

# LEVELS OF ORGANOHALOGEN CONTAMINANTS AND PARASITIC INFECTION IN FINLESS PORPOISES FROM JAPAN

Isobe T<sup>1</sup>, Oshihoi T<sup>1</sup>, Hamada H<sup>1</sup>, Yamada TK<sup>3</sup>, Tajima Y<sup>3</sup>, Nakayama K<sup>1</sup>, Tanabe S<sup>1</sup>

<sup>1</sup> Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matuyama 790-8577, Japan; <sup>2</sup> Senior Research Fellow Center, Ehime University; <sup>3</sup> National Museum of Nature and Science, Hyakunin-cho 3-23-1, Shinjuku-ku, Tokyo 169-0073, Japan

## Introduction

Brominated flame retardants (BFRs) such as PBDEs and HBCDs have been extensively used in electrical and electronic equipments to reduce their flammability. Concern over PBDEs and HBCDs has increased in recent years due to their physico-chemical and toxicological similarities to organochlorine compounds (OCs). Although many reports on the environmental behavior and fate of PBDEs have been published <sup>1-3</sup>, only limited information on HBCDs in wildlife is available so far <sup>4-6</sup>. Marine mammals are considered to be particularly susceptible to the exposure of contaminants because they feed high in the food chain and have long life span and low metabolic capacity. In the present study, we collected stranded finless porpoises (*Neophocaena phocaenoides*) from Seto Inland Sea and Omura Bay in Japan, and measured concentrations of BFRs (PBDEs and HBCDs) concentrations and OCs (PCBs, DDTs, CHLs, HCB and HCHs). From the levels of chemicals, their contamination status, accumulation feature and temporal trend were investigated. In addition, relationship between concentrations of organohalogens and infection status of lung nematodes and liver trematodes was investigated to evaluate the adverse effects of organohalogen contaminants on the health of porpoises.

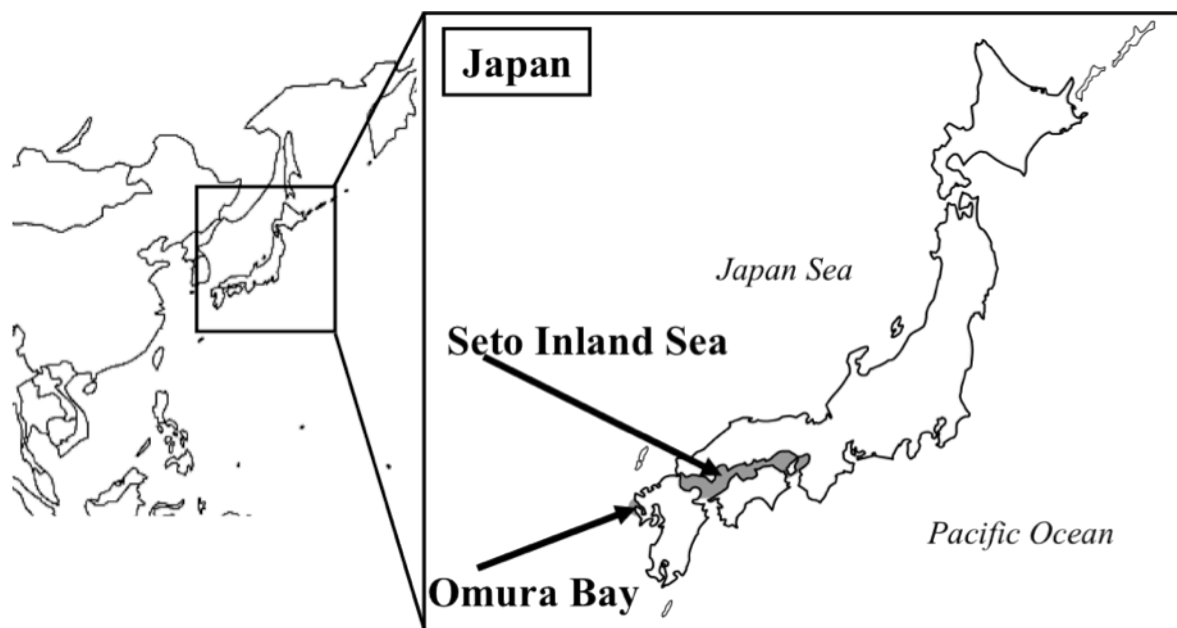
## Materials and Methods

### *Samples*

Finless porpoises (*Neophocaena phocaenoides*) samples, either stranded or by-caught, were collected from Seto Inland Sea (2000-2007; males  $n=31$ , females  $n=14$ ) and Omura Bay (2005-2007; males  $n=9$ ), Japan (Figure 1). Blubber samples were excised from the animals, and stored at the Environmental Specimen Bank (es-BANK) of Ehime University at -25°C until chemical analysis.

### *Chemical analysis*

Analysis of BFRs (PBDEs and HBCDs), PCBs and organochlorine pesticides (OCPs - DDTs, CHLs, HCB



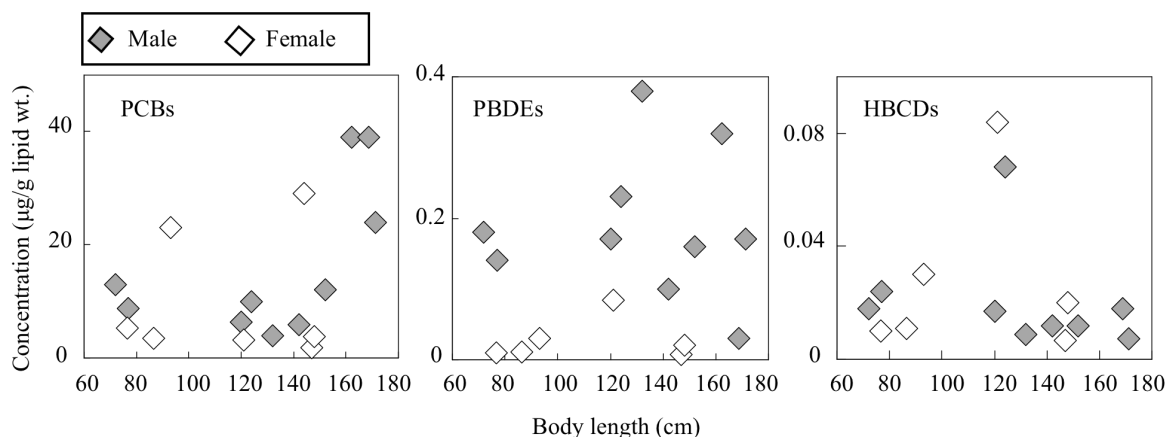
**Figure 1.** Sampling locations of finless porpoises in the present study

and HCHs) were carried out following previous reports <sup>7-9</sup>. Briefly, 2-10 g of tissue sample was extracted with hexane/acetone (1:1, v/v) using a high-speed solvent extractor. For PCBs, PBDEs and HBCDs analysis, the extract was spiked with surrogates (<sup>13</sup>C<sub>12</sub>-PCBs, <sup>13</sup>C<sub>12</sub>-PBDEs and <sup>13</sup>C<sub>12</sub>-HBCDs) and purified with gel permeation chromatography (GPC) and fractionated with an activated silica gel column chromatography. The fraction containing PCBs and PBDEs was spiked with <sup>13</sup>C<sub>12</sub>-BDE-139 as an internal standard and subjected to GC-MS analysis. The HBCDs fraction was evaporated and spiked with HBCDs-*d*<sub>18</sub> prior to LC-MS/MS analysis. For analysis of OCPs including DDTs, HCHs, CHLs, and HCB, another aliquot of the Soxhlet extract was purified and fractionated using a GPC and an activated florisil column. Identification and quantification of OCPs was performed using a GC-ECD without recovery correction. Concentrations of analytes were expressed as ng/g lipid weight unless stated otherwise.

## Results and Discussion

### Contamination Status

PBDEs and HBCDs were detected in all the blubber samples analyzed in the present study, suggesting ubiquitous contamination by BFRs. To our knowledge, this is the first report on HBCDs in finless porpoises from Seto Inland Sea and Omura Bay. Levels of PCBs, DDTs and CHLs were significantly higher than HCHs, HCB, PBDEs and HBCDs. OCs and BFRs levels in Seto Inland Sea were in the same ranges as in Omura Bay.

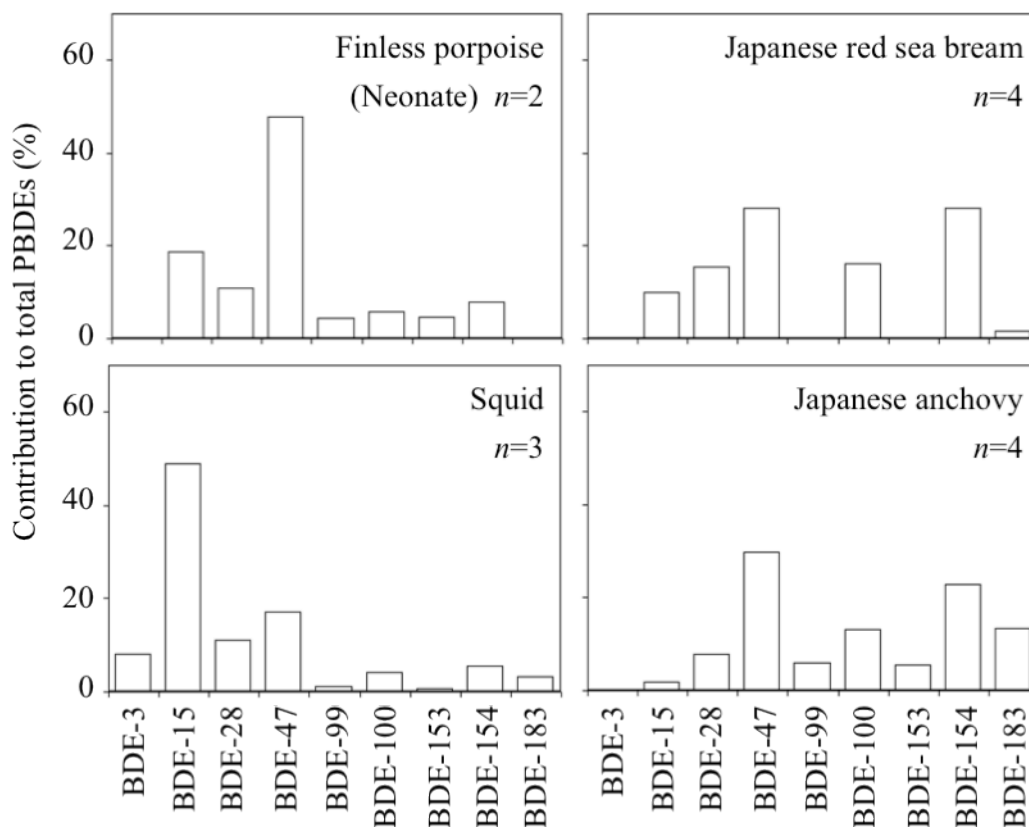


**Figure 2.** Relationship between contaminant levels in liver and body length

For blubber samples, concentrations of PBDEs and HBCDs, as well as OCs in males increased with body length ( $p < 0.05$ ), while those in females did not show any trend, probably due to excretion of these contaminants from their bodies via lactation and gestation. On the other hand, no increasing trend with body length was observed for concentrations of analytes in liver samples (Figure 2).

Among analyzed 14 PBDE congeners, BDE-47 was the dominant in all stages of growth, i.e., neonate, juvenile and adult (Figure 3), which is consistent with other reports on cetaceans<sup>10-14</sup>. The proportions of lower brominated congeners found in neonates and juveniles were higher than those in adults. This suggests that lower brominated congeners are easily transferred from mother to neonate due to the relatively smaller molecular size and higher water solubility of the lower brominated congeners<sup>15,16</sup>. BDE-15 was detected in finless porpoises from Seto Inland Sea and its proportion in total PBDEs was 19 and 9 % in neonate and juvenile, respectively, while this isomer was not found in the specimens from Omura Bay. Furthermore, low level or below the detection limit of BDE-15 in cetaceans from Japanese coastal waters was previously reported<sup>5,16,17</sup>. Full scan mode analysis was performed to confirm the identification of BDE-15. As a result, we found complete agreement of mass spectrum for the respective peak in each sample with that in standard solution. In addition, we also analyzed fish species from Seto Inland Sea on which porpoises feed, and BDE-15 was detected in those samples also (Figure 3). Those data suggests the presence of specific contamination sources of BDE-15 in Seto Inland Sea rather than metabolism in the bodies of porpoises.

To elucidate the temporal trends of contamination by BFRs and OCs, blubber samples of males collected during 2000-2007 were analyzed. In this study, concentrations of BFRs and OCs in males increased with body length as described above. To normalize the concentrations of analytes in blubber with body length, we calculated multiple regression models as following equations.



**Figure 3.** Congener profiles of PBDEs in finless porpoises and fishes from Seto Inland Sea

$$\text{Log} [\Sigma \text{PCBs}] = 0.0092 \times [\text{Body Length}] - 0.0068 \times [\text{Sampling Year}] + 16 \quad (R^2=0.54)$$

$$\text{Log} [\Sigma \text{DDTs}] = 0.0094 \times [\text{Body Length}] - 0.077 \times [\text{Sampling Year}] + 160 \quad (R^2=0.54)$$

$$\text{Log} [\Sigma \text{CHLs}] = 0.0094 \times [\text{Body Length}] - 0.046 \times [\text{Sampling Year}] + 94 \quad (R^2=0.63)$$

$$\text{Log} [\Sigma \text{HCHs}] = 0.0094 \times [\text{Body Length}] - 0.046 \times [\text{Sampling Year}] + 94 \quad (R^2=0.47)$$

$$\text{Log} [\Sigma \text{HCB}] = 0.00081 \times [\text{Body Length}] - 0.057 \times [\text{Sampling Year}] + 120 \quad (R^2=0.32)$$

$$\text{Log} [\Sigma \text{PBDEs}] = 0.0098 \times [\text{Body Length}] - 0.027 \times [\text{Sampling Year}] + 55 \quad (R^2=0.58)$$

$$\text{Log} [\Sigma \text{HBCDs}] = 0.010 \times [\text{Body Length}] + 0.0068 \times [\text{Sampling Year}] - 13 \quad (R^2=0.19)$$

Concentrations of DDTs, CHLs, HCB and HCHs decreased continuously during the study period ( $p < 0.05$ ). These trends imply the decrease in the usage and consequent environmental discharge of organochlorine contaminants into the Seto Inland Sea. On the other hand, concentrations of PBDEs and HBCDs did not show any temporal trend from 2000 to 2007, suggesting continuous usage and environmental contamination by

PBDEs and HBCDs. This phenomenon was also observed in cetaceans stranded around Japan <sup>5</sup>. The present increasing usage of BFRs in the world may further accelerate the contamination of ecosystems. No significant decreasing trend for PCBs suggest that this compound has been continuously released into the environment, mainly from old transformers and capacitors, although the production of PCBs has been banned in Japan since about 40 years ago.

#### *Relationships between contaminants concentrations and parasitic infection*

The immunotoxic effects of environmental contaminants on marine mammals have been reported elsewhere <sup>18-22</sup>. Bull et al. (2006) found a significant positive correlation of PCBs levels in the blubber with nematode burden in the cardiac stomach of harbor porpoises. In a previous study, we also reported significant association between butyltin (BTs) levels and parasitic infectious status of lung nematode in finless porpoises from Seto Inland Sea <sup>23</sup>. Therefore, the relationships between levels of BFRs and OCs and status of parasitic infection status in finless porpoise were examined. Lung nematode (*Halocercus* sp.) and hepatic trematode (*Campulla* sp.) were observed pathologically in 15 individuals from Seto Inland Sea. The animals of the present study were categorized into two classes based on the infectious status as follows: *infected* (considered to have serious levels of parasitic infection) or *not infected* (absent or negligible levels of parasites). Although the difference in the concentrations between infected and not infected groups was not statistically significant, the highest concentrations as well as mean values were detected in the specimens categorized as lung nematode infected group (Table 1). This may suggest that porpoises with high levels of contaminants could have high levels of parasitic infection. A previous study revealed adverse effects of PCBs on infectious status

**Table 1.** Parasitic infection status and contaminant levels in blubber (ng/g lipid wt.)

Gorups	n	Year	length(cm)	PCBs	DDTs	CHLs	PBDEs	HBCDs
<b>Lung nematode</b>								
Not-infected	7	2000-2007	100 (72-144)	6600 (3400-8300)	3500 (860-5700)	1800 (500-3300)	150 (66-290)	160 (14-740)
Infected	8	2000-2007	130 (79-178)	13000 (5400-46000)	10000 (710-57000)	2600 (500-8700)	400 (34-1000)	240 (7.2-1200)
<b>Liver trematode</b>								
Not-infected	9	2000-2007	110 (72-159)	7800 (3400-17000)	9400 (860-57000)	1900 (500-4400)	280 (66-820)	140 (14-740)
Infected	6	2002-2007	120 (79-178)	14000 (2000-46000)	3400 (710-7100)	2800 (500-8700)	300 (34-1000)	310 (7.2-1200)

using the results of analysis of 224 specimens<sup>22</sup>, while we could get only 15 individuals. Therefore, further studies on the relationship between contamination levels and porpoise health with large number of specimens to elucidate the adverse effects of chemicals are warranted.

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