ORGANOHALOGEN CONTAMINANTS AND METABOLITES IN KILLER WHALE (*ORCINUS ORCA*) AND MELON-HEADED WHALE (*PEPONOCEPHALA ELECTRA*) FROM JAPANESE COASTAL WATER

Koichi Haraguchi[1], Yousuke Hisamichi[2], Sachie Moriki[1], and Tetsuya Endo[2]

[1] *Daiichi College of Pharmaceutical Sciences, 22-1 Tamagawa-cho, Minami-ku, Fukuoka 815-8511, Japan*

[2] *Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, 1757, Ishikari-Tobetsu, Hokkaido 061-0293, Japan*

Introduction

Organochlorine pollutants such as PCBs in the marine environment represent a threat to marine organisms. Some of PCBs are metabolized to persistent methylsulfonyl PCBs ($MeSO_2$-CBs), which accumulate in blubbers of mammals such as seals[1] and polar bears[2]. In recent years, on the other hand, new bioaccumulative organohalogen compounds, proposed to be of natural origin, have been detected in blubbers of mammals throughout the world. Some of them are mixed halogenated dimethyl bipyrroles (HDBPs)[3] heptachloromethyl bipyrrole (Q1)[4], methoxy brominated diphenyl ethers (MeO-BDE)[5] and dimethoxy tetrabromobiphenyl (diMeO-BB)[6]. Among them, HDBPs have been detected in the same concentration range as anthropogenic PCBs in blubbers of small cetaceans from Japanese coastal water[7]. Killer whale (*Orcinus orca*) represents the top of many marine food webs and accumulates relatively high levels of anthropogenic contaminants, but the levels and profiles of PCB metabolites and natural persistent organohalogens have not been reported. The present study was therefore intended to investigate the levels and profiles of anthropogenic contaminants (PCBs, DDTs, CHLs and $MeSO_2$-CBs) as well as natural occurring products in blubber samples of killer whales and melon-headed whales (*Peponocephala electra*) stranded in Japanese coastal water in 2005-2006. Thus, the ratios of HDBP/PCB and metabolite/parent PCB were compared in both species..

Materials and Methods

Sampling. Blubber samples were obtained from killer whales (one mature male and five mature females) stranded off Rausu, Hokkaido, 2005, and from melon-headed whale (n=13, body length 250-260 cm) stranded off Chiba, 2006.

Chemicals. HDBP congeners were synthesized according to the method of Gribble et al[8]. Q1 was synthesized by the method of Wu et al[9]. The spectroscopic data for both HDBPs and Q1 were identical in all respects to the data reported previously. 6-MeO-BDE47 and 2,2'-diMeO-BB80 were

kindly donated by Dr. G. Marsh, Stockholm University. $MeSO_2$-CB congeners were synthesized as described previously[10]. Tris(4-chlorophenyl)methanol (TCPMe) was purchased from AccuStandard Inc. USA).

Sample clean-up. The procedure was performed according to a modification of our previous method[7]. The lipids were removed by gel permeation chromatography (Bio-Beads, SX-3, Bio-Rad Laboratories), with elution with *n*-hexane/dichloromethane (1:1). The eluate was concentrated to dryness and dissolved in *n*-hexane (1 mL) and was applied to an activated silica gel S-1 column (1 g, Wako Pure Chemical Industries Ltd.), eluting with *n*-hexane (10 mL, first fraction), with another *n*-hexane (10mL, second fraction), with *n*-hexane : dichloromethane (DCM) (9:1, 10 mL, third fraction) and DCM (20 mL, fourth fraction). Each eluate was reduced to 500 µL and subjected to GC/MS.

Identification and quantification. Analyses of contaminants in each fraction were performed using a gas chromatograph (Agilent GC-6980N) equipped with a mass-selective detector (5973*i*) in electron-ionization and selected ion monitoring mode (EI-SIM). An HP-5 column (30 m × 0.25 mm, i.d., J&W Scientific) was installed in the GC. In the full scan EI mode, *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 total PCB concentration (∑PCBs) was the sum of 12 PCB congeners. ∑DDT was the sum of *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD. ∑CHLs was the sum of oxychlordane, trans- and cis-chlordanes and trans- and cis-nonachlor. ∑HDBPs was the sum of Br_4Cl_2-, Br_3Cl_2- and Br_3Cl_3-DBPs. ∑$MeSO_2$-CBs was the sum of 18 $MeSO_2$-CB congeners.

Results and Discussion

Anthropogenic and natural persistent organohalogens were separated into four fractions by activated silica gel (1g) column. The concentrations of major components in blubbers of killer whales and melon-headed whales are listed in Table 1.

First silica fraction eluted with *n*-hexane (10 mL) contained mainly PCBs, DDTs and chlordanes (CHLs). Their concentrations were up to 4-fold higher in killer whales than in melon-headed whales. The higher concentrations in killer whales indicate the different dietary habits from melon-headed whales (i.e. fish and/or cetacean-eating killer whales and fish-eating melon-headed whales)[11].

Second fraction eluted with another *n*-hexane (10 mL) contained natural occurring organohalogens such as heptachloromethyl bipyrrole (Q1) and mixed halogenated dimethyl bipyrroles (HDBPs). Most abundant congener among eight HDBPs detected in blubbers was Br_4Cl_2-DBP, followed by Br_3Cl_2-DBP and Br_3Cl_3-DBP. The distribution of Q1 and HDBPs has been observed in the other mammals[3,4]. Despite higher contamination of PCBs in killer whales, the

concentrations of Q1 and HDBPs were relatively low and thus the ratio of natural HDBPs and anthropogenic PCBs (i.e, Br_4Cl_2-DBP/CB153) were higher in melon-headed whales (ratio: 7.98) than in killer whales (ratio: 1.48). The results suggest the different dietary source of halogenated pyrrole derivatives from those of anthropogenic PCBs.

Third fraction eluted successively with n-hexane: dichloromethane (DCM) (9:1, 10mL) contained mainly two natural products, 6-methoxy-2,2',4,4'-tetrabromodiphenyl ether (6-MeO-BDE47) and 2,2'-dimethoxy-3,3',5,5'-tetrabromobiphenyl (diMeO-BB80). Their concentrations were at the ppb ranges in both species. Since diMeO-BB80 and Br_4Cl_2-DBP in the second fraction were nearly co-eluted on HP-5 capillary column (30m), both components should be separately determined by GC/MS-EI/SIM (*m/z* 530 and *m/z* 544 ions) after silica gel separation.

Fourth fraction eluted with DCM (20mL) contained methylsulfonyl PCBs ($MeSO_2$-CBs), 3-$MeSO_2$-*p,p'*-DDE and tris(4-chlorophenyl)methanol (TCPMe). Among 18 $MeSO_2$-CBs detected, major PCB metabolites were identified as 3'- and 4'-$MeSO_2$-CB49, 3'- and 4'-$MeSO_2$-CB101, 3'- and 4'-$MeOS_2$-CB87 and 3- and 4-$MeSO_2$-CB149. Although the congener profiles in this fraction did not differ between both species, and from the other mammals such as grey seals, their concentrations were two orders of magnitude higher in killer whales. As the metabolite/parent PCB ratios were calculated, the ratios of $MeSO_2$-CB101/ PCB 101 were 0.034 in killer whales, whereas 0.007 in melon-headed whales. The results may suggest the differences in metabolic capacity to CB101 in both species. It is well known that cetaceans have lower ability to metabolize some PCBs with adjacent non-chlorinated *meta* and *para* carbons, due to the specific mode of cytochrome P-450 enzyme system[12]. Moreover, another contaminant, TCPMe[13], was detected in this fraction and distributed at similar concentration ranges to $MeSO_2$-CB congeners in blubbers of both species.

In conclusion, blubbers in two mammalian species from Japanese marine ecosystem accumulate high concentrations of anthropogenic and natural persistent products, together with $MeSO_2$-CBs. Although health significance of these natural compounds is unclear, the temporal tend and bioaccumulation process of these persistent contaminants should be further investigated in marine food webs.

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References

1. Bergman Å., Norstrom R.J., Kuroki H., and Beland P. (1994). *Environ. Toxicol. Chem.* **13**, 121-128.
2. Sandala G.M., Sonne-Hansen C., Dietz R., Muir D.C., Valters K., Bennett E.R., Born E.W., and Letcher R.J. (2004). *Sci. Total Environ.* **331**, 125-141.

3. Tittlemier S., Borrell A., Duffe J., Duignan P.J., Fair P., Hall A., Hoekstra P., Kovacs K.M., Krahn M.M., Lebeuf M., Lydersen C., Muir D., O'Hara T., Olsson M., Pranschke J., Ross P., Siebert U., Stern G., Tanabe S., and Norstrom R**.** (2002). *Arch. Environ. Contam. Toxicol.* **43**, 244-255.
4. Vetter W., Jun W., and Althoff G. (2003). *Chemosphere* **52**, 415-422.
5. Teuten E.L., Xu L., and Reddy C.M. (2005). *Science*, **307**, 917-920.
6. Marsh G., Athanasiadou M., Athanassiadis I., Bergman Å., Endo T., and Haraguchi K. (2005). *Environ. Sci. Technol*. **15**, 8684-8690.
7. Haraguchi K., Hisamichi, Y., and Endo T. (2006). *Arch. Environ. Contam. Toxicol.* **51**, 135-141.
8. Gribble G.W., Blank D.H., and Jasinski J.P. (1999). *Chem. Commun*. 2195-2196.
9. Wu J., Vetter W., Gribble G.W., Schneekloth J.S., Blank DH Jr., and Görls H. (2002) *Angew. Chem. Int. Ed*. **41**, 1740-1742.
10. Haraguchi K., Kuroki H., and Masuda Y. (1987). *J. Agric. Food Chem*. **13**, 121-128.
11. Haytens D.L., and Duffield D.A. (2000). *Mar. Pollut. Bull.* **40**, 558-561.
12. Tanabe S., Watanabe S., Kan H., and Tatsukawa R. (1998). *Mar. Mammal Sci*. **4**, 103-124.
13. Minh T.B., Watanabe M., Tanabe S., Miyazaki N., Jefferson T.A., Prudente M.S., Subramanian A., and Karuppiah S. (2000). *Environ. Pollut.* **110**, 459-468.

Table 1. Concentrations of persistent halogenated compounds detected in blubbers of killer whales and melon-headed whales from Japanese coastal water.

Components	Concentration (μg/g, lipid)*	
	killer whale n=5	melon headed whale n=13
1st fraction		
ΣPCBs	25.0 (14.0-42.3)	12.1 (7.31-15.5)
ΣDDT	46.0 (36.3-50.5)	13.5 (9.40-17.3)
ΣCHLs	21.8 (15.5-28.5)	4.80 (3.37-5.76)
2nd fraction		
ΣHDBPs	13.4 (10.6-18.6)	16.2 (13.3-18.9)
Q1	0.73 (0.38-1.21)	4.70 (2.70-6.51)
3rd fraction		
6-MeO-BDE47	0.58 (0.42-0.88)	0.92 (0.62-1.21)
diMeO-BB80	0.51 (0.35-0.72)	0.78 (0.55-1.03)
4th fraction		
Σ$MeSO_2$-CBs	0.77 (0.53-0.92)	0.006 (0.003-0.008)
3-$MeSO_2$-DDE	0.05 (0.03-0.07)	0.001 (0.001-0.002)
TCPMe	0.52 (0.40-0.73)	0.12 (0.08-0.15)
Ratio		
Br_4Cl_2-DBP/CB153	1.48 (0.63-4.64)	7.98 (6.67-10.1)
$MeSO_2$-CB101**/CB101	0.034 (0.028-0.039)	0.007 (0.003-0.009)

* means (ranges),

** sum of 3'- and 4'-$MeSO_2$-CB101