CONTAMINATION STATUS OF BROMINATED FLAME RETARDANTS IN HARBOR (*PHOCOENA PHOCOENA*) AND DALL'S (*PHOCOENOIDES DALLI*) PORPOISES FROM HOKKAIDO, JAPAN

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Introduction

Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) have been extensively used in electrical and electronic equipments to reduce their flammability. Environmental contamination by BFRs is of public concern in recent years because of their similar physicochemical and toxicological characteristics to those of persistent organic pollutants (POPs). In 2009, Penta- and Octa- PBDE commercial mixtures were listed as POPs due to the growing evidence of their bioaccumulation potential and toxicity. Cetaceans are known to be susceptible to the effects of contaminant exposure because they feed high in the food web, have a long life span and low metabolic capacity. Although there are some studies on organochlorine compounds (OCs) and BFRs in dolphins and porpoises [1-8], information on accumulation features of PBDEs and HBCDs in small cetaceans from Japanese coastal waters is still limited. Harbor porpoise (*Phocoena phocoena*) is a small coastal porpoise endemic to northern temperate and subarctic waters whereas Dall's porpoise (*Phocoenoides dalli*) inhabits continental shelf and slope and offshore waters of the temperate North Pacific. Although the number of reports on stranding and incidental mortality by fishing is increasing in the recent years, information on ecotoxicological effects of BFRs as well as POPs is scarce so far. In the present study, stranded or by-caught harbor and Dall's porpoises were collected from Hokkaido, Japan to investigate the contamination status, accumulation features and temporal trends of BFRs (PBDEs and HBCDs) and PCBs.

Materials and methods

Sample collection

Harbor porpoise (1985-2010; male *n*=33, female *n*=5, unknown *n*=4) and Dall's porpoise (1980-2010; male *n*=46, female *n*=6), either stranded or by-caught, were collected from Hokkaido and northern part of Japan (Figure 1). Blubber was excised from the animal, and stored at –25°C in Environmental Specimen Bank (*es*-BANK) of Ehime University until chemical analysis.

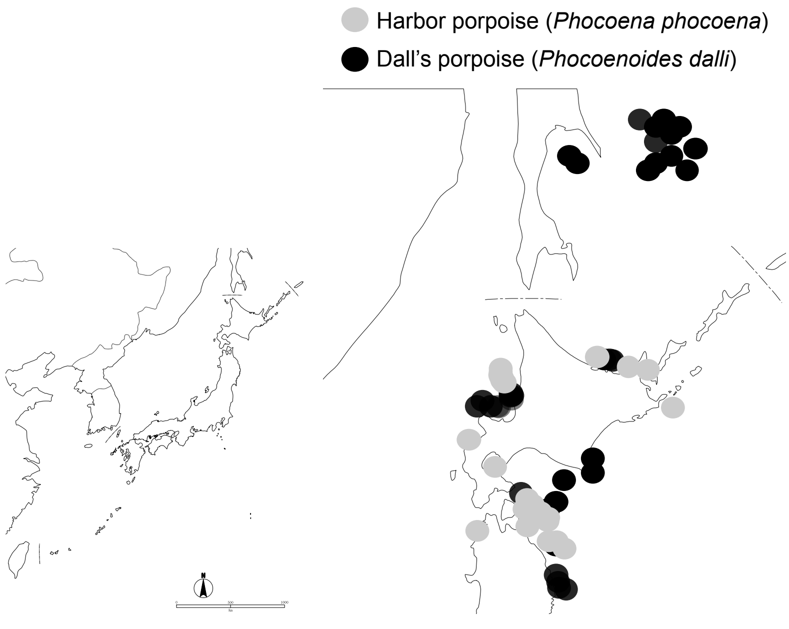

Figure 1. Sampling locations of harbor and Dall's porpoises

Chemical analysis

Analysis of BFRs (PBDEs and HBCDs) and PCBs were carried out following the procedures described in the previous studies [3, 9]. Briefly, 2-5 g of blubber was extracted with hexane/acetone (1:1, v/v) using a

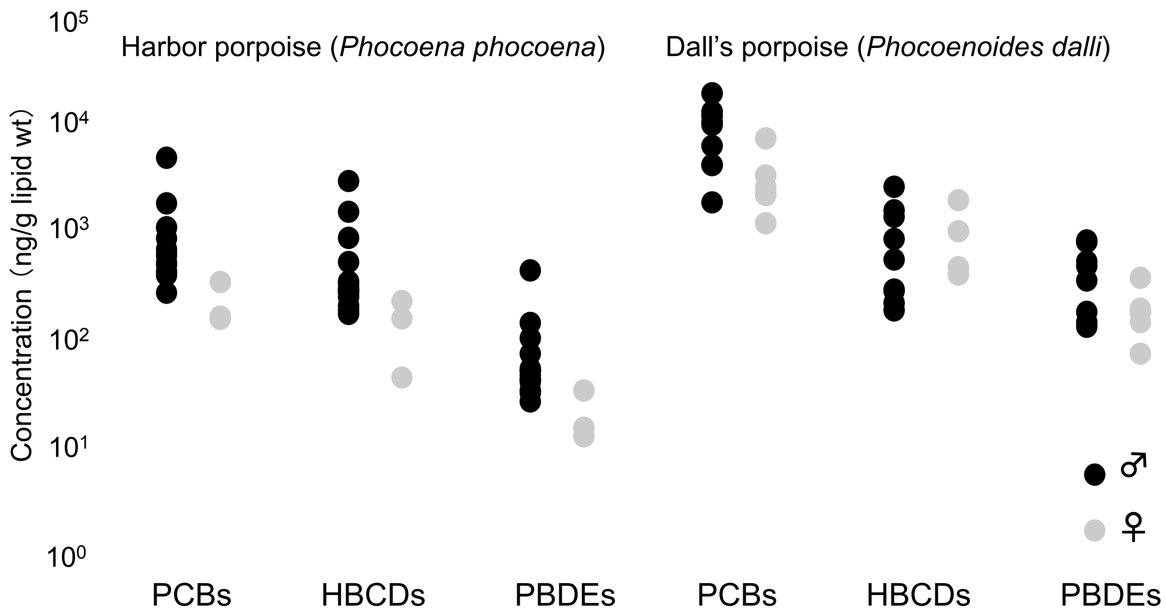

Figure 2. Concentrations of PCBs, HBCDs and PBDEs in harbor and Dall's porpoises stranded along the coast of Hokkaido, Japan in 2007-2008

high-speed solvent extractor (SE-100, Mitsubishi Chemicals). For PCBs, PBDEs and HBCDs analysis, the extract was spiked with surrogates ($^{13}C_{12}$-PCBs, $^{13}C_{12}$-PBDEs and $^{13}C_{12}$-HBCDs) and purified with a gel permeation chromatography (Bio-Beads S-X3, Bio-Rad) and fractionated with an activated silica gel column chromatography (Wako gel DX, Wako Pure Chemicals). The fraction containing PCBs and PBDEs was spiked with $^{13}C_{12}$-BDE-139 as an internal standard and subjected to GC-MS analysis. The HBCDs fraction was evaporated and spiked with HBCDs-d_{18} prior to LC-MS/MS analysis. Concentrations of analytes were expressed as ng/g lipid weight unless stated otherwise.

Results and discussion

Contamination status

All the target compounds were detected in all the blubber samples of harbor and Dall's porpoises stranded along Hokkaido, Japan. Concentrations of analytes in the specimens stranded in 2007–2008 are summarized in Fig. 2. Among the three contaminants, PCBs ranked first followed by HBCDs and PBDEs. Levels of PCBs and PBDEs were in the same range as those reported for other cetaceans from Japanese and Asian coastal waters [1, 2, 4]. On the other hand, HBCDs levels in harbor and Dall's porpoises were higher than those in other Asian coastal dolphins and porpoises. The extensive usage and subsequent discharge into the environment in Japan may be a possible reason for the high HBCD levels found in the present study. Between the two target species of this study, the levels of PCBs, PBDEs and HBCDs in Dall's porpoise were higher than those in harbor porpoise ($p < 0.05$). Since Dall's porpoise is an oceanic species and migrates mainly from Okhotsk to Japan Sea, this species could be exposed to contaminants released from countries surrounding Japan Sea. Considering these facts, the existence of pollution sources of PBDEs in the Asian region, apart from those in Japan is suspected. The observed levels of PCBs and PBDEs in harbor and Dall's porpoises were lower than finless porpoise (*Neophocaena phocaenoides*) from the Seto Inland Sea [1]. Since finless porpoise usually inhabits coastal and enclosed waters close to cities and industrial areas, they might be exposed to a variety of chemicals and accumulate lipophilic compounds. Harbor porpoise in this study, however, had lower HBCD, PBDE and PCB levels than those in Dall's porpoise even though they inhabit in shallow coastal waters without long range migration. Although the reason for the low level of organohalogen contaminants in harbor porpoise is still unclear, the fact that the areas where they were found stranded are not characterized by heavy human activities may partially account for this difference. Interestingly, levels of HBCD in Dall's porpoise were significantly higher than finless porpoises from the Seto Inland Sea (Mann-Whitney's U-test). Possible contamination source of HBCDs to Dall's and harbor porpoise could be located in northern region of Japan or surrounding countries of Japan Sea.

Temporal trend

To evaluate the temporal trends of contamination by PBDEs, HBCDs and PCBs, blubber samples of harbor and Dall's porpoises collected in 1980–2010 and archived in *es*-BANK of Ehime University were analyzed. To avoid the effect of gender and age, only adult male specimens were selected for the discussion. Concentrations of PCBs in both harbor and Dall's porpoises did not change significantly during this period. Reports suggest that the temporal trends in PCB levels vary among locations and media. Borrell et al (2007) [10] and Braune et al (2005) [11] reported that PCB levels decreased from 1970s to 2000s. On the other hand, there are some reports that suggest no significant trend in PCBs concentrations [1, 7, 12]. Relatively constant levels in this study may suggest continuous discharge of PCBs into the aquatic ecosystem. Although the production and use of PCBs were banned about 40 years ago in Japan, small leakage of PCBs from stockpile of old transformers and capacitors is reported. Levels of PBDEs in the harbor porpoise did not show an increasing trend, whereas levels in Dall's porpoise significantly increased from 3-11 ng/g lipid in 1980 to 123-375 ng/g lipid in 2008. The significant temporal increase of PBDE levels in Dall's porpoises indicates the continuous use and subsequent environmental load of PBDEs in recent decades. In addition, harbor porpoise showed higher levels of PBDEs even in the year of 1985, whereas the levels in Dall's porpoise were lower in 1987 than harbor porpoise and increased continuously until 2008. This result implies that the difference in habitat and feeding behavior affects the pollution levels. The percentage contribution of Hexa-BDE, which was defined as the ratio of BDE-153+154 to total PBDEs, increased during the study period, while the contribution of lower brominated congeners decreased. This might be due to the increase in demand of the higher brominated BDE commercial mixture. Similar PBDE congener profile was observed in northern fur seals (*Callorhinus ursinus*) from Japan [13], and Japanese human adipose tissue [14]. HBCDs levels showed continuous increasing trends during the study period for both porpoises. Interestingly, the slope of linear regression line for HBCD concentrations in Dall's porpoises was greater than that of harbor porpoises. In Japan, HBCDs have been extensively used since the mid-1980s and the consumption is still increasing. This may indicate the higher transportability of HBCDs than PBDEs from pollution sources to open ocean.

Acknowledgements:

Sample collection was carried out under cooperation of Stranding Network Hokkaido. This research was financially supported by Grant-in-Aid for Scientific Research (S: 20221003, B: 21310043, young scientist B: 23710077) from Japan Society of the Promotion of Science, "Global Center of Excellence Program" and "Promotion of Environmental Improvement for Independence of Young Researchers" under the Special Coordination Funds for Promoting Science and Technology by the Ministry of Education, Culture, Sports, Science and Technology, and the Targeted Project of National Museum of Nature and Science, "Marine Mammal Researches Utilizing Stranded Carcasses".

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