NATURAL AND ANTHROPOGENIC POPs IN BLUEFIN TUNA FROM THE JAPANESE MARKET

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Abstract
Anthropogenic and naturally occurring organohalogens have been found to coexist in the lipids of marine mammals. To further understanding of the global distribution of natural persistent organic pollutants (POPs), we analyzed bluefin tuna and grouper in Japanese market. Two methoxylated tetraBDEs (MeO-BDEs), one dimethoxylated tetrabromobiphenyl (diMeO-BB), one heptachlorinated methylbipyrrole (Cl_7-MBP) and three mixed halogenated dimethyl bipyrroles (HDBPs) were identified. The component profiles were different, mainly depending on habitats. HDBPs were localized in tuna from the North Pacific, whereas Cl_7-MBP was extremely abundant in Oceania. MeO-BDEs were more abundant in grouper than in bluefin tuna from Okinawa, Japan.

Introduction
In recent years, new bioaccumulative organohalogen compounds, proposed to be of natural origin, have been detected in marine biota from different sites throughout the world. In some cases, the levels were comparable to those of anthropogenic POPs. Major natural lipophilic components described to date are heptachlorinated methylbipyrrole (Cl_7-MBP, referred to Q1)[1], mixed halogenated dimethylbipyrroles (HDBPs)[2], methoxylated tetrabromodiphenyl ethers (MeO-BDEs)[3] and dimethoxylated tetrabromobiphenyl (diMeO-BB).[3] Cl_7-MBP has been found at the ppm range in marine mammals and at the ppb range in fish, seafood and even in human milk.[4] HDBPs have also been determined at the ppm range in marine mammals[5,6] and at the ppb range in canned fish composites in Canadian markets.[7] On the other hand, MeO-BDEs have been found in algae, sponge, fish and marine mammals.[8] diMeO-BB have recently been detected in whale products from Japan[3] and in dolphins from Australia.[9] Their sources are thought to be marine bacteria.[8] Due to their similar lipophilicity to PCBs, they are likely bioconcentrated in higher trophic animals including humans via the food chain.

Bluefin tuna (*Thunnus thynnus*) inhabits coastal waters to open seas and is distributed in tropical and temperate regions almost all over the world. Tuna fish has been recognized as a predator able to concentrate large amounts of POPs. This characteristic makes it possible to use them as "bioindicators" of aquatic pollution. Tuna products are frequently and widely eaten in Japan, so their xenobiotic content including natural compounds could be of concern to human health. The present study was performed to investigate contamination trend of anthropogenic PBDEs and natural POPs in bluefin tuna available in Japanese market. The collected materials were classified by different habitat (countries). The contaminant levels were compared between samples from Japanese coastal waters and from the other countries, and between bluefin tuna (farmed) and grouper in Okinawa, Japan.

Materials and Methods
Sampling: Fish materials were collected from retail outlets across Japan during 2003-2004. Purchases were conducted simultaneously to control for potential differences in seasonal availability of local by-catch or imported materials. Fresh tuna samples (n=238) were derived into wild and farmed ones, fatty flesh and red meat. In this study, we selected fatty flesh samples (lipid contents, >20%, n=49), which were divided into four categories from Asia (Japanese coastal water), North America (Canada), Oceania (Australia) and Europe (Spain). For comparison, groupers (n=10) were collected in Okinawan market.
Chemicals: Two internal standards, 2,3,4,2',4',5'-hexabromodiphenyl ether (BDE138) and 4'-MeO-2,4,6,3',5'-pentabromodiphenyl ether (4'-MeO-BDE121) were used for the determination of PBDEs and natural organohalogens, respectively. Three HDBP congeners (Br_4Cl_2-, Br_3Cl_3- and Br_3Cl_2-DBPs) were prepared according to the method of Gribble *et al*. Cl_7-MBP was by the method of Wu *et al*. Standards of five

methoxylated compounds (2'-MeO-BDE68, 6-MeO-BDE47, 4'-MeO-BDE121, 2',6-diMeO-BDE68 and 2,2'-diMeO-BB80) were donated by Dr. G. Marsh (Stockholm University).
Sample clean-up: Accurately weighed samples (10 g) were cut into small species and mixed with a 10-fold amount of anhydrous sodium sulfate. The mixtures spiked with three internal standards were wet-packed with dichloromethane/*n*-hexane (1:1) into a glass column. The filtered extracts were concentrated and the lipid contents were determined gravimetrically. The lipids were then removed by gel permeation chromatography (Bio-Beads, SX-3). The eluate was concentrated and purified by silica gel column (Wako gel S-1). The eluate (20% dichloromethane in *n*-hexane) was reduced to 500 µL and subjected to GC/MS.
Identification and quantification: A gas chromatograph (Agilent 6980N) equipped with a mass-selective detector (5973*i*) in electron-capture negative ionization and selected ion monitoring mode (ECNI-SIM). An HP-5MS column (30 m × 0.25 mm, 0.25 µm-film thickness) was installed in the GC. In the full scan ECNI 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 and methane as a regent gas. The injector and transferline temperatures were 250ºC 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). Natural organohalogens were measured in the full-scan mode for identification, but in the SIM mode for quantification.

Results and Discussion
Four classes of natural organohalogens were detected in bluefin tuna and quantified by the ECNI-SIM mode, using *m/z* 79, 81 and 161 for brominated compounds, *m/z* 386 for Cl_7-MBP and *m/z* 358, 362, 396 and 430 for PCBs. Fig. 1 shows the profile and concentration of major components in bluefin tuna from four regions (Asia, n=28; Australia, n=10; Europe, n=7; and North America, n=4).

PBDEs: Congeners were dominated by BDE47 (46-55%), BDE100 (7-13%), BDE99 (9-16%), BDE153 (5-10%) and BDE154 (4-8%) in most cases. The total levels were higher in tuna imported from other countries (56 ng/g lipid, n=21) than in tuna from Japanese coastal water (24 ng/g, n=28). The mean concentrations of PBDE levels in bluefin tuna were similar to the results from skipjack tuna (up to 53 ng/g lipid)[10]. Among those from Japanese coastal waters, the levels were higher in wild tuna (39-80 ng/g, n=4) than in farmed tuna (6-45 ng/g n=24). Among farmed tuna, the levels were relatively high in those from Spain, followed by from Japanese coastal waters, and the lowest from Australia.

MeO-BDEs/MeO-BB: Four signals in the SIM mode (*m/z* 79) were identified as 2'-MeO-BDE68 (17.54 min), 6-MeO-BDE47 (18.09 min), 2',6-diMeO-BDE68 (19.21 min) and 2,2'-diMeO-BB80 (17.73 min). Total concentrations of four methoxylated compounds in bluefin tuna were slightly lower than those in whale products from Japanese coastal waters[3], but higher than those in fish products from Swedish waters.[11] The levels were the highest in tuna products from Canada, followed by those from Japanese coastal waters, and Australia. The ratios of 2'-MeO-BDE68/ 6-MeO-BDE47 were higher in tuna from Japanese coastal water, whereas the ratio was the lowest in products from Canada. This finding suggests that 2'-MeO-BDE68 and 6-MeO-BDE47 have different sources. The former has been isolated from marine algae in Okinawan waters[13] and accumulated at the ppm levels in marine wildlife (e.g. pygmy sperm whale) from Australia[9], whereas the latter has been found in red algae from the Baltic sea.[12] There were no correlation between 6-MeO-BDE47 and BDE47, indicating that 6-MeO-BDE47 are not derived from metabolites of BDE47. In this study, 2,6'-diMeO-BDE68 was also detected in most samples, ranging from 4 to 78 ng/g lipid, and abundant in Okinawan samples. The concentration range was comparable to the range in whale products from the Pacific.[3] 2,2'-diMeO-BB80 ranged from 18 to 58 ng/g lipid in tuna samples. In particular, higher levels were observed in those from Asia and Canada. This finding suggests that 2,2'-diMeO-BB80 is likely localized in the Pacific. In fact, diOH-BB80 has been isolated from bacteria in the sea water from Ogasawara area in the Pacific.[14]

MBP/HDBPs: Cl_7-MBP was detected in the highest concentrations (490-1260 ng/g lipid) in tuna from Australia. Even in the other samples, this component was widely distributed at the ppb range. Actually, Cl_7-MBP has been reported at the ppm range in mammals from Australia and Africa, at the ppm range in human milk from Faroe

Islands[4]. On the other hand, HDBPs were highly distributed in products from Japanese coastal waters (80-260 ng/g) and from Canada (80-320 ng/g), but not detected in the other samples. HDBP composition was dominated by Br_4Cl_2-DBP (70-95 %), followed by Br_3Cl_3-DBP(5-15 %) and Br_3Cl_2-DBP (2-8 %). The full scan ECNI and EI mass spectra were in agreement with those from whale products in Japanese market[5]. As a result, HDBP appear to be localized in the North Pacific. HDBPs may be produced by a marine chromobacterium (*e.g. Psuedoalteromonas*), as it was shown to produce a structurally similar compound, hexabromo- 2,2'-bipyrrole[15].

Natural POPs in groupers from Okinawa: To compare the profiles and levels of natural POPs in bluefin tuna with those in the other fish products, we analyzed groupers (*Serranidae*) inhabiting in coral reefs of Okinawa (southern Japan). As shown in Table 1, four methoxylated organohalogens were distributed at one order of magnitude higher concentrations in groupers than in bluefin tuna. In some cases, 2'-MeO-BDE68 and 6-MeO-BDE47 were detected with concentrations exceeding 1 ppm, although the levels of both Cl_7-MBP and Br_4Cl_2-DBPs were lower in groupers. We hypothesize that these methoxylated organohalogens are major components emerged in coral reefs, where these components may be bioconcentrated to higher organisms via the food chain. Both bluefin tuna and groupers are likely the most critical source of natural POPs, since they usually exhibit predatory behaviour, higher longevity and lower metabolic rates and belong to higher trophic levels.

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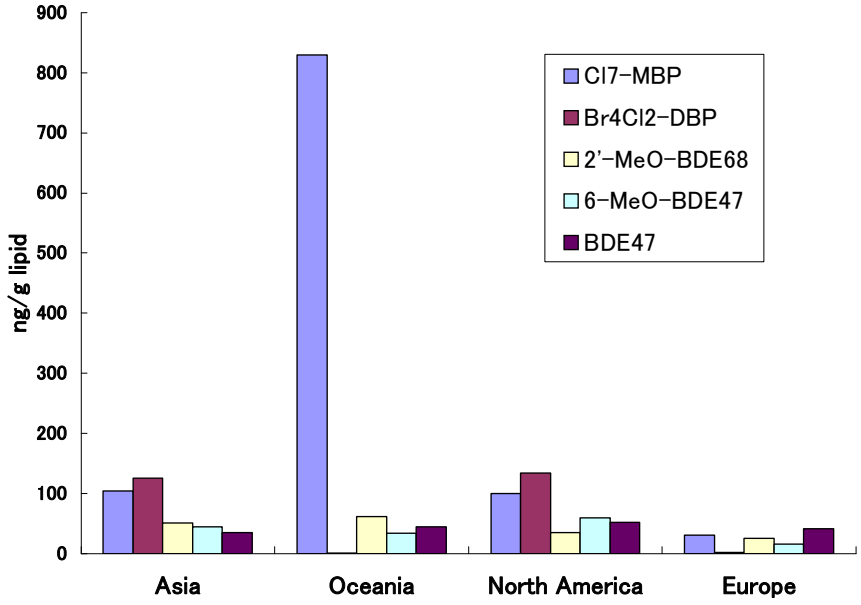

Fig. 1. Comparison of the levels of natural POPs and BDE47 in bluefin tuna imported from different area in Japanese market.

Table 1. The concentration of natural POPs in bluefin tuna and grouper from Okinawan market in Japan.

	Concentration (ng/g lipid)			
	bluefin tuna (n=8)		grouper (n=10)	
Cl_7-MBP	74	(50-506)*	32	(23-68)
Br_4Cl_2-DBP	45	(24-180)	5	(nd -9)
2'-MeO-BDE68	54	(26-78)	450	(103-1290)
6-MeO-BDE47	62	(15-80)	480	(220-1420)
2',6-diMeO-BDE68	8	(nd-15)	101	(52-540)
2,2'-MeO-BB80	18	(8-33)	200	(30-850)
Total	261		1268	
ratio				
6-MeO-BDE47/BDE47	12		76500	

* range, nd = not detected (<0.2 ng/g)

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