Environ. Sci. Technol. **2010,** *44,* 6068–6073

Contribution of Synthetic and Naturally Occurring Organobromine Compounds to Bromine Mass in Marine Organisms

YI WAN,[†] PAUL D. JONES,[‡] STEVE WISEMAN,[†] HONG CHANG,[†] DAVE CHORNEY,[§] KURUNTHACHALAM KANNAN,[||] KUN ZHANG,[⊥] JIAN-YING HU, JONG SEONG KHIM,[#] SHINSUKE TANABE,[▽] MICHAEL H. W. LAM,[○] AND JOHN P. GIESY*[,†,○,◆,¶,+]

Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada, School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5C8B3, Canada, Radiochemistry & SLOWPOKE Reactor, SRC Analytical Laboratories, Saskatoon, Saskatchewan, Canada, Wadsworth Center, New York State Department of Health and Department of Environmental Health Sciences, School of Public Health, State University of New York, Empire State Plaza, Albany, New York 12201-0509, Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China, Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-713, Korea, Center for Marine Environmental Studies, Ehime University, Matsuyama, Japan, Department of Biomedical Veterinary Sciences, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada, Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong, SAR China, School of Biological Sciences, University of Hong Kong, Hong Kong, SAR, PR China, and Department of Zoology and Center for Integrative Toxicology, Michigan State University, East Lansing, Michigan

Received March 22, 2010. Revised manuscript received June 17, 2010. Accepted July 8, 2010.

An extraction, separation, and purification method was developed for the identification and quantification of total bromine (TBr), extractable organobromine (EOBr), and five classes of identified EOBrs. Instrumental neutron activation analysis (INAA) was utilized to quantify EOBr and TBr. The method was then applied to liver samples of tuna, albatross, and polar bear collected from remote marine locations. Polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), bromophenols (BRPs), hydroxylated (OH-) and methoxylated (MeO-) PBDEs were analyzed as identified EOBr. The majority of the bromine in these marine organisms was nonextractable or inorganic, with EOBr accounting for 10−28% of the TBr. Of the identified EOBr, in tuna and albatross, naturally occurring compounds, including MeO-PBDEs, OH-PBDEs, and BPRs, were prevalent. However, the identifiable EOBr in polar bears consisted primarily of synthetic compounds, including PBDEs and PBBs. Overall, 0.08−0.11% and 0.008−0.012% of EOBr and TBr, respectively, were identified. The proportion of EOBr that was identified in marine organisms was relatively small compared to the proportions for organofluorine and organochlorine compounds. This could be related to the great diversity of naturally occurring organobromine compounds in the environment. Naturally occurring brominated fatty acids were estimated to be the predominant compounds in the EOBr fraction.

Introduction

Due to their persistence, bioaccumulation, and toxicity, organohalogen compounds are of concern as contaminants in aquatic and terrestrial ecosystems. Some well-known synthetic organohalogens such as perfluorooctanesulfonate (PFOS), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) have become ubiquitous environmental pollutants (*1*−*3*). In addition to the known synthetic organohalogens there are a number of unidentified organohalogens in the environment (*4*, *5*). Identification and quantification of these novel organohalogen compounds is essential for the assessment of potential risks to exposed organisms.

Synoptic quantification of individual organohalogen compounds along with quantification of total halogens is an effective method to estimate the mass balance of identified and unidentified organohalogen compounds in the environment. Several studies have examined the contributions of known organohalogens to extractable organohalogens and total organohalogens in abiotic and biological matrices (*4*−*8*). Mass balance studies of chlorine in samples collected near a former chloralkali facility indicated that the identified organochlorines accounted for 48% and 1−35% of the extractable organic chlorine (EOCl) in sediment and biota, respectively (*4*). Methods for the mass balance analysis of fluorine have been developed and applied to environmental samples (*5*, *8*). In these studies, most of the fluorine (>50%) was in the nonextractable fraction and the identified perfluorinated compounds (PFCs) contributed 30−85% of extractable organic fluorine (EOF) in human blood and wildlife tissues (*5*, *8*).

Brominated flame retardants (BFRs) have recently emerged as contaminants of concern. Among BFRs, PBDEs and polybrominated biphenyls (PBBs) are of primary interest due to their high production volume, widespread use, and ubiquitous occurrence in the environment (*9*, *10*). The occurrence of hydroxylated (OH-) and methoxylated (MeO-) PBDEs has been investigated in aquatic ecosystems (*11*), and concentrations of MeO-PBDEs were greater than those of

* Corresponding author address: Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada; tel (direct): 306-966-2096; tel (secretary): 306-966-4680; fax: 306-966-4796; mobile: 517-614-6123; e-mail: jgiesy@aol.com.
† Toxicology Centre, University of Saskatchewan.
‡ School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan.
§ Radiochemistry & SLOWPOKE Reactor, SRC Analytical Laboratories.
|| Wadsworth Center, New York State Department of Health and Department of Environmental Health Sciences.
⊥ Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University.
Division of Environmental Science and Ecological Engineering, Korea University.
▽ Center for Marine Environmental Studies, Ehime University.
○ Department of Biomedical Veterinary Sciences University of Saskatchewan.
◆ Department of Biology and Chemistry, City University of Hong Kong.
¶ School of Biological Sciences, University of Hong Kong.
+ Department of Zoology and Center for Integrative Toxicology, Michigan State University.

10.1021/es100914r

Published on Web 07/22/2010

TABLE 1. Concentrations of Bromine in Five Groups of Identified Organic Brominated Compounds, Identified EOBr, EOBr, and TBr Extracted from Liver Samples Collected from Species Inhabiting Remote Marine Locations[a]

species name	water (%)	lipid (%)	MeO-PBDEs	OH-PBDEs	BRPs	PBDEs	PBBs	identified EOBr	EOBr	TBr
tuna	67 ± 3.7 (58−72)	5.0 ± 2.7 (1.9−9.0)	0.31 ± 0.10 (0.17−0.47)	0.014 ± 0.005 (0.008−0.03)	0.45 ± 0.21 (0.14−0.67)	0.13 ± 0.05 (0.063−0.22)	0.003 ± 0.006 (N.D.−0.014)	0.9 ± 0.3 (0.5−1.3)	1100 ± 540 (600−2000)	10700 ± 3400 (8300−17200)
albatross	60 ± 2.2 (55−64)	10 ± 3.5 (4.3−15)	0.65 ± 0.85 (0.08−2.8)	0.34 ± 0.24 (0.11−0.90)	0.021 ± 0.033 (N.D.−0.10)	0.19 ± 0.21 (0.05−0.85)	0.049 ± 0.057 (N.D.−0.21)	1.3 ± 1.1 (0.2−3.8)	1700 ± 950 (810−3500)	11300 ± 2600 (7000−14400)
polar bear	60 ± 5.2 (54−71)	7.1 ± 2.2 (4.7−12)	0.014 ± 0.01 (0.003−0.03)	0.004 ± 0.005 (0.001−0.02)	0.11 ± 0.12 (N.D.−0.39)	0.49 ± 0.16 (0.19−0.68)	0.34 ± 0.29 (0.064−1.0)	1.0 ± 0.5 (0.5−1.9)	2800 ± 2300 (310−6100)	12400 ± 6100 (6200−21800)

[a] ng/g ww, mean ± SD (range in parentheses). N.D. Not detected.

PBDEs in some samples (*12*). Bromophenols (BRPs), which are structurally similar to PBDEs, are key natural flavor components of some marine fish. However, synthetic BRPs have also been produced and widely used as flame retardants (2,4,6-triBRP) with a worldwide production of 9500 t in 2001 (*13*).

Extractable organic bromine (EOBr) has been investigated in a variety of environmental samples (*4, 6, 14−16*). However, few studies have determined the proportion of EOBr that could be identified, and the contribution of natural and synthetic compounds to EOBr concentrations in environmental samples is unknown. In this study, the relative contribution of natural and synthetic organobrominated compounds to EOBr and total bromine (TBr) was determined. Both EOBr and TBr were determined by instrumental neutron activation analysis (INAA) of either crude samples or organic solvent extracts of samples. PBBs, PBDEs, and related brominated compounds were analyzed as identified EOBr (*9, 11, 17, 18*). An extraction and cleanup method for TBr, EOBr, and five classes of identified EOBr was developed. The method was applied to determine concentrations of EOBr and TBr as well as the concentrations of individual congeners of PBBs, PBDEs, OH-PBDEs, MeO-PBDEs, and BRPs in livers of tuna (*Katsuwonus pelamis*), five species of albatrosses (*Thalassarche chlororhynchos*, *Phoebetria palpebrata*, *Thalassarche chrysostoma*, *Thalassarche cauta*, and *Thalassarche melanophrys*) and polar bear (*Ursus maritimus*) collected from remote marine locations. Absolute and relative contributions of identified EOBr to EOBr and TBr were determined, and the predominant forms of brominated compounds in the marine organisms are suggested.

Materials and Methods

Tissue Collection. Livers from fifteen albatross, ten tuna, and ten polar bear were used for quantification of TBr, total EOBr, and identified EOBr. Albatross were collected from the Indian and South Atlantic Oceans, polar bear were collected from Northern and Western Alaska, and tuna were collected from the North Pacific Ocean in 1992−2002 (Table 1) (*11*). All samples were stored at −20 °C from the time of collection until analysis.

Sample Preparation. An efficient fractionation method, requiring a single extraction of 5 g ww of liver tissue that allows for the simultaneous analysis of TBr, EOBr, and five groups of identified EOBr was developed (Figure 1). The identified EOBr included 10 PBBs, 21 PBDEs, 12 MeO-PBDEs, 10 OH-PBDEs, and 16 BRPs. Details of chemicals and standards are provided in Supporting Information. To estimate the concentrations of organobromines in the environment, EOBr has been quantified in various matrixes, such as sediment, atmosphere, and aquatic biota (*4, 6, 15*). The current study is the first to compare TBr, EOBr, and identified EOBr in tissues (Figure 1) (*4*). Due to the limited sample mass available for the analysis of multiple target compounds, direct quantifications of TBr in tissues were not attempted. In contrast to the method used to quantify total

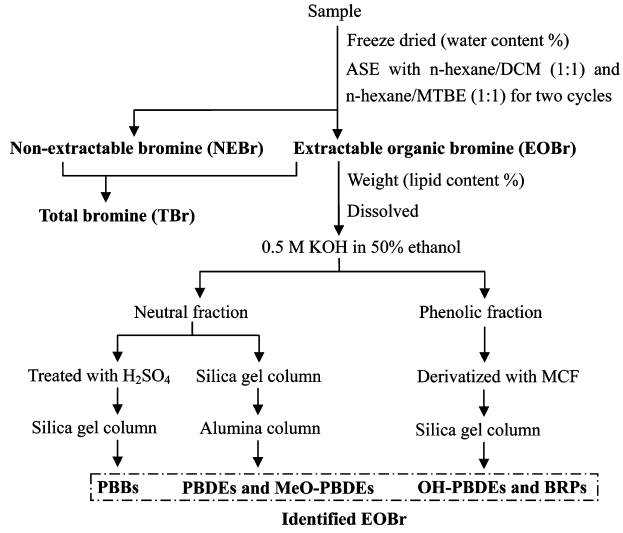

FIGURE 1. Flow diagram for the procedure used to fractionate total bromine, extractable organic bromine, and known extractable organic bromines in liver tissue samples.

chlorine (TCl) and total fluorine (TF), TBr was quantified by summing EOBr and nonextractable bromine (NEBr), and the concentrations of NEBr were determined by analyzing the residues from solvent extracted samples and normalized to lipid and water contents. The calculation is based on an assumption that negligible amounts of bromine (Br) were lost during extraction.

Samples (approximately 5 g wet weight (ww)) were homogenized and freeze-dried. Water content was measured gravimetrically. Sample extraction was conducted using an accelerated solvent extractor (Dionex ASE-200, Sunnyvale, CA). Two kinds of solvents were used for the extraction: (1) *n*-hexane/dichloromethane (DCM) (1:1) at 100 °C and 1500 psi, and (2) *n*-hexane/methyl *tert*-butyl ether (MTBE) (1:1) at 60 °C and 1000 psi. Two extraction cycles (10 min each) were performed for each solvent per sample (about 50 mL for each solvent), and both extraction fractions were combined. The residual materials after solvent extraction were air-dried and used for quantification of NEBr. The solvent extract was reduced to 10 mL by rotary evaporation and 2 mL was used for the determination of EOBr.

Lipid content of each extract was determined gravimetrically by evaporating the remaining extract to constant weight. Extracts were then spiked with a mixture of ^{13}C-labeled PBB, PBDE, and BRP surrogates for analysis of PBBs, PBDEs, MeO-PBDEs, OH-PBDEs, and BRPs. Thus, the spiking solutions did not influence quantification of Br EOBr, and the details of the analysis of PBDEs, MeO-PBDEs, OH-PBDEs, and BRPs have been reported previously (*11*). The method was modified to accommodate simultaneous identification and quantification of PBBs (Figure 1). The neutral and phenolic fractions in extracts were separated with 0.5 M potassium hydroxide (KOH) in 50% ethanol. The methods for purification of PBDEs,

MeO-PBDE, BRPs, and OH-PBDEs have been reported previously (*11*). For analysis of PBBs, the extract was treated with sulfuric acid because PBBs, unlike MeO-PBDEs and PBDEs, are not degraded by concentrated sulfuric acid. Extracts were passed through a column packed with 2 g of sodium sulfate and 8 g of acidified silica (50 g of silica gel mixed with 27 mL of concentrated sulfuric acid). After application of the sample, the column was eluted with 15 mL of *n*-hexane and 10 mL of DCM. Eluates were concentrated to 40 μL for quantification of PBBs. Instrument analyses of identified EOBr are in Supporting Information.

Calculations of Bromine Concentrations. Concentrations of EOBr (ng Br/g ww) were determined (eq 1)

$$C_{\mathrm{EOBr}} = \frac{C_{\mathrm{Br\ in\ extract}} \times 10}{W_{\mathrm{wet}}} \times 1000 \quad (1)$$

where $C_{\mathrm{Br\ in\ extract}}$ is the concentration of bromine in the extract (μg/mL), 10 is the final volume of extract (mL), and W_{wet} is the wet weight of sample (g). The residual materials after solvent extraction were used for quantification of Br to determine the nonextractable Br content (NEBr). Concentrations of NEBr (ng Br/g ww) were calculated (eq 2)

$$C_{\mathrm{NEBr}} = C_{\mathrm{Br\ in\ RSP}} \times (1 - \mathrm{Wat}\%) \times (1 - \mathrm{Lipid}\%) \times 1000 \quad (2)$$

where $C_{\mathrm{Br\ in\ RSP}}$ is the concentration of bromine in the residual sample powders (RSP) (μg/g), Wat% is the water content of the samples (%), and Lipid% is the lipid content of the samples (%). Thus, the concentration of TBr, expressed as ng Br/g ww, was the sum of concentrations of EOBr and NEBr (eq 3).

$$C_{\mathrm{TBr}} = C_{\mathrm{EOBr}} + C_{\mathrm{NEBr}} \quad (3)$$

Identifiable EOBr, including PBBs, PBDEs, MeO-PBDEs, OH-PBDEs, and BRPs, were quantified in liver samples. Masses of Br contributing to total EOBr were determined by multiplying the concentration of each compound identified by its mole fraction of Br. Concentrations of identified EOBr were the sum Br concentrations of all detected compounds (eq 4).

$$C_{\mathrm{known\ EOBr}} = \sum_{i=1}^{m} \left(\frac{80 \times N_i}{\mathrm{MW}_i} \times C_i \right) \quad (4)$$

where C_i is the concentration of organic brominated compound i (ng/g ww), N_i is the number of Br atoms contained in the compound i, MW_i is the molecular weight of compound i, 80 is the molecular weight of Br, and m is the number of detected organobromine compounds.

Bromine Analysis. Concentrations of Br were determined by INAA. Samples were activated for 15 min in 1.5 mL NAA-grade polyvials at a neutron flux of 5.0×10^{11} (n/cm^2)/s in the SLOWPOKE 2 nuclear reactor (Saskatchewan Research Council Analytical Laboratories, Saskatoon, SK, Canada). After activation, γ-rays from ^{80}Br were measured by γ-ray spectrometry by use of an Ortec model GMX20P4 HPGe solid-state detector and Ortec DSPEC Pro 8192 channel digital gamma ray spectrometer for peak area calculations. Quantification was based on γ-peaks from ^{80}Br ($t_{1/2}$ = 17.6 min, E_γ = 616 keV). The count time was 15 min. Known concentrations of bromoform ($CHBr_3$) dissolved in toluene were used as standards for the quantification of EOBr and TBr using INAA.

Quality Assurance and Quality Control (QA/QC). Counting efficiency of INAA was determined for EOBr and TBr for each type of sample geometry. This was done by analyzing a set of four standards and calculating counts per second per microgram of bromine (cps/μg). The average efficiency factor was used to calculate the EOBr and TBr concentrations. To smooth out minor fluctuations that occur in individual standards or batches, each time a new set of calibration standards was quantified, a new average was calculated from all standards run and maintained as a running average.

The QA/QC for the analysis of PBDEs, MeO-PBDEs, OH-PBDEs, and BRPs has been reported previously (*11*). A laboratory blank and a matrix spike were analyzed for every batch of 15 samples. Beef liver was used for matrix spike samples, and the spiking solutions were added before accelerated solvent extraction. Recoveries of spiked materials from samples were 80−127%, 81−126%, 87−128%, 81−123%, and 65−126% for PBBs, MeO-PBDEs, PBDEs, OH-PBDEs, and BRPs, respectively, in the entire analytical procedure. Concentrations quantified in spiked samples were within 20% of the spiked concentrations. Thus, both accuracy and precision of the analysis were deemed to be acceptable. Quantification of PBBs, PBDEs, and BRPs was performed with ^{13}C-PBBs, ^{13}C-PBDEs, and ^{13}C-BRPs as surrogates. Concentrations of OH-PBDEs were quantified relative to 2,3,4,6-^{13}C-TeBRPs, and MeO-PBDEs were quantified relative to ^{13}C-PBDEs with the same number of Br atoms.

The instrumental limits of quantification for Br analysis were 2 μg Br/g dw for solid samples and 0.08−0.5 μg Br/mL for liquid samples. Method detection limits (MDL) were defined to be mean plus three times the standard deviation of concentrations in the blank. MDLs for compounds that were not detected in the blank were set to be the instrumental minimum detectable amounts. The MDLs were 0.4 pg/g ww for MeO-PBDEs, 0.2−10.1 pg/g ww for PBDEs, 2−4 pg/g ww for OH-PBDEs, 2.0−10 pg/g ww for BRPs, and 2−50 pg/g ww for PBBs. For those results that were less than the MDL, a sensitivity analysis was conducted and no statistically significant differences could be found by using different values ranging from 0 to the MDL. Thus, a value of 0 was assigned to avoid missing values in statistical analyses.

Results and Discussion

Bromine Fractions. Concentrations of TBr, EOBr, and identified EOBr in liver of tuna, albatross, and polar bear are presented (Table 1). Mean (±SD) concentrations of TBr were 10 700 ± 3400, 11 200 ± 2600, and 12 400 ± 6100 ng/g ww in tuna, albatross, and polar bear, respectively. Concentrations of EOBr were approximately 3- to 9-fold less than those of NEBr, which ranged from 1100 ± 540 (tuna) to 2800 ± 2300 ng/g ww (polar bear). Among the species analyzed, polar bear liver contained the greatest concentrations of both TBr and EOBr. However, concentrations of identified EOBr were relatively small, ranging from 0.9 ± 0.3 (tuna) to 1.3 ± 1.1 ng/g ww (albatross) and accounted for only a small proportion of the TBr.

Few studies have investigated concentrations of EOBr in marine biota. The mean concentration of EOBr in harbor porpoise (1700 ng/g ww) from the Baltic Sea (*16*) was comparable to concentrations in tuna and albatross observed in this study. However, concentrations of EOBr in marine organisms in the present study were greater than those in Atlantic herring from the Baltic Sea (120−240 ng/g ww) (*16*) and northern pink shrimp from the North Atlantic Ocean (60−1030 ng/g ww) (*19*). Overall, the reported concentrations of EOBr in the organisms analyzed in this study ranged from of 60 to 6100 ng/g ww. Similar variation in EOBr concentrations has been reported for tissues of terrapins, birds, and fish collected from coastal waters of Georgia, U.S., which ranged from 110 to 3700 ng/g ww. Although the underlying reasons for this variation are unknown, it has been suggested that concentrations of EOBr are species- and location-specific (*4*).

Concentrations of bromine associated with identified EOBr compounds are presented (Figure 2 and Table 1). The

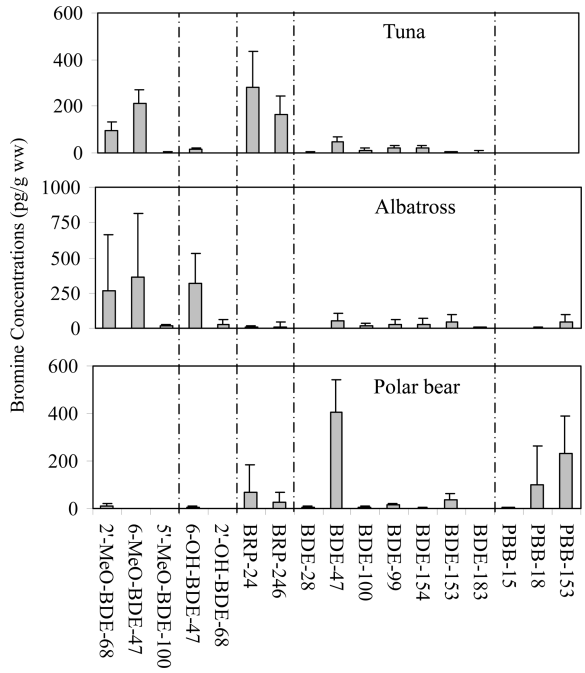

FIGURE 2. Concentrations of major organic brominated compounds detected in liver samples collected from species inhabiting remote marine locations.

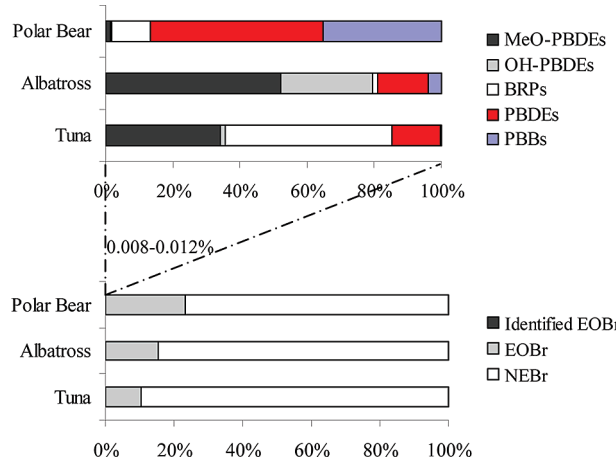

FIGURE 3. Relative contribution (%) of identified EOBr, EOBr, and NEBr to TBr concentration in livers of polar bear, albatross, and tuna from remote marine locations.

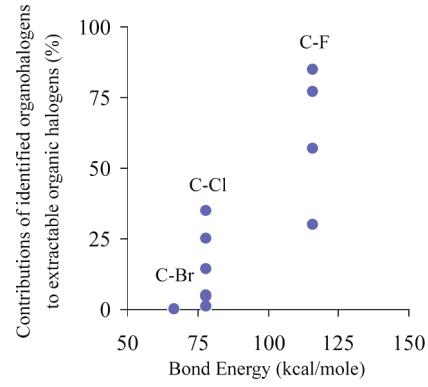

FIGURE 4. Relationship between carbon−halogen bond energies and contributions of identified anthropogenic organohalogens to extractable organic halogens. Bond energies were referenced from *26*.

proportion of Br contributed by MeO-PBDEs was relatively large in tuna (0.31 ± 0.10 ng/g ww) and albatross (0.65 ± 0.85 ng/g ww). The greatest contributions of OH-PBDEs to identified EOBr were found in albatross (0.34 ± 0.24 ng/g ww), and the greatest bromine contributions from BRPs were observed in tuna (0.48 ± 0.21 ng/g ww). In these two species, the predominant congeners of MeO-PBDEs, OH-PBDEs, and BRPs were 6-MeO-BDE-47, 6-OH-BDE-47, and BRP-24, respectively. Previous studies have demonstrated that these compounds originate primarily from natural synthesis by marine algae (*11, 12, 20, 21*). Thus, most of the identified compounds contributing to concentrations of identified EOBr in tuna and albatross are from naturally occurring compounds. Contributions of Br from the naturally occurring MeO-PBDEs (0.014 ± 0.01 ng/g ww) and OH-PBDEs (0.004 ± 0.005 ng/g ww) were small in polar bear, relative to those in tuna and albatross. The relative contributions of organobromine compounds of human origin in polar bear liver (PBDEs: 0.49 ± 0.16 ng/g ww and PBBs: 0.34 ± 0.29 ng/g ww) were greater than those in tuna or albatross. Profiles of relative proportions of identified compounds including PBDEs and PBBs in polar bears were dominated by BDE-47 and PBB-153, respectively. Similar profiles of PBDEs and PBBs have been reported for other marine organisms (*17, 22*). Because point sources play only a minor role in regional contamination of the Arctic marine ecosystem (*23*), the relatively great concentrations of PBDEs and PBBs in polar bear are likely due to atmospheric transport and deposition of these anthropogenic compounds (*24*).

Proportions of Identified Compounds in EOBr and TBr. A mass balance analysis of Br composition showed that NEBr accounted for a major proportion of TBr (72 ± 24% to 90 ± 4%) in all samples (Figure 3). Similar to the results reported here, the major proportions of total fluorine (TF) found in human blood and dolphin livers were nonextractable organic fluorine (NEOF) and inorganic fluoride (IF) (*5, 8*). The EOBr accounted for a relatively small fraction of TBr in each species, ranging from 10 ± 4% in tuna to 28 ± 24% in polar bear. Although the contributions of EOBr to TBr in polar bear liver were variable (2−53%), the large percentages are comparable to the contributions of extractable organic fluorine (EOF) to TF measured in livers of marine mammals in Hong Kong such as the Indo-pacific humpback dolphin: 58%, and finless porpoise: 27% (*8*).

Identified EOBr accounted for only 0.08−0.11% and 0.008−0.012% of EOBr and TBr, respectively (Figure 3) in the three species, all of which are at the top of the food chain. Contributions of identified EOBr to the total concentrations of EOBr were small compared to the ratios of identified EOF to total EOF (Indo-pacific humpback dolphin: 30%, finless porpoise: 30%, and human blood: higher than 60%; refs *5, 8)* and identified EOCl to total EOCl (fish: 5−25%; blue crab: 35%, birds: 1−14%, and terrapin: 4.2%; refs *4, 14)*. The small proportion of identified EOBr contributing to total EOBr concentration is probably due to the diversity of naturally occurring organobromine compounds. Previous studies have shown the existence of nearly 3200 known naturally occurring organohalogen compounds in the environment, with more than 1600 containing bromine (*25*). The predominance of naturally occurring organobrominated compounds is likely a result of the relatively low energy requirement for the formation of the C−Br bond. The stronger halogen−carbon bonds are less likely to be derived from naturally biological processes. The relationship between identifiable synthetic and unidentified organohalognes is given in Figure 4. Since the C−F bond is relatively strong, it is much less likely to be formed naturally and there are likely to be fewer F atoms in naturally occurring organic molecules. Therefore, most of the organically bound fluorine is attributable to identifiable, synthetic, per- and polyfluorinated compounds.

TABLE 2. Concentrations of Organobromine Compounds Reported in Previous Studies[a]

		brominated flame retardants				originated from both sources			naturally occurring brominated compound				
sample	ref	PBDEs	PBBs	TBBPA	HBCD	PBDD/Fs	BRPs	OH-PBDEs	MeO-PBDEs	PBHDs	TBA	MHC-1	BRI
glaucous gull	*18*	1325			21.9			26.5	54.3				
polar bear	*18*	533			N.D.			N.D.	N.D.				
eel	*27*	24.6	0.1	N.D.	57.5								
cormorant	*9*	1419	14.4			0.1							
predatory birds	*17*	1753	63.0	N.D.−0.26									
deep sea fish	*28*	16.9			<1.5				28.9	530	0.3	9.7	
deep sea fish	*28*	5.1			<1.5				6.5	7040	<0.2	0.6	
food web	*29*	6.4−115.4			1.0−3.0				0.7−110.1	4.7−146	11.7−30.1	0.1−1.6	
wild tuna	*30*	58−74							134−167	3580−5241	3.1−4.2	26−30	
whale oil	*31*								0.1				0.008
sediment	*32*						13.3−360.8						0.4−118
fish and mussels	*33*									<5−1140		<1−3	
tuna	*b*	5.7	0.2				18.8	0.6	13.4				
albatross	*b*	2.6	0.6				0.4	6.7	12.3				
polar bear	*b*	11.0	0.8				2.1	0.6	0.4				

[a] (ng/g lw (lipid weight). Number underlined indicates the predominant brominated compounds detected. The concentrations of EOBr in tuna, albatross, and polar bear in current study were 23 000, 17 000, and 40 000 ng/g lw, respectively. TBBPA: tetrabromobisphenol A; HBCD: hexabromocyclododecane; PBDD/Fs: polybrominated dibenzo-p-dioxins and dibenzofurans; PBHDs: polybrominated hexahydroxanthene derivatives; TBA: tribrominated anisole; BRI: brominated indole. Locations of references: (*18*), Norwegian Arctic; (*27*), Irish waters; (*9*), Japan; (*17*), Norway; (*28, 30*), and (*33*), Mediterranean Sea; (*29*), Sydney Harbour; (*31*), historical sample; (*32*), North and Baltic Sea. [b] Current study.

Estimating the Major Organobromine Compounds in EOBr. There is a variety of synthetic and natural organobrominated compounds that were not quantified in this study that could have contributed to the EOBr of these samples (Table 2). Since the masses of samples available for this study were limited and standards are not available for many of the classes of brominated compounds that could have contributed to the EOBr, an assessment of the potential for those unquantified compounds to contribute to the EOBr was made from information available in the literature (Table 2). PBDEs were generally the predominant compounds among brominated flame retardants, which is also consistent with the fact that they are used in the greatest quantities globally. Among naturally occurring brominated compounds, concentrations of MeO-PBDEs, BRPs, and polybrominated hexahydroxanthene derivatives (PBHDs) were generally greater than other brominated compounds of natural origin (*28*−*30*). In the current study, PBHDs were not analyzed, but concentrations of PBHDs have been reported to be as great as 5000 ng/g lipid weight (lw) in some deep-ocean fishes (*28*). Since authentic PBHD standards were not commercially available, PBHDs were identified and quantified based on previously reported methods (*28*). While MeO-PBDEs in the sample extracts can be detected by GC-EI-MS, no obvious peaks that would have corresponded to masses of PBHDs were observed (Supporting Information Figure S1). This result suggests that concentrations of PBHDs in the samples studied were not significant contributors to TBr. The presence of PBHDs and other organobromine compounds were further studied by GC-ECNI-MS, and no brominated compounds with concentrations greater than approximately 1 ng/g ww could be detected (Supporting Information Figure S2). In addition, concentrations of the detected organobromine compounds reported previously were very small compared with those of EOBr (Table 2). Including the estimated concentrations of these additional brominated compounds, the identified proportion of the EOBr would not exceed 1%.

Brominated fatty acids (BFAs) could be the predominant compounds in EOBr, since BFAs have been reported to occur at concentrations ranging from 2.2 to 82 μg/g ww in marine fishes and invertebrates (*34*). These concentrations are comparable to those of EOBr determined in this study (1.1−2.8 μg/g ww). However, reported concentrations of BFAs were based on the analysis of bromine content. As one of the most interesting groups among the naturally occurring halogen compounds, BFAs comprise a relatively large and diverse class of compounds (*35, 36*). BFAs with different structures have been identified in marine algae and invertebrates, and some compounds contain as many as 28 carbon atoms (*35, 36*). However, none of the studies has attempted to quantify BFAs. In the current study concentrations of saturated BFAs (C2−C21) were estimated by use of GC-EI-MS after derivatization of the extracts with *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide with 1% *t*-BDMS-chloride (MTBSTFA) (FigureS3 in Supporting Information). However, the chain lengths of BFAs in marine organisms were generally greater than 16 (*35, 36*), and standards for BFAs of this chain length are not commercially available. A number of possible peaks with relatively great concentrations were observed (Figure S4 in Supporting Information), but without authentic standards, it was difficult to accurately identify or quantify the individual BFAs. Further studies should focus on the analysis and potential effects of these compounds.

Acknowledgments

The research was supported by a Discovery Grant from the National Science and Engineering Research Council of Canada (Project 326415-07) and a grant from Western Economic Diversification Canada (Projects 6971 and 6807). J.G. was supported by the Canada Research Chair program and an at large Chair Professorship at the Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong. We acknowledge the support of an instrumentation grant from the Canada Foundation for Infrastructure. We thank Thomas Evans, U.S. Fish and Wildlife Service, Anchorage, Alaska, for providing polar bear livers.

Supporting Information Available

Detailed information on chemicals, standards, instrument analysis, and analysis of saturated brominated fatty acids; GC-EI-MS and GC-NCI-MS chromatography of sample extracts. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Safe, S. H. Polychlorinated-biphenyls (PCBs) - environmental-impact, biochemical and toxic responses, and implications for risk assessment. *Crit. Rev. Toxicol.* **1994**, *24*, 87–149.

(2) Giesy, J. P.; Kannan, K. Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **2001**, *35*, 1339–1342.

(3) Hites, R. A. Polybrominated diphenyl ethers in the environment and in people: A meta-analysis of concentrations. *Environ. Sci. Technol.* **2004**, *38*, 945–956.

(4) Kannan, K.; Kawano, M.; Kashiwa, Y.; Matsui, M.; Giesy, J. P. Extractable organohalogens (EOX) in sediment and biota collected at an estuarine marsh near a former chloralkali facility. *Environ. Sci. Technol.* **1999**, *33*, 1004–1008.

(5) Yeung, L. W. Y.; Miyake, Y.; Taniyasu, S.; Wang, Y.; Yu, H.; So, M. K.; Jiang, G.; Wu, Y.; Li, J.; Giesy, J. P.; Yamashita, N.; Lam, P. K. S. Perfluorinated compounds and total and extractable organic fluorine in human blood samples from China. *Environ. Sci. Technol.* **2008**, *42*, 8140–8145.

(6) Loganathan, B. G.; Kannan, K.; Watanabe, I.; Kawano, M.; Irvine, K.; Kumar, S.; Sikka, H. C. Isomer-specific determination and toxic evaluation of polychlorinated biphenyls (PCBs), polychlorinated/brominated dibenzo-p-dioxins (PCDDs/PBDDs), dibenzofurans (PCDFs/PBDFs), polybrominated biphenyl ethers (PBBEs) and extractable organic halogen (EOX) in carp from the Buffalo River, New York. *Environ. Sci. Technol.* **1995**, *29*, 1832–1838.

(7) Miyake, Y.; Yamashita, N.; So, M. K.; Rostkowski, P.; Taniyasu, S.; Lam, P. K. S.; Kannan, K. Trace analysis of total fluorine in human blood using combustion ion chromatography for fluorine: A mass balance approach for the determination of known and unknown organofluorine compounds. *J. Chromatogr., A* **2007**, *1154*, 214–221.

(8) Yeung, L. W. Y.; Miyake, Y.; Wang, Y.; Taniyasu, S.; Yamashita, N.; Lam, P. K. S. Total fluorine, extractable organic fluorine, perfluorooctane sulfonate and other related fluorochemicals in liver of Indo-Pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*) from South China. *Environ. Pollut.* **2009**, *157*, 17–23.

(9) Watanabe, K.; Senthilkumar, K.; Masunaga, S.; Takasuga, T.; Iseki, N.; Morita, M. Brominated organic contaminants in the liver and egg of the common cormorants (*Phalacrocorax carbo*) from Japan. *Environ. Sci. Technol.* **2004**, *38*, 4071–4077.

(10) Wan, Y.; Hu, J. Y.; Zhang, K.; An, L. H. Trophodynamics of polybrominated diphenyl ethers in the marine food web of Bohai Bay, North China. *Environ. Sci. Technol.* **2008**, *42*, 1078–1083.

(11) Wan, Y.; Wiseman, S.; Chang, H.; Zhang, X. W.; Jones, P. D.; Hecker, M.; Kannan, K.; Tanabe, S.; Hu, J. Y.; Lam, M. H. W.; Giesy, J. P. Origin of Hydroxylated Brominated Diphenyl Ethers: Natural Compounds or Man-Made Flame Retardants. *Environ. Sci. Technol.* **2009**, *43*, 7536–7542.

(12) Teuten, E. L.; Xu, L.; Reddy, C. M. Two abundant bioaccumulated halogenated compounds are natural products. *Science* **2005**, *307*, 917–920.

(13) IUCLID. *Data set for 2,4,6-tribromophenol.* Ispra, European Chemicals Bureau; International Uniform Chemicals Information Database; 2003.

(14) Wesén, C.; Calberg, G. E.; Martinsen, K. On the identity of chlorinated organic substances in aquatic organisms and sediments. *Ambio* **1990**, *19*, 36–38.

(15) Xu, D.; Dan, M.; Song, Y.; Chai, Z.; Zhuang, G. Concentration charateristics of extracable organohalogens in PM2.5 and PM10 in Beijing, China. *Atmos. Environ.* **2005**, *39*, 4119–4128.

(16) Kawano, M.; Falandysz, J.; Morita, M. Instrumental neutron activation analysis of extractable organohalogens in marine mammals, harbour porpoise (*Phocoena phocoena*) and its feed, Atlantic herring (*Clupea harengus*), from the Baltic Sea. *J. Radioanal. Nucl. Chem.* **2008**, *278*, 263–266.

(17) Herzke, D.; Berger, U.; Kallenbron, R.; Nygård, T.; Vetter, W. Brominated flame retardants and other organobromines in Norwegian predatory bird eggs. *Chemophere* **2005**, *61*, 441–449.

(18) Verreault, J.; Gabrielsen, G. W.; Chu, S.; Muir, D. C.; Andersen, M.; Hamaed, A.; Letcher, R. J. Flame retardants and methoxylated and hydroxylated polybrominated diphenyl ethers in two Norwegian Arctic top predators: Glaucous gulls and polar bears. *Environ. Sci. Technol.* **2005**, *39*, 6021–6028.

(19) Bottaro, C. S.; Kiceniuk, J. W.; Chatt, A. Spatial distribution of extractable organohalogens in northern pink shrimp in the North Atlantic. *Biol. Trace Elem. Res.* **1999**, *71−72*, 149–166.

(20) Ueno, D.; Kajiwara, N.; Tanaka, H.; Subramanian, A.; Fillmann, G.; Lam, P. K. S.; Zhang, G. J.; Muchitar, M.; Razak, H.; Prudente, M.; Chung, K. H.; Tanabe, S. Global pollution monitoring of polybrominated diphenyl ethers using Skipjack tuna as a bioindicator. *Environ. Sci. Technol.* **2004**, *38*, 2312–2316.

(21) Chung, H. Y.; Ma, W. C. J.; Ang, P. O.; Kim, J. S.; Chen, F. Seasonal variations of bromophenols in brown algae (*Padina arborescens*, *Sargassum siliquastrum* and *Lobophora variegata*) collected in Hong Kong. *J. Agric. Food Chem.* **2003**, *51*, 2619–2624.

(22) Zhu, L. Y.; Hites, R. A. Temporal trends and spatial distributions of brominated flame retardants in archived fishes from the Great Lakes. *Environ. Sci. Technol.* **2004**, *38*, 2779–2784.

(23) Borgå, K.; Fisk, A. T.; Hoekstra, P. F.; Muir, D. C. G. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in Arctic marine food webs. *Environ. Toxicol. Chem.* **2004**, *10*, 2367–2385.

(24) Unson, M. D.; Holland, N. D.; Faulkner, D. J. A brominated secondary metabolite synthesized by the cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. *Mar. Biol.* **1994**, *119*, 1–11.

(25) Gribble, G. W. The diversity of naturally occurring organobromine compounds. *Chem. Sov. Rev.* **1999**, *28*, 335–346.

(26) Glockler, G. Carbon-halogen bond energies and bond distances. *J. Phys. Chem.* **1959**, *63*, 828–832.

(27) McHugh, B.; Poole, R.; Corcoran, J.; Anninou, P.; Boyle, B.; Joyce, E.; Foley, M. B.; McGovern, E. The occurrence of persistent chlorinated and brominated organic contaminants in the European eel (*Anguilla anguilla*) in Irish waters. *Chemosphere* **2010**, *79*, 305–313.

(28) Covaci, A.; Losada, S.; Roosens, L.; Vetter, W.; Santos, F. J.; Neels, H.; Storelli, A.; Storelli, M. M. Anthropogenic and naturally occurring organobrominated compounds in two deep-sea fish species from the Mediterranean Sea. *Environ. Sci. Technol.* **2008**, *42*, 8654–8660.

(29) Losada, S.; Roach, A.; Roosens, L.; Santos, F. J.; Galceran, M. T.; Vetter, W.; Neels, H.; Covaci, A. Biomagnification of anthropogenic and naturally-produced organobrominated compounds in a marine food web from Sydney Harbour, Australia. *Environ. Int.* **2009**, *35*, 1142–1149.

(30) Pena-Abaurrea, M.; Weijs, L.; Ramos, L.; Borghesi, N.; Corsolini, S.; Neels, H.; Blust, R.; Covaci, A. Anthropogenic and naturally-produced organobrominated compounds in bluefin tuna from the Mediterranean Sea. *Chemosphere* **2009**, *76*, 1477–1482.

(31) Teuten, E. L.; Reddy, C. M. Halogenated organic compounds in archived whale oil: A pre-industrial record. *Environ. Pollut.* **2007**, *145*, 668–671.

(32) Reineke, N.; Biselli, S.; Franke, S.; Francke, W.; Heinzel, N.; Huhnerfuss, H.; Iznaguen, H.; Kammann, U.; Theobald, N.; Vobach, M.; Wosniok, W. Brominated indoles and phenols in marine sediment and water extracts from the North and Baltic Seas - concentrations and effects. *Arch. Environ. Contam. Toxicol.* **2006**, *51*, 186–196.

(33) Melcher, J.; Janussen, D.; Garson, M. J.; Hiebl, J.; Vetter, W. Polybrominated hexahydroxanthene derivatives (PBHDs) and other halogenated natural products from the Mediterranean sponge *Scalarispongia scalaris* in marine biota. *Arch. Environ. Contam. Toxicol.* **2007**, *52*, 512–518.

(34) Lunde, G. Analysis of arsenic and bromine in marine and terrestrial oils. *J. Am. Oil Chem. Soc.* **1972**, *49*, 44–47.

(35) Dembitsky, V. M.; Srebnik, M. Natural halogenated fatty acids: their analogues and derivatives. *Prog. Lipid Res.* **2002**, *41*, 315–367.

(36) Gribble, G. W. The natural production of organobromine compounds. *Environ. Sci. Pollut. Res.* **2000**, *7*, 37–47.

ES100914R