# Persistent natural organohalogens in marine samples from Japanese coastal water

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## Introduction

Studies of pollutant impact on the marine environment have assumed that organic contaminants are anthropogenic in origin. In recent years, however, new bioaccumulative organohalogen compounds, proposed to be of natural origin, have been detected in marine biota from different sites throughout the world. One of them is mixed halogenated dimethyl bipyrroles (HDBPs) which have been first determined in seabird eggs from Canada in 1999[1] and then in marine mammals such as Dall's porpoises (*Phocoenoides dalli*) and harbor seals (*Phoca vitulina*)[2] as well as canned fish composites in Canadian markets[3]. Although the geographical source of HDBPs is poorly understood, these compounds were more abundant in marine samples from the North Pacific Ocean than the Atlantic Ocean[2]. In Japan, small cetaceans, i.e., odontocetes (beaked whales, dolphins, and porpoises) and mystecetes (mainly minke whales), have been hunted locally and their products are sold for human consumption[4]. In the present study we report the levels and profiles of HDBP congeners accumulated in Dall's porpoise (*Phocoenoides dalli*) collected from Sanriku, killer whales (*Orcinus orca*) stranded in Hokkaido and tuna fish from Japanese coastal water. The quantitative results of HDBPs were compared with those of major anthropogenic PCBs and polybrominated diphenyl ethers (PBDEs).

## Materials and Methods

**Sampling**. The blubber of Dall's porpoise (n=6) and tuna fish (n=4) were collected from retail outlets across Japan between 2001 and 2003. The blubber of killer whales was obtained from nine individuals (two males and seven females) stranded in northern Japan in 2005.

**Chemicals**. Two HDBP congeners, 1,1'-dimethyl-3,3',4,4'-tetrabromo-5,5'-dichloro-2,2'-bipyrrole ($Br_4Cl_2$-DBP) and 1,1'-dimethyl-3,3',4,4',5,5'-hexabromo-2,2'-bipyrrole ($Br_6$-DBP) were synthesized according to the method of Gribble *et al.*[1]. The spectroscopic data for both HDBPs were identical in all respects to the data reported previously[1]. $Br_3Cl_2$-, $Br_3Cl_3$-, and $Br_5Cl$-DBPs were obtained as synthetic byproducts during the chlorination or bromination process of 1,1'-dimethyl-2,2'-bipyrrole. 2,3,4,5,6,3',4',5'-Octachlorobiphenyl (CB205, AccuStandard, Inc.) was used as an internal standard (IS) for quantification of HDBPs in this study.

**Sample clean-up**. The procedure was performed according to a modification of our previous method[5]. The lipids were removed by gel permeation chromatography (Bio-Beads, S-X3, Bio-Rad Laboratories), with elution with *n*-hexane/dichloromethane (1:1) for organohalogen residues. The eluate containing organohalogens was concentrated to dryness and dissolved in *n*-hexane (1 mL), which was applied to an activated silica gel S-1 column (1 g, Wako Pure Chemical Industries Ltd., Japan), with elution with *n*-hexane (12 mL) for HDBPs, together with PCBs and PBDEs. The eluate was reduced to 500 µL and subjected to GC/MS.

**Identification and quantification.** Analyses of HDBPs, PCBs and PBDEs were performed using a gas chromatograph (Agilent 6980N) equipped with a mass-selective detector (5973*i*) in electron-ionization (EI) and selected ion monitoring mode for whales, whereas in electron capture negative ionization (ECNI) – selected ion (m/z 79, 81, 161 and 544) monitoring for tuna fish. An HP-5 column (30 m x 0.25 mm, i.d., 0.25 µm film thickness, J&W Scientific) was installed in the GC. In the full scan EI and ECNI modes, *m/z* 50 to 650 were recorded. Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min. The injector and transferline temperatures were 250°C and 280ºC, respectively. The GC oven program was as follows: After injection at 70ºC (1.5 min), the temperature was increased at 20ºC /min to 230ºC (2 min), then at 4ºC /min to 280ºC (20 min). Total run time was 35 min. The quantifications of $Br_4Cl_2$-DBP, BDE47 and CB153 were based on the relative response factors to IS.

## Results and Discussion

Figure 1 shows the GC/EI-MS total ion chromatogram of HDBP fraction from the blubber of Dall's porpoise collected in northern Japanese coastal water. By EI mass spectra, eight HDBP congeners were detected and identified as $Br_6$-, $Br_5Cl$-, $Br_4Cl_2$-, $Br_4Cl$-, $Br_3Cl_3$-, $Br_3Cl_2$-, $Br_2Cl_3$- and $Br_2Cl_2$-DBPs. The major congeners were $Br_4Cl_2$-DBP, contributing 75-85% of total HDBPs, followed by $Br_3Cl_2$-DBP. In the blubber of killer whales stranded in Hokkaido, similar congener patterns of HDBPs were observed. The ECNI ion monitoring at *m/z* 79 and 81 showed that several brominated contaminants were present in tuna fish from Japan and major components were $Br_4Cl_2$-DBP, followed by BDE47 and BDE100, as shown in Figure 2. Table 1 shows the levels of natural $Br_4Cl_2$-DBP and anthropogenic BDE47 and CB153. The mean level of $Br_4Cl_2$-DBP was the highest in killer whales, followed by Dall's porpoises and the lowest in tuna fish. Marine samples from different regions have benn shown to be contaminated with other natural products, namely 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole[6], 6-methoxy-BDE47 and 2'-methoxy-BDE68[7]. We found that these natural compounds were less distributed in Japanese marine samples investigated, although there were large variations in the levels, probably depending on the sampling location. Our recent survey indicated that HDBPs were not detected in the blubber of Baird's beaked whales (*Berardius bairdii*) from the Sea of Japan, as compared to the blubber of these whales from the Pacific Ocean which were highly contaminated HDBPs. Naturally occurring HDBPs may be produced by a marine bacterium (e.g. *Psuedoalteromonas*), as it was shown to produce a structurally similar compound 3,4,5,3',4',5'-hexabromo-2,2'-bipyrrole[8]. Mixed halogenation is rare for industrial synthesis of semivolatileorganohalogens but common for natural marine products, and the geographical distribution was dissimilar from that of PCBs. Further investigation may be required to understand the potential sources and biological effects of HDBPs.

Figure 1. GC/EI-MS chromatogram of HDBPs in the blubber of Dall's porpoise.

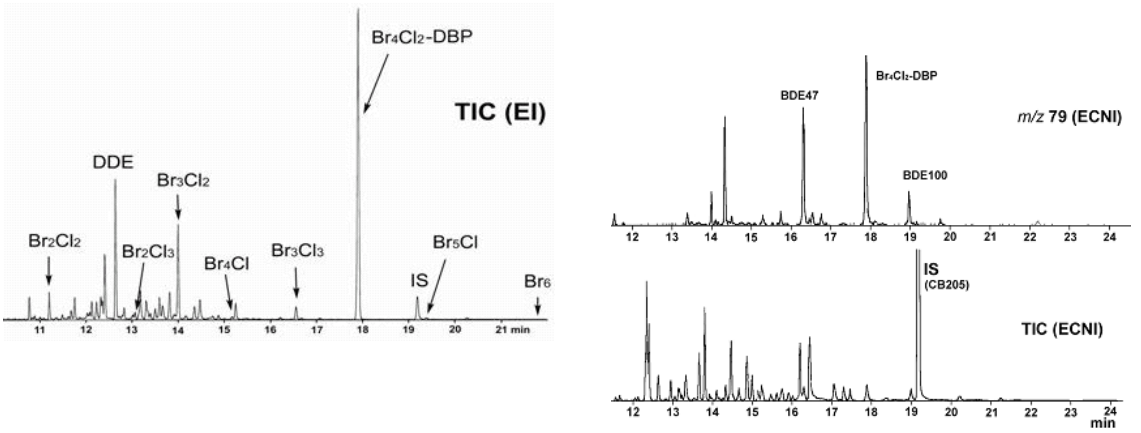


Figure 2. GC/ECNI-MS chromatograms of organohalogens in tuna fish

Table 1. Quantification of major organohalogens in Dall's porpoise, killer whale and tuna fish from Japanese coastal water.

| | Concentration (μg/g lipid)* | | |
|---|---|---|---|
| | $Br_4Cl_2$-DBP | BDE47 | CB153 |
| Killer whale (n=9) | 8.03 (3.29 – 20.1) | 0.86 (0.36 – 2.11) | 11.1 (3.10 – 17.4) |
| Dall's porpoise (n=6) | 3.55 (1.46 – 5.21) | 0.17 (0.07 – 0.22) | 0.39 (0.17 – 0.54) |
| Tuna fish (n=4) | 0.03 (0.01 – 0.06) | 0.04 (0.01 – 0.08) | 0.13 (0.06 – 0.15) |

*Values represent means (ranges).

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