Contamination Status and Body Distribution of Organohalogen Compounds in Striped Dolphins (*Stenella Coeruleoalba*) Stranded at Gogo-shima Island, Matsuyama, Japan

Tomohiko Isobe [1], Yoko Ochi [1], Karri Ramu [1], Takahito Yamamoto [2], Shin Takahashi [1], and Shinsuke Tanabe [1]

[1] Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan
[2] Department of Natural History, Ehime Prefectural Science Museum, 2133-2 Ohjohin, Niihama 792-0060, Japan

Introduction

Mass mortality of seven adult striped dolphins (*Stenella coeruleoalba*) was found at Gogo-shima Island, Matsuyama, Japan in 2003. Some parasites were found in all the six analyzed specimens by pathological diagnosis, although the main cause of death was undetermined. A goiter was also observed in three of the six individuals, suggesting thyroid hypofunction by anthropogenic contaminants accumulated in the dolphins (Makara et al. 2006). It is well known that some of the organochlorines (OCs), brominated flame retardants (BFRs) and their metabolites have immunotoxic or endocrine disrupting potential. OCs including PCBs and organochlorine pesticides such as DDTs were extensively used until the early-1970s in Japan. Because of persistence and lipophilicity of these compounds, their ubiquitous contamination and extreme biomagnification in marine mammals have been reported. Concern about environmental contamination by BFRs, especially by polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs), have increased in recent years due to their persistence, bioaccumulative nature, and possible adverse effects on humans and wildlife. Both chemicals are used as additive flame retardants in a wide variety of commercial products such as plastics, textiles, and electronic appliances including computers, and televisions. Statistical data demonstrated that Asian countries shared about 40% and 23% of the global PBDEs and HBCDs consumption in 2001, respectively (BSEF 2003). Although many reports on the environmental behavior and fate of PBDEs have been published (Hites 2004, Kajiwara et al. 2004, Rahman et al. 2001, Ueno et al. 2004), only limited information on other BFRs such as HBCDs are available so far (Covaci et al. 2006). In this regard, OCs and BFRs were analyzed in striped dolphins found stranded at Gogo-shima Island to evaluate the present status of contamination and toxicological risks by organohalogen compounds.

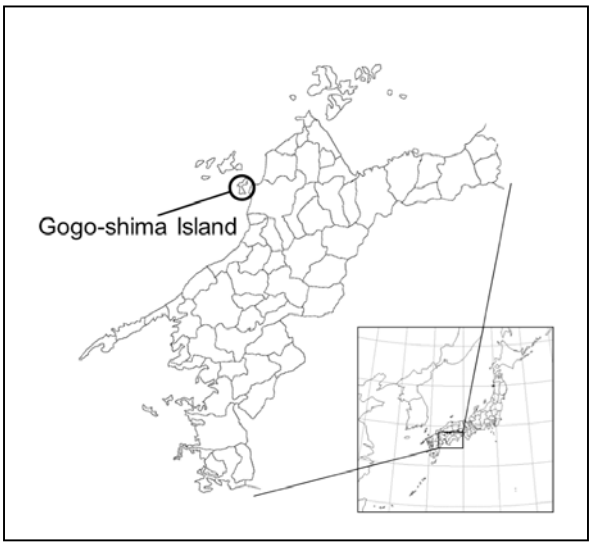

Figure 1. Sampling location

Table 1. Concentrations of organochlorines and brominated flame retardants in striped dolphins collected from Gogo-shima Island, Matsuyama, Japan

	SC-1	SC-2	SC-3	SC-4	SC-5	SC-6
Sex	M	F	M	M	M	M
BL (cm)	231	223	238	232	231	237
BW (kg)	155	105	150	155	135	150
Fat (%) in blubber	56	56	58	50	44	54
Moisture (%) in liver	67.3	70.2	68.1	69.0	69.9	68.9
Organohalogens in blubber (ng/g-lipid)						
PBDEs	520	84	530	850	650	610
HBCDs	700	290	580	940	700	620
PCBs	28000	3200	26000	42000	32000	36000
DDTs	52000	3500	44000	88000	56000	67000
CHLs	8700	730	7300	12000	9100	11000
HCB	410	53	340	540	440	620
HCHs	400	40	300	490	380	520

Materials and Methods

Samples

Five male and one female striped dolphin specimens analyzed in this study were found stranded at Gogo-shima Island, Matsuyama, Japan in 2003 (Fig. 1). Biological data of the animals analyzed are given in Table 1. Blubber samples were excised from the dead animals, wrapped in aluminum foil, and stored at –20 ˚C until chemical analysis.

Chemical analysis

Analysis of OCs and BFRs were performed following the procedures described elsewhere with slight modifications. Briefly, 2-10 g (wet wt) of the sample was ground with anhydrous sodium sulfate and Soxhlet extracted with diethyl ether/hexane (3:1, v/v) for 7-8 h. An aliquot of the extract, after spiking $^{13}C_{12}$-BDEs ($^{13}C_{12}$-BDE-3, 15, 28, 47, 99, 153, 154, 183, 197, 207, 209) and $^{13}C_{12}$-HBCDs (α-, β-, γ-$^{13}C_{12}$-HBCD), was loaded to a gel permeation chromatography (GPC, Bio-Beads S-X3, Bio-Rad, CA, 2 cm i.d., x 50 cm) column for lipid removal. The GPC fraction containing BFRs was concentrated and subjected to an activated silica gel column (Wakogel DX, 4 g, Wako Pure Chemicals, Tokyo) for clean-up and fractionation. PBDEs were eluted with 80 ml of dichloromethane/hexane (5:95, v/v) from the silica gel column and HBCDs were eluted with following 100 ml of dichloromethane/hexane (25:75, v/v). $^{13}C_{12}$-BDE-139 was spiked as an internal standard and subjected to GC-MS analysis. Concentrations of all the targeted BDE congeners, except BDE-209, were summed to obtain concentration of ΣPBDEs. The HBCDs fraction was evaporated and spiked with HBCD-d_{18} (α-, β-, γ-HBCD-d_{18}) prior to LC-MS-MS analysis. The diastereoisomer specific analysis was performed on the basis of the reported analytical method (Tomy et al., 2005). For analysis of OCs including PCBs, 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 OCs were performed using a GC-ECD. Concentrations of analytes were expressed as ng/g in lipid weight unless stated elsewhere.

Table 2. Reported concentrations of organohalogens in blubber of marine mammals from Asia (μg/g-lipid)

Species*	PCBs**	DDTs**	PBDEs**	HBCDs**	
Japan					
Striped dolphin (2003)	27 (3.2-42)	52 (3.5-88)	0.54 (0.084-0.85)	0.64 (0.29-0.94)	This study
Finless porpoise (1998-2000)					
Seto Inland Sea	120 (44-370)	76 (19-270)	0.73 (0.41-1.3)	na	Kajiwara et al. 2006a
Pacific Coast	29 (13-45)	32 (11-52)	0.047 (0.042-0.052)	na	
Harbor porpoise (1999)	2.2 (0.86-3)	3.3 (1.1-5.0)	0.087	na	
Dall's porpoise (2000)					
truei-type	9.0 (6.8-13)	11 (7.0-18)	0.087 (0.083-0.089)	na	
dalli-type	18 (12-23)	31 (21-41)	0.086 (0.080-0.099)	na	
Melon-headed whale (2001)	24 (15-30)	27 (18-33)	0.068 (0.063-0.074)	0.39 (0.33-0.46)	
Pacific white-sided dolphin (1999)	8.7 (1.3-16)	14 (1.5-26)	0.069 (0.046-0.084)	na	
Stejneger's beaked whale (2000/1)	19 (17-20)	110 (83-140)	0.071 (0.049-0.092)	na	
Killer whale (2005, mature male)	57	220	0.27	na	Kajiwara et al. 2006b
Northern fur seal (1998)	2.2 (1.5-3.0)	1.3 (0.62-2.7)	0.030 (0.022-0.038)	0.024 (0.012-0.033)	Kajiwara et al. 2004
Hong Kong					
Finless porpoise (2000/1)	13 (1.4-28)	120 (10-260)	0.6 (0.23-0.98)	0.013 (0.021-0.055)	Ramu et al. 2006
Indo-Pacific humpback dolphin (97-01)	45 (9.4-83)	190 (51-470)	1.9 (0.28-6.0)	0.11 (0.028-0.38)	

* collected year in parenthesis

** range in parenthesis

na; not analyzed

Results and Discussion

Contamination status

Concentrations of OCs and BFRs detected in the blubber of striped dolphins are listed in Table 1. Concentrations of all the analytes were significantly lower in the female dolphin than those in males, suggesting transfer of these contaminants from mother to calf during lactation. Among the organohalogen compounds analyzed in this study, DDTs and PCBs were the predominant contaminants in the striped dolphins, ranging from 3,200 to 42,000 and 3,500 to 88,000 ng/g lipid wt., respectively. Levels of OCs and BFRs were in the same range of other marine mammals collected around Japan as shown in Table 2 (Kajiwara et al. 2004, 2006a, 2006b, Ramu et al. 2006). Concentrations of PCBs detected in striped dolphins exceeded the threshold levels for immunosuppression in harbor seal (*Phoca vitulina*) suggesting some adverse effect in the dolphins, although there was no direct evidence of this (de Swart et al. 1995). Concentrations of PBDEs and HBCDs ranged from 84 to 850 and 290 to 940 ng/g lipid wt., respectively. Levels of PBDEs in striped dolphins were higher than those in other cetaceans reported from Japan, with an exception of finless porpoises (*Neophocaena phocaenoides*) from the Seto Inland Sea. HBCDs levels in the specimens of the present study were also higher than those in cetaceans from other regions in Asia-Pacific, probably due to intensive usage in recent years and high consumption in Japan among Asian countries. An interdisciplinary approach from environmental chemistry, toxicology, and pathology is essential to elucidate the reasons for mass stranding of striped dolphins.

Body distribution

Body distribution of PBDEs and HBCDs in the striped dolphins was evaluated. No significant difference between the concentrations in blubber, liver and muscle was observed, whereas concentrations in brain were significantly lower than those in other tissues. This may be due to the existence of the blood-brain barrier.

Congener specific accumulation

Of the fourteen PBDE congeners analyzed, a total of thirteen congeners from di- to deca-BDE were identified in all the tissues of striped dolphins. Contributions of BDE-154 and BDE-153 to total PBDEs were higher than those generally reported in biological samples (Ikonomou et al. 2002). This might be due to the fact that octa-BDE mixture was used more extensively than tetra-BDE mixture in Japan. On the other hand, α-HBCD was the predominant isomer among the three HBCD isomers. Most of the previous studies, which analyzed isomeric pattern of HBCDs in biological samples from high trophic levels, also reported a higher proportion of α-HBCD as summarized in a review paper (Covaci et al. 2006).

Acknowledgments

Dissection of stranded dolphins was conducted with the cooperation of Drs T. K. Yamada and Y. Tajima (National Science Museum) and many other scientists and volunteers. This research was partly supported by the Global Environment Research Fund (RF-064) and the Waste Management Research Grant (K1821) from the Ministry of the Environment, Japan and by Scientific Research (Project No. 12308030) of Japan Society for the Promotion of Science (JSPS). Financial support was also brought by Grant-in-Aid for Scientific Research (A) (No. 16201014) and (B) (No. 18310046) and 21st Century COE program of the Japanese Ministry of Education, Science, Sports, Culture and Technology and JSPS.

References

BSEF. 2003. http://www.bsef.com/.

Covaci A, Gerecke AC, Law RJ, Voorspoels S, Kohler M, Heeb NV, Leslie H, Allchin CR, De Boer J. 2006. Environ Sci Technol 40, 3679-88.

de Swart RL, Ross PS, Timmerman HH, Hijman WC, de Ruiter EM, Liem AK, Brouwer A, van Loveren H, Reijnders PJ, Vos JG, Osterhaus ADME. 1995. Chemosphere 31, 4289-306.

Hites RA. 2004. Environ Sci Technol 38, 945-56.

Ikonomou MG, Rayne S, Fischer M, Fernandez MP, Cretney W. 2002. Chemosphere 46, 649-63.

Kajiwara N, Kamikawa S, Ramu K, Ueno D, Yamada TK, Subramanian A, Lam PK, Jefferson TA, Prudente M, Chung KH, Tanabe S. 2006a. Chemosphere 64, 287-95.

Kajiwara N, Kunisue T, Kamikawa S, Ochi Y, Yano S, Tanabe S. 2006b. Mar Pollut Bull 52, 1066-1076.

Kajiwara N, Ueno D, Takahashi A, Baba N, Tanabe S. 2004. Environ Sci Technol 38, 3804-9.

Makara M, Shimada A, Tajima Y, Yamada TK, Tanabe S. 2006. In 17th The Cetology Study Group of Japan, Shinjuku, Japan, p 18.

Rahman F, Langford KH, Scrimshaw MD, Lester JN. 2001. Sci Total Environ 275, 1-17.

Ramu K, Kajiwara N, Lam PK, Jefferson TA, Zhou K, Tanabe S. 2006. Environ Pollut 144, 516-523.

Ueno D, Kajiwara N, Tanaka H, Subramanian A, Fillmann G, Lam PK, Zheng GJ, Muchitar M, Razak H, Prudente M, Chung KH, Tanabe S. 2004. Environ Sci Technol 38, 2312-6.