.04–0.7% and 0.06–0.3% of EOCl in PM2.5 and PM10, respectively.[12] This contribution is similar to our result, although we could not compare the result for EOX because of the difference in unit (ng m^{-3} and µg/g). Considering these results, high concentrations of unidentified organochlorine compounds may be common in aerosols. Volatile halogenated organic compounds (e.g., chlorofluorocarbons and other halocarbons) have been reported to be ubiquitous in the atmosphere.[13] EOCl-L seems to include these halocarbons (as an example).

In house dust, 58 PCB congeners and 34 chlorinated pesticides contributed 8.8% to EOCl-L. This suggests that the impact of PCBs and pesticides is greater in house dust than in other samples. The remaining 91.2% of EOCl-L may be derived from crushed fiber (e.g., polyvinyl chloride; PVC) and some unmeasured (e.g., chlorinated paraffin[14]) or unknown organochlorine compounds. The bromine mass balance in house dust was remarkable: nearly 100% of the EOBr-L could be explained by PBDEs, indicating that PBDEs are virtually the only organobromine compounds in house dust.

In fly ash and bottom ash, 12 PCB congeners and PCDD/DFs contributed 0.87% and 0.05%, respectively, to EOCl-L (12 μg/g). Because there is a lower possibility of naturally produced organochlorine compounds existing in ash, the unidentified Cl, accounting for more than 99% of the EOCl-L, is mainly derived from unmeasured (e.g., Cl-PAHs, PXDD/DFs, chlorinated benzenes) or unknown organochlorine compounds produced after combustion. Matsui et al. reported that Cl derived from identified dioxins accounts for less than 9% of the EOCl in fly ash and bottom ash. From these results, the combustion process is suggested to be an important source of unidentified organochlorine compounds.

In finless porpoise, 12 PCB congeners and PCDD/DFs contributed 2.7% to EOCl-L. This contribution is 13.5-fold greater than that of sediment (0.2%). In addition, EOCl-L was also present in greater amounts in finless porpoise blubber than in sediment. This indicates that both identified and unidentified organochlorine compounds bioaccumulate in blubber. PBDEs and other brominated flame retardants (BFRs) contributed 17% of the EOBr-L in blubber. This contribution is lower than that of sediment (61%). This may be because PBDEs and other BFRs are metabolized before accumulating in blubber. Metabolites of PBDEs and other naturally produced brominated compounds (e.g., bromophenols) are known to be accumulated in marine organisms.[2, 15] Kawano et al. reported that known EOCl (PCBs, DDTs, HCHs, and CHLs) accounted for 35% of EOCl in harbor porpoise in the Baltic Sea, whereas known EOCl (12 PCBs and PCDD/DFs) accounted for only for 1.7% of the EOCl-L+H in this study. This suggests that pesticides are more important for EOCl accumulation in porpoise. Kannan et al. reported that known EOBr (polybrominated biphenyls [PBBs], MeO-PBDEs, PBDEs, OH-PBDEs, and bis(diphenyl phosphates) [BRPs]) accounted for 0.08–0.1% of EOBr in livers of polar bear, albatross, and tuna, whereas known EOBr (PBDEs, tetrabromobisphenol A [TBBPA], hexabromocyclododecane [HBCD], 2,4,6-tribromophenol) accounted for 5.9% of EOBr-L+H in this study. This suggests that blubber accumulates known organobromine compounds more easily and has lower metabolizing ability than liver.

Conclusion: In this study, we showed that EOX-L is a more realistic value for assessing the potential of POP-like OHCs. By investigating the mass balance of identified/unidentified OHCs in EOX-L, we evaluated the impacts of known and unidentified POP-like OHCs in several environments. The potential of unidentified POP-like organochlorine compounds is high in urban PM, house dust, fly ash, and finless porpoise blubber, and the potential of unidentified POP-like organobromine compounds is high in finless porpoise blubber. To obtain a more comprehensive understanding of unknown OHCs and their ecotoxicological risks, we need to (1) analyze more individual compounds, (2) divide EOX-L into smaller fractions by its chemical and physical properties, and (3) measure the toxic potencies of each fraction.

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