Synthetic musk fragrances in marine mammals and other aquatic organisms from Japanese coastal waters

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Introduction

Synthetic musk fragrances have been used in a wide variety of personal care products, detergents, and household cleaners in the world. In recent years, it has been reported that several nitro and polycyclicmusks are present in aquatic environment, such as water, sediment, crustacean, mussel and fish samples[1-3]. These results suggest that synthetic musks have a potential for bioaccumulation in aquatic system. However, little investigation has focused on the residue levels of synthetic musks in top predators of the marine food web, such as marine mammals.

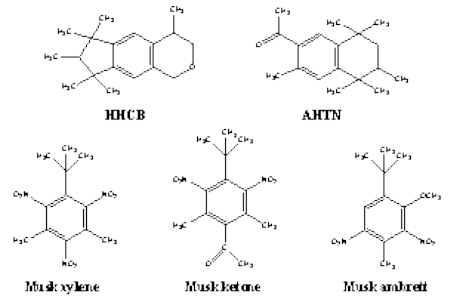

Fig. 1 Chemical structure of synthetic musk fragrances analyzed in this study

In this study, the occurrence of synthetic musk fragrances, such as two polycyclicmusks (HHCB [1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta [g]-2-benzopyrane] and AHTN [7 -acetyl-1,1,3,4,4,6-hexamethyltetrahydronaphthalene]) and three nitro musks (musk xylene, musk ketone, musk ambrett) (Fig. 1) were examined in marine mammals (finless porpoises) collected from Japanese coastal waters. To understand the body distribution of musks in marine mammals, twelve tissues and organs of an adult finless porpoise were also analyzed. In addition, several marine organisms, such as clams, crustaceans, fish, and seabirds were analyzed in order to evaluate the bioamgnification of musk fragrances in a marine food web.

Materials and Methods

Samples: Finless porpoises including a fetus were collected from the Ariake Sea and Yatsushiro Sea, western Japan during 1999 and 2002. All of animals were stranded along the coastal area or accidentally caught by fishing net. The marine organisms, such as clam, crustacean (shrimp, mantis shrimp, crab), fish (right eye flounder, mullet, eagle ray, hammerhead shark etc.), and seabird (mallard, black-headed gull) samples were also collected in the Ariake Sea. All samples were stored at –20℃ until analysis. The blubber of finless porpoises was analyzed in this study. For seabirds, rays, sharks and mullets, the liver tissues were analyzed. The whole bodies homogenates of other fish samples, crustaceans and clams were used for the analysis. Twelve tissues of an adult female finless porpoise, such as blubber, liver, kidney, stomach, muscle, lung, blood, brain, heart, small intestine, ovary, and placenta were also analyzed to understand the body distribution of musks.

Chemical Analysis: Synthetic musk fragrances were analyzed according to the methods described previously[4]. Briefly, approximately 1-4 g of tissues were ground with sodium sulfate and extracted with mixed solvents of dichloromethane and hexane using a Soxhlet apparatus. A d_{10}-phenantherene was spiked into an aliquot of the extract as a surrogate standard. Lipid in the sample extract was removed by gel permeation chromatography (GPC) using a Bio -beads S-X3 (Bio-Rad Laboratories, Hercules, CA, USA) packed glass column. The eluted solvent was concentrated, and passed through a 1.5 g activated silica gel (Wako-gel S-1, Wako Pure Chemical Co. Ltd, Japan) packed glass column for further fractionation. The fraction including musk fragrances was micro-concentrated and injected into a gas chromatograph interfaced with a mass spectrometer (GC -MSD, Agilent 6890 and 5973 Series). The GC column used was DB1 (J&W Scientific Inc. USA) fused silica capillary column (30 m x 0.25 mm i.d., 0.25 mm of filr thickness). The oven temperature was programmed from 80℃ to 160℃ at a rate of 10℃/min and held for 10 min, the temperature was increased to 300℃ at a rate of 3℃/min, with a final hold time of 20 min. The temperatures of injector and detector were set at 270 an 300℃, respectively. Helium was used as a carrier gas.

Quality Control: A standard mixture containing all synthetic musks of interest was used to determine general recovery rates of the compounds through the analytical procedure. Salad oil, that did not contain detectable quantities of synthetic musks, was spiked with 50 ng of the standard mixture. Three replicate analyses were performed, and the average recoveries of musk fragrances ranged fror 92 % for AHTN to 108 % for musk -ambrett. In addition to the recovery test, the reproducibility of synthetic musk concentrations in samples was examined. Furthermore, the blubbers of three finless porpoises were analyzed in duplicate. As a result, the average concentrations of HHCB were less variable, suggesting thatthe analytical method is suitable for routine analysis of synthetic musks ir biological samples. A procedural blank was analyzed with every set of six samples to confirm interfering peaks in chromatograms and to correct sample values, if necessary. The detection limits for HHCB, AHTN, musk xyene, musk ketone, and musk ambrett in samples were 8.8, 9.1, 1.9, 2.3, and 2.5 ng/g, respectively.

Results and Discussion

Levels: GC-MS chromatograms and fragmentation patterns of HHCB in finless porpoise and standard mixture are shown in Fig. 2. The retention time and fragmentation patterns of HHCB in finless porpoise blubber agreed well with those in standard mixture.

HHCB was detected in blubber samples of all finless porpoises (n=8), ranging in concentrations from 13 ng/g to 149 ng/g on a wet wt. basis (Table 1). The highest concentration was found in an adult female. In spite of the occurrence of HHCB in finless porpoise, AHTN was detected only in an adult female, and the concentration was low, 9.6 ng/g wet wt. basis, close to the limit of detection. Concentrations of musk xylene, musk ketone, and musk ambrett were all below the detection limits in finless porpoises. To our knowledge, this is the first report on the accumulation of synthetic musk fragrances in marine mammals.

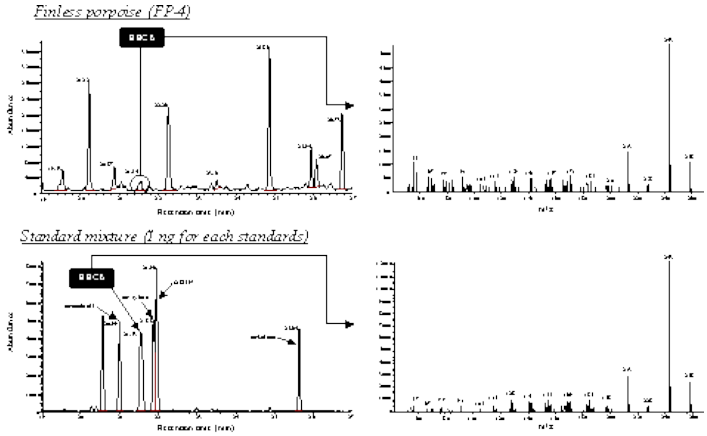

Fig. 2 GC-MS chromatograms (SCAN mode) and fragment patterns of HHCB in the blubber of finless porpoises and a standard mixture

Table 1 Concentrations of musk fragrances (ng/g wet wt.) in blubber of finless porpoises including a fetus collceted from Japanese coastal waters.

	FP-1	FP-2	FP-3	FP-4	FP-5	FP-6	FP-7	FP-8	FPF*
Sex	F	F	F	F	M	M	M	M	M
Length (cm)	110	NA	151	140	117	99	123	123	80
Weight (kg)	23.4	NA	53	41.2	22.8	19	21.4	37.5	5.8
Lipid (%)	84	88	81	83	86	86	85	86	76
HHCB	22	39	117	149	72	15	50	13	26
AHTN	<9.1	<9.1	<9.1	9.6	<9.1	<9.1	<9.1	<9.1	<9.1
Musk xylene	<1.9	<1.9	<1.9	<1.9	<1.9	<1.9	<1.9	<1.9	<1.9
Musk ketone	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
Musk ambrett	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5

*: Fetus of FP-2 sample. NA: not available.

Maternal Transfer: HHCB was detected in blubber of a finless porpoise fetus (FPF) at a concentration of 26 ng/g wet wt. basis. Th level was comparable to that in the mother (FP -2, 39 ng/g), suggesting transplacental transfer of HHCB during pregnancy. To understand the transfer pattern of HHCB in marine mammals, concentration ratio in the blubber of fetus to pregnant female was calculated. The concentration ratio of HHCB in finless porpoise was 0.67, which is similar to those reported for persistent organochlorines, such as PCBs (0.38), DDTs (0.46), HCHs (0.86) and HCB (0.97) in striped dolphins[5]. In contrast, the concentration ratio of organotins in the mother -fetus pair of a killer whale was quite low, at 0.015 [6]. These observations imply that transplacental transfer of HHCB is similar to those of organochlorine contaminants, and this phenomenon might be relevant to other mammals, such as human.

Tissue Distribution: Among twelve tissues and organs of a finless porpoise analyzed in this study, the highest HHCB concentration was found in blubber (149 ng/g wet wt.), followed by kidney (9.3 ng/g). This result indicates that HHCB accumulation is dependent on the affinity to lipids in tissues and organs, which similar to the profiles of persistent organochlorines, PCBs and DDTs [7]. This may be related to the physico -chemical properties, especially lipophilicity, of these compounds. It has been reported that octanol/water coefficients (log *K*ow) of HHCB is 5.9[8] which value is almost similar to those of tri- to tetra-chlorinated biphenyls.

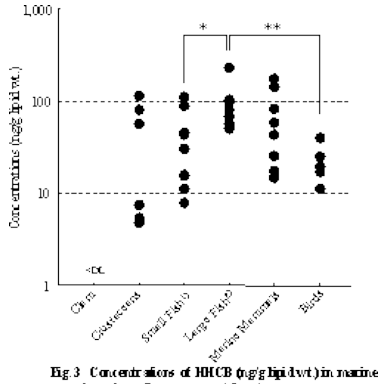

Fig. 3 Concentrations of HHCB (ng/g lipid wt.) in marine organisms from Japanese coastal waters.

1) Sole, Goby, Rockfish, Pufferfish, Flatfish, Seaperch, Sweetlips, Mullet.
2) Sea bream, Mackerel, Shark.
*: $p<0.05$, **: $p<0.01$

Biomagnification of HHCB: HHCB was detected in most marine organisms analyzed, at the concentrations ranging from several to two hundred ng/g (lipid wt. basis) (Fig. 3). In general, large variations of HHCB levels were found in organisms although HHCB concentrations in large fish were significantly higher than those in small fish ($p<0.05$) and birds ($p<0.01$).

While no data of HHCB concentrations in seawater from the Ariake Sea is available, it ha been reported that HHCB levels in river water in Japan ranged from 0.7 to 100 ng/L (average: 55 ng/L) [9]. These results imply that HHCB is accumulated in fish and other marine organisms approximately >1000 times higher than in water. However, little biomagnification was observed for HHCB through food chain in this study. In particular, HHCB levels in birds were generally lower than those in other organisms. These observations may suggest that HHCB were metabolized to some polar metabolites in marine organisms in a relatively short time and then excreted to water phase.

Acknowledgements

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