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Polybrominated Diphenylethers and Methoxylated Tetrabromodiphenylethers in Cetaceans from the Mediterranean Sea

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Abstract. Eight tetrabrominated to hexabrominated diphenylethers were present at ppb levels in liver from cetaceans found stranded on the beaches of the Mediterranean Sea, Italy. The highest concentration was found in striped dolphin (sum polybrominated diphenyl ethers [PBDE] 8133 ng/g l.w.) and the lowest concentration in bottlenose dolphin (sum PBDE 66 ng/g lipid weight [l.w.]). The predominant congener in all samples was 2,2′,4,4′-tetraBDE (PBDE # 47) followed by, in decreasing order, the pentaPBDE # 99 and 100 and the hexaPBDE # 154 and 153. In 12 of the 14 analyzed samples, 3 different methoxylated PBDEs (MeO-PBDE # 1, 2, and 3) were detected at semiquantitatively calculated concentration ranges of 2 to 14 ng/g l.w.; 5 to 167 ng/g l.w.; and 7 to 628 ng/g l.w., respectively. In addition, several unidentified bromine compounds were seen when screening the samples in negative-chemical ionization (NCI) mode monitoring m/z 79 and 81, which illustrates the importance of running both electron-impact ionization and NCI when analyzing environmental samples. Electron-impact ionization is more specific for monitoring the molecular ion compared with NCI, which might overestimate the concentration of certain PBDE congeners.

Polybrominated diphenylethers (PBDEs) are a class of brominated flame retardants that have been used extensively in electronic equipment such as computers and televisions and also in textiles and polyurethane foam in furniture and cars. The amount of PBDE present in flame-retarded products ranges from 5% to 30% (EHC 1994). Commercially used PBDEs are produced in three different formulations—pentaBDE, octaBDE, and decaBDE—named after the most prominent bromine congener in the different formulations. The world consumption of technical PBDE formulations in 2001 was 67,390 tons, of which the dominating deca formulation contributed 56,100 ton, the octa formulation contributed 3790 ton, and the penta formulation contributed 7500 tons (BSEF 2003). The chemical properties of PBDEs have made them ubiquitous environmental contaminants. As with polychlorinated biphenyls (PCBs), the PBDEs are found at relatively high concentrations in the aquatic environment. In 1981, PBDEs were first identified in pike, bream, eel, and sea trout from the Viskan-Klosterfjorden water system south of Gothenburg, Sweden (Andersson and Blomkvist 1981), and its presence in the aquatic environment has since been confirmed by other studies (Jansson *et al.* 1993; Jansson and Asplund 1987; Law *et al.* 2002; Lindström *et al.* 1999b; Sellström *et al.* 1993; Watanabe *et al.* 1987). The tendency of PBDEs to accumulate in sediment and biomagnify in the aquatic environment has been shown in the Baltic Sea (Haglund *et al.* 1997; Nylund *et al.* 1992; Sellström *et al.* 1999). The most prominent PBDE congener in sediment was found to be TeBDE # 47 followed by PeBDE # 99 (numbered according to the International Union of Pure and Applied Chemistry nomenclature for PCBs) (Nylund *et al.* 1992). TeBDE # 47 has also been shown to biomagnify in the Baltic Sea food chain, and the highest concentrations have been found in seals and fish-eating birds (Darnerud *et al.* 2001; Haglund *et al.* 1997).

In the marine environment, top predators such as dolphins display high levels of PBDEs in their fatty tissues (de Boer *et al.* 1998a, 1998b). In whitebeaked dolphins (*Lagenorhynchus albirostris*), a total PBDE concentration $>$ 7000 ng/g lipid weight (l.w.) was found in an animal living mainly on fish from the Wadden Sea and the North Sea; however, deep-feeding sperm whales (*Physeter macrocephalus*) also display similar levels of PBDE contamination (de Boer *et al.* 1998a). This indicates that even the deep-water marine environment is contaminated with PBDEs, which was confirmed when 19 PBDE congeners were identified in long-finned pilot whales (*Globicephala melas*) feeding at considerable depths in the Atlantic Ocean (Lindström *et al.* 1999b; van Bavel *et al.* 1999). Lindström *et al.* (1999a) also showed that younger male and female whales had a higher total concentration of PBDE (3160 and 3038 ng/g l.w., respectively) than the adult animals. Adult female whales showed the lowest concentrations of PBDEs (1047 ng/g l.w.), indicating a lactational transfer of these compounds from female whales to their offspring.

No complete toxicologic evaluation is available for PBDEs (Darnerud *et al.* 2001), and little is known about the toxicity and metabolism of PBDEs in cetaceans. The P450 enzyme

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system is central to the metabolism of exogenous substances including environmental contaminants. The P450 enzyme system consists of subfamilies, of which CYP1A and CYP2B are important in the metabolism of environmental pollutants. Examples of contaminants that induce CYP1A are dioxins, coplanar PCBs, and polycyclic aromatic hydrocarbons, but PBDEs can also induce CYP1A, although not as effectively as dioxins and PCBs (Bunce *et al.* 2001; Pettersson *et al.* 2001). In rats, microsomal enzyme induction was studied for TeBDE # 47 and the PCB mixture Aroclor 1254. It was shown that TeBDE # 47 induced pentoxyresorufin-*O*-deethylase (PROD) to the same extent as did Aroclor 1254. Induction of PROD is known to describe CYP2B activity, which suggests that PBDE metabolism may be linked to CYP2B (Hallgren and Darnerud 1998). However, in cetaceans, studies have shown that the activity of CYP2B is low compared with terrestrial mammals (Tanabe *et al.* 1988; White *et al.* 1994), yielding low or no effect on PBDE metabolism (Boon *et al.* 1992, 2000; Norstrom *et al.* 1992). Lack of metabolism was also observed by Boon *et al.* (2000), who showed that microsomes isolated from sperm whale were unable to metabolize PBDE when tested in an *in vitro* assay.

The aim of this study was to identify and quantify PBDEs in cetaceans from the Mediterranean Sea, of which little is known today. Second, the samples were scanned for possible methoxylated PBDEs (MeO-PBDEs) and other bromine-containing compounds because studies have shown MeO-PBDEs in marine mammals (Haglund *et al.* 1997; Vetter *et al.* 2001). The analysis was done using gas chromatography/mass spectrometry (GC/MS) electron impact ionisation (EI) and negative chemical ionization (NCI) techniques.

Material and Methods

Sampling

The samples analyzed were liver tissue from 5 species of cetaceans including fin whale (*Balaenoptera physalus*), pilot whale (*Globicephala melas*), bottlenose dolphin (*Tursiops truncatus*), striped dolphin (*Stenella coeruleoalba*), and Risso's dolphin (*Grampus griseus*) from the Mediterranean Sea. All sampled animals were found stranded along the coast of the Tyrrhenian, Adriatic, and Ligurian Seas during the period 1990 to 1992. The Centro Studi Cetacei (Milan) authorized and supervised the collection and transport of the carcasses. All liver samples were frozen and stored at −20°C until analysis. A total of 14 samples were collected and analyzed. Specific data on the samples are listed in Table 1.

Sample Extraction

The sample preparation and extraction method has been reported elsewhere (van Bavel *et al.* 1999) and was used with slight modifications. Sample preparation included homogenization of the liver tissue with sodium sulphate (1:5). The homogenized sample, approximately 3 g, was then packed in standard supercritical fluid extraction (SFE) vessels and covered with basic aluminium oxide, ca 4.5 g, to avoid lipid carryover. Internal standard ^{13}C-labeled PBDE # 47, 99, and 153 were added to the samples before extraction. Extraction was performed using a Supprex SFE with carbon dioxide as the supercritical fluid. A supercritical density of 0.9 g/mL at a flow rate of 2 mL/min was achieved by applying a pressure of 281 bar and a chamber temperature of 40°C during the 25-minute extraction. The analytes were trapped on a C18 solid sorbent column (octadecylsilica) with nozzle and trap temperatures set at 45°C and 40°C, respectively. After completion of the extraction, the analytes were desorbed from the trap using 2 mL hexane followed by 2 mL dichloromethane at a rate of 2 mL/min. A recovery standard, ^{13}C-labeled PCB # 128 in tetradecane, was added to the samples, and the volume was decreased to 30 μL. Lipid determination was performed gravimetrically by applying a part of the sample (approximately 1 g) to a miniature column and quantitatively extracting the lipids with a 1:1 mixture of hexane and dichloromethane.

GC/MS Analysis

The extracts, 2 μL, were analyzed on a GC/MS system (HP 6890 gas chromatograph coupled to an HP 5973 low-resolution mass spectrometer) using both EI and negative-chemical ionization (NCI) on an HP-5MS (5% phenyl methyl siloxane) capillary column. The following temperature system was used: initial temperature 180°C, 15°C/min to 250°C, followed by 4°C/min to 325°C. When using single-ion monitoring (SIM), the two most abundant ions of the molecular ion cluster were monitored for TeBDE (m/z 483.7 and 485.7), PeBDE (m/z 563.6 and 565.6), and HxBDE (m/z 643.5 and 645.5). For the MeO-TeBDE, the two most abundant ions of the molecular ion cluster as reported by Haglund *et al.* (1997) (m/z 515.7 $(M + 4)^+$ and 517.7 $(M + 6)^+$) were chosen. In addition, the following masses were used for the ^{13}C-labeled internal standards: TeBDE (m/z 497.8), PeBDE (m/z 575.7), and HxBDE (m/z 655.6).

For NCI analysis, chromatographic separation was achieved by splitless injection of 2μL on the same HP-5MS (5% phenyl methyl siloxane) capillary column. In the NCI mode, methane was used as reagent gas at a pressure of $3.4 \cdot 10^{-4}$ torr in the ion source. PBDEs were monitored at m/z 79 and 81.

Identification and Quantification of PBDE

To detect PBDEs in the samples, GC/MS EI was run in the SIM mode. Running samples in SIM mode strongly enhances the detection limit because the noise is decreased compared with the full-scan mode. The PBDEs were identified by comparison of retention time (RT) to a standard solution. In addition, the isotope ratio of the two most abundant ions of the molecular ion cluster should be within 15% of the theoretical ratio for positive identification in EI. For the NCI GC/MS runs, the ratio between m/z 79 and 81 should be within 15%.

Quantification was achieved against the standard mixture containing internal standard (^{13}C-PBDEs # 47, 99, and 153); recovery standard (^{13}C-PCB # 128); and a quantification mix containing one PBDE of each bromination level (^{12}C-PBDEs # 47, 99 and 153). Results for MeO-TeBDE were calculated semiquantitatively using the relative response factor of the closest eluting PBDE at the same bromination level.

The recoveries of the internal standards in all samples were between 70% and 106% (Table 2), except for the fin whale sample, which had a recovery of 48% for TeBDE # 47 and PeBDE # 99 and 33% for HxBDE # 153.

Results and Discussion

The most abundant PBDE congener was TeBDE # 47, which was found in all samples at a concentration range of 47 to 3208 ng/g l.w. (Table 2). The second most abundant congener was

Table 1. Data available on the specimens studied[a]

Species	Sample Code	Sampling Site	Sex	Lipid Weight (%)
Fin whale (*Balaenopthera physalus*)	M10	Tyrrhenian (T)	Female	64
Pilot whale (*Globicephala melaena*)	M9	Ligurian (L)	Male	3
Bottlenose dolphin (*Tursiops truncatus*)	M8	Adriatic (A)	Male	8
Bottlenose dolphin (*T. truncatus*)	M6	Adriatic (A)	Male	13
Bottlenose dolphin (*T. truncatus*)	M5	Adriatic (A)	Female	32
Bottlenose dolphin (*T. truncatus*)	M4	Tyrrhenian (T)	Male	2
Risso's dolphin (*Grampus griseus*)	M2	Tyrrhenian (T)	Male	5
Risso's dolphin (*G. griseus*)	M1	Tyrrhenian (T)	Female	6
Risso's dolphin (*G. griseus*)	M3	Ligurian (L)	Male	11
Striped dolphin (*Stenella coeruleoalba*)	L2	Tyrrhenian (T)	Male	9
Striped dolphin (*S. coeruleoalba*)	L5	Tyrrhenian (T)	Female	2
Striped dolphin (*S. coeruleoalba*)	L6	Tyrrhenian (T)	Male	8
Striped dolphin (*S. coeruleoalba*)	L3	Ligurian (L)	Female	8
Striped dolphin (*S. coeruleoalba*)	L4	Ligurian (L)	Male	11

[a] Sample code, sampling site, sex, and lipid weight are shown. Note that the lipid weight of animals M10 and M5—64% and 32%, respectively—are high and could be a result of metabolic disorder, but this cannot be confirmed because the samples were taken from stranded dead animals.

Table 2. Concentrations (ng/g lipid) of PBDEs and MeO-TeBDEs in cetacean liver samples from the Mediterranean Sea, Italy

	Fin whale	Pilot whale	Bottlenose dolphin				Risso's dolphin			Striped dolphin				
	M10 (T)[a]	M9 (L)	M8 (A)	M6 (A)	M5 (A)	M4 (T)	M2 (T)	M1 (T)	M3 (L)	L2 (T)	L5 (T)	L6 (T)	L3 (L)	L4 (L)
TeBDE														
# 47	570	214	47	260	56	170	1001	977	685	316	543	2498	56	3208
PeBDE														
# "a"	5	<10[b]	<2	<2	<3	<9	<24	<4	<2	<2	<11	27	<2	135
# 100	94	62	9	150	29	81	351	359	214	99	184	1006	14	1414
# 99	142	134	10	17	<3	48	703	700	379	159	297	1573	36	1315
HxBDE														
# "a"	14	<27	<5	23	<10	<26	154	141	107	30	102	536	<6	348
# 154	50	73	<5	<46	<10	<26	557	515	294	83	165	1492	23	1106
# 153	11	<27	<5	22	<10	<26	232	225	99	39	107	606	<6	607
# 138	<3	<27	<5	<5	<10	<26	<10	<10	<5	<6	<35	<6	<6	<4
Total BDEs	886	483	66	518	85	299	2998	2917	1778	726	1398	7738	129	8133
MeO-TeBDE														
# "a"	8	<4	2	<1	<2	<5	14	13	6	3	<6	8	<1	6
# "b"	34	<4	5	9	52	<5	62	167	<1	13	36	24	<1	36
# "c"	93	81	79	12	45	<5	258	628	7	31	88	122	<1	152
Total MeO-TeBDE	135	81	86	21	97	<15	334	808	13	47	124	154	<3	194
Recovery ^{13}C-BDE (%)														
BDE # 47	48	95	106	97	99	99	83	83	93	96	92	71	95	67
BDE # 99	48	79	104	100	89	94	83	80	90	87	81	79	87	83
BDE # 153	33	70	98	94	78	86	83	80	90	86	68	83	81	85

[a] Capital letters in parentheses indicate sampling site: T = Tyrrhanian Sea; A = Adriatic Sea; L = Ligurean Sea.
[b] < ng/g lipid weight as 3 × S/N.
HxBDE = Hexabromodiphenylether.
MeO-TeBDE = Methoxylated tetrabromodiphenylether.
PBDE = Polybrominated diphenylether.

PeBDE # 99 with a concentration range of 10 to 1573 ng/g l.w., followed by PeBDE # 100 with a concentration range of 9 to 1414 ng/g l.w. In addition, an unidentified PeBDE was found at RT 15.464, eluting before # 100 and 99, in three of the samples. No standard for this PeBDE was available at the time of analysis, hence it was denoted PeBDE # "a" as in Lindström *et al.* (1999b). The calculation of the unknown PBDE, of which no standard was available, was achieved by using the same response factor as the closest eluting PBDE at the same bromination level, i.e., semiquantitatively. PeBDE # "a" was found at low concentrations in fin whale and in two of the striped dolphin samples (5, 27, and 135 ng/g l.w., repectively).

It can be observed from Table 2 that the bottlenose dolphin M6 displayed a ratio of approximately 9 for PeBDE # 100 and

#99, which is in contrast to all of the other cetaceans studied. This phenomenon was reported earlier in studies of polar cod (*Boreogadus saida*) and beluga whale (*Delphinapterus leucas*), however, the ratio was not as high as in the bottlenose dolphin (3.1 and 1.3 to 3.3, respectively; Wolkers *et al.* 2004). Three of the four sampled bottlenose dolphins had accumulated higher concentrations of PeBDE # 100 than # 99. The average concentrations of each congeners are listed on a cetacean-species basis in Figure 1.

The HxBDE congener # 154 was the fourth most abundant congener at a concentration of 23 to 1106 ng/g l.w., which was somewhat higher than for HxBDE # 153 at 11 to 607 ng/g l.w. An isomer was also found among the HxBDEs—denoted # "a" as in Lindström *et al.* (1999b) and with an unknown structure—in 9 of the 14 samples. The lowest concentration was 14 ng/g l.w. in the fin whale sample, and the highest concentration, 536 ng/g l.w., was found in one of the striped dolphin samples. Both PeBDE # "a" and HxBDE # "a" were identified earlier in pilot whales from the Atlantic Ocean at similar concentrations as for the fin whale sample (Lindström *et al.* 1999b; van Bavel *et al.* 1999). The striped dolphin sample with the highest concentration of HxBDE # "a" had a concentration that was approximately 8 to 20 times higher than pilot whales in the Atlantic Ocean. In Table 2, the concentrations of the 8 identified PBDE congeners are listed in ng/g l.w. for the 14 different samples grouped by species.

All of the analyzed samples displayed the same congener pattern for the most abundant PBDEs: TeBDE # 47 >PeBDE # 99 and 100 >HxBDE # 154 and 153 (Fig. 1). This pattern can also be seen in the technical product Bromkal 70-5DE with one exception; HxBDE # 154 is less abundant than HxBDE # 153 in Bromkal 70-5DE as shown by Sjödin *et al.* (1998). This difference in congener pattern has been seen earlier in cetacean samples and also in other marine species (Boon *et al.* 2002; Lindström *et al.* 1999b; van Bavel *et al.* 1999). It has been suggested by Lindström *et al.* (1999b) that this pattern indicates that Bromkal 70-5DE is not the only source of exposure to cetaceans.

As shown in Table 2 the dolphin species are the cetacean samples that display the highest concentration of PBDEs. This was shown previously by de Boer *et al.* (1998a). The bottlenose dolphins found stranded along the coasts of the Adriatic Sea had the lowest concentration of PBDEs for all the analyzed samples (range sum PBDE 66 to 518 ng/g l.w. compared with 129 to 8133 ng/g l.w. for the other dolphin species), with one exception of a striped dolphin from the Ligurian Sea. Our data suggest that the Adriatic Sea seems to be less polluted with PBDEs than the Ligurian and Tyrrhenian Seas (Fig. 2). These results are in agreement with what was shown earlier for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in the same samples (Jimenez *et al.* 2000). Examination of the sex of the animals in relation to PBDE contamination shows that male animals had a slightly higher body burden of PBDE than female animals, which as shown previously for pilot whales (Lindström *et al.* 1999b; van Bavel *et al.* 1999). However, no information exists about the age of the animals in this study, which might influence the differences seen in PBDE concentrations concerning the sex of the animals.

Methoxylated PBDEs

In Figure 3, four mass traces are shown from one of the striped dolphin sample chromatograms (i.e., L6 in Tables 1 and 2) acquired when running GC/MS EI (SIM). The TeBDEs are identified at mass 483.7 (PBDE # 47), the PeBDEs at mass 563.7 (PBDE # "a," # 100, and # 99) and finally the HxBDEs at mass 643.5 (PBDE # "a," # 154, and # 153). At mass 515.7, the molecular mass of the $(M + 4)^+$ ion of MeO-TeBDE, three peaks were identified corresponding to MeO-TeBDE-1, -2, and -3 as described by Haglund *et al.* (1997). These three peaks are probably the same three MeO-TeBDEs identified by Marsh *et al.* (2004) as 6′-MeO-BDE-49, 2′-MeO-BDE-68, and 6′-MeO-BDE-47; however, without internal standards no definite conclusions can be drawn.

In this study, the most abundant methoxyBDE was MeO-TeBDE # 3 with a concentration range of 7 to 628 ng/g l.w. The concentration reported by Haglund *et al.* (1997) for MeO-TeBDE # 3 was 95 ng/g l.w. and 158 ng/g l.w. in blubber from grey seal and ringed seal, respectively. The corresponding levels in liver tissue from the same animals were 1.5 ng/g l.w. and 1.8 ng/g l.w., respectively. Compared with the cetacean samples, these seal samples have a concentration 5 to 350 times lower in liver tissue than the cetaceans. Haglund *et al.* (1997) also identified MeO-PeBDEs, which were not seen in the cetacean samples.

As can be seen in Figure 4 and Table 2, the relationship between the total amount of PBDEs compared with the total amount of MeO-TeBDEs does not support the hypothesis that the animal with the highest concentration of PBDEs would consequentially also have the highest amount of MeO-TeBDEs because of metabolism of PBDEs. For example, in the group of striped dolphins displaying the highest total concentration of PBDEs in this study, the total amount of MeO-TeBDEs represents only 3% of the total amount of PBDEs, whereas in the bottlenose dolphin and Risso's dolphin groups, the MeO-TeBDEs represent 13% and 15% of the total PBDE concentration, respectively. In fin and pilot whales, the corresponding percentage is 15% and 17%, respectively.

It has been suggested that PBDEs are metabolized in mammals by cytochrome P450 2B (Hallgren and Darnerud 1998) and that this specific enzyme isomer is lacking or underdeveloped in most studied cetacean families (Boon *et al.* 2000; Tanabe *et al.* 1988, White *et al.* 1994). Boon *et al.* (2000) also showed that microsomes isolated from sperm whale were unable to metabolize PBDEs when tested in an *in vitro* assay. It is therefore unlikely that the three MeO-TeBDEs found in this study are metabolites of PBDEs. The source of MeO-PBDEs has been suggested to be natural production by marine sponges in symbiosis with cyanobacteria (Carté and Faulkner 1981; Fu *et al.* 1995; Handayani *et al.* 1997) and marine red algae (Asplund *et al.* 2001). No reports are available on any industrial production or byproduct formation of HO-PBDEs or MeO-PBDEs (Haglund *et al.* 1997; Marsh *et al.* 2001). Several studies have reported HO-PBDEs (Asplund *et al.* 2001; Marsh *et al.* 2001) and MeO-PBDEs in environmental samples (Haglund *et al.* 1997; Marsh *et al.* 2004; Olsson *et al.* 2000; Vetter 2001; Vetter *et al.* 2001; Vetter *et al.* 2002), suggesting that they are ubiquitous compounds. Vetter *et al.* (2001, 2002)

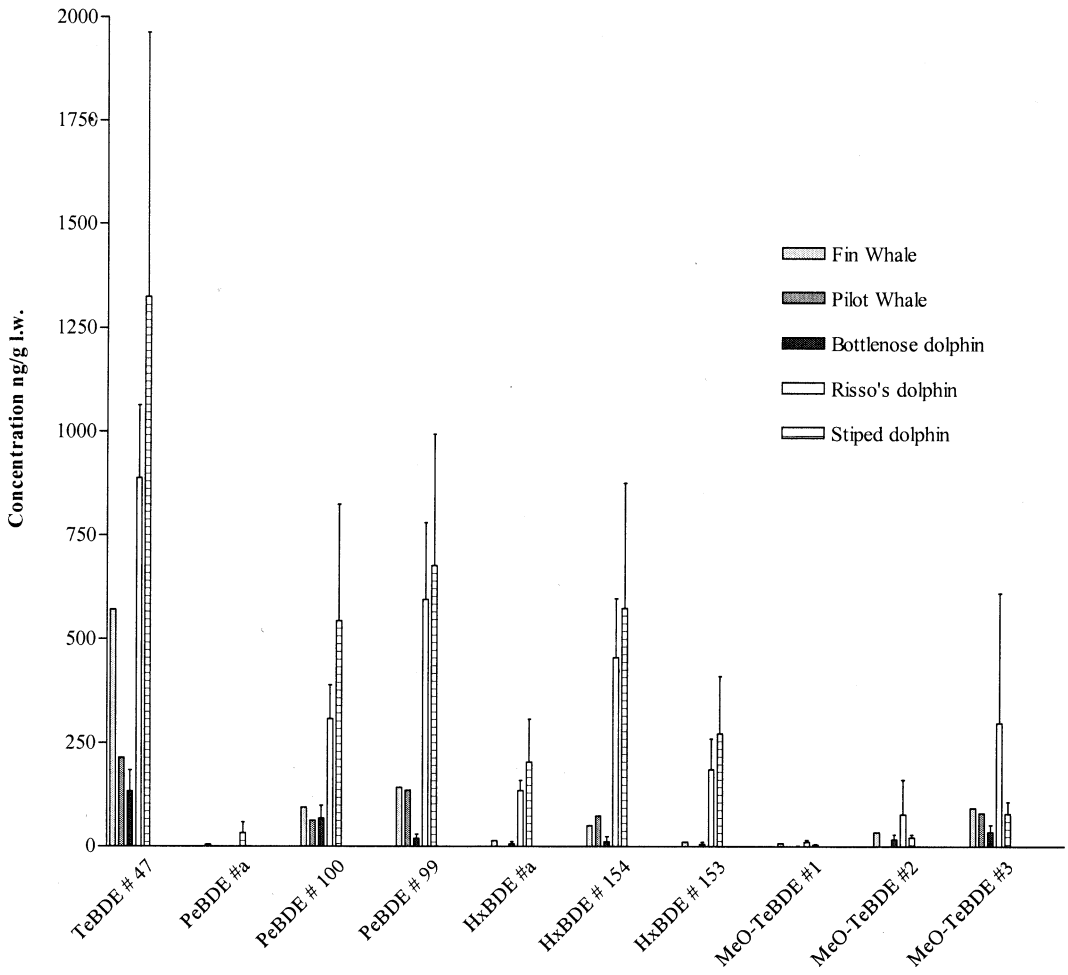

Fig. 1. Bar graph displaying the concentration (ng/g l.w.) for the different PBDE congeners as well as the three MeO-TeBDEs, as a function of cetacean species. l.w. = lipid weight; MeO-TeBDEs = methoxylated tetrabromodiphenylethers

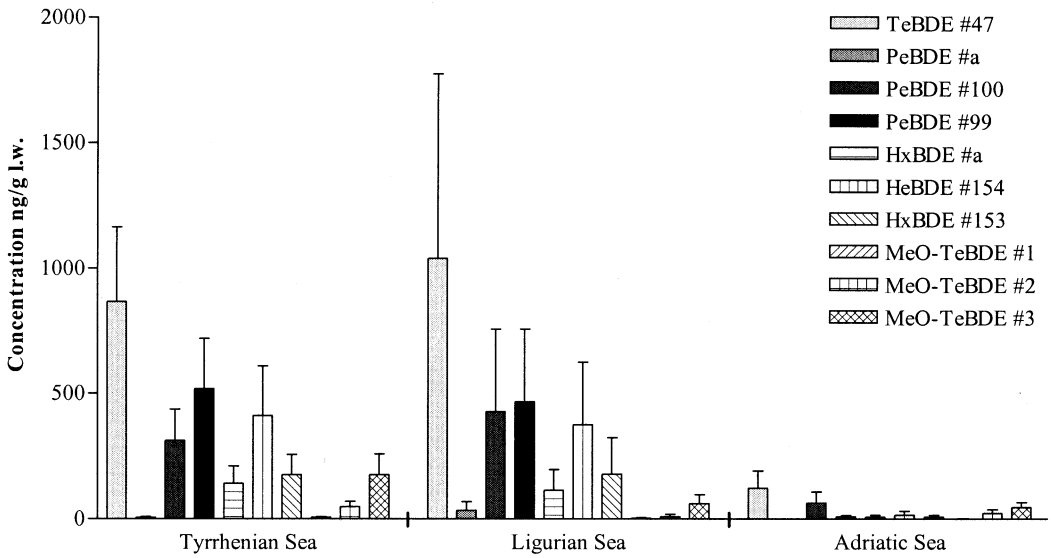

Fig. 2. Regional differences between the average amount of PBDEs and MeO-TeBDEs (ng/g l.w.) in the cetacean samples. l.w. = lipid weight; MeO-TeBDEs = methoxylated tetrabromodiphenylethers; PBDEs = polybrominated diphenylethers

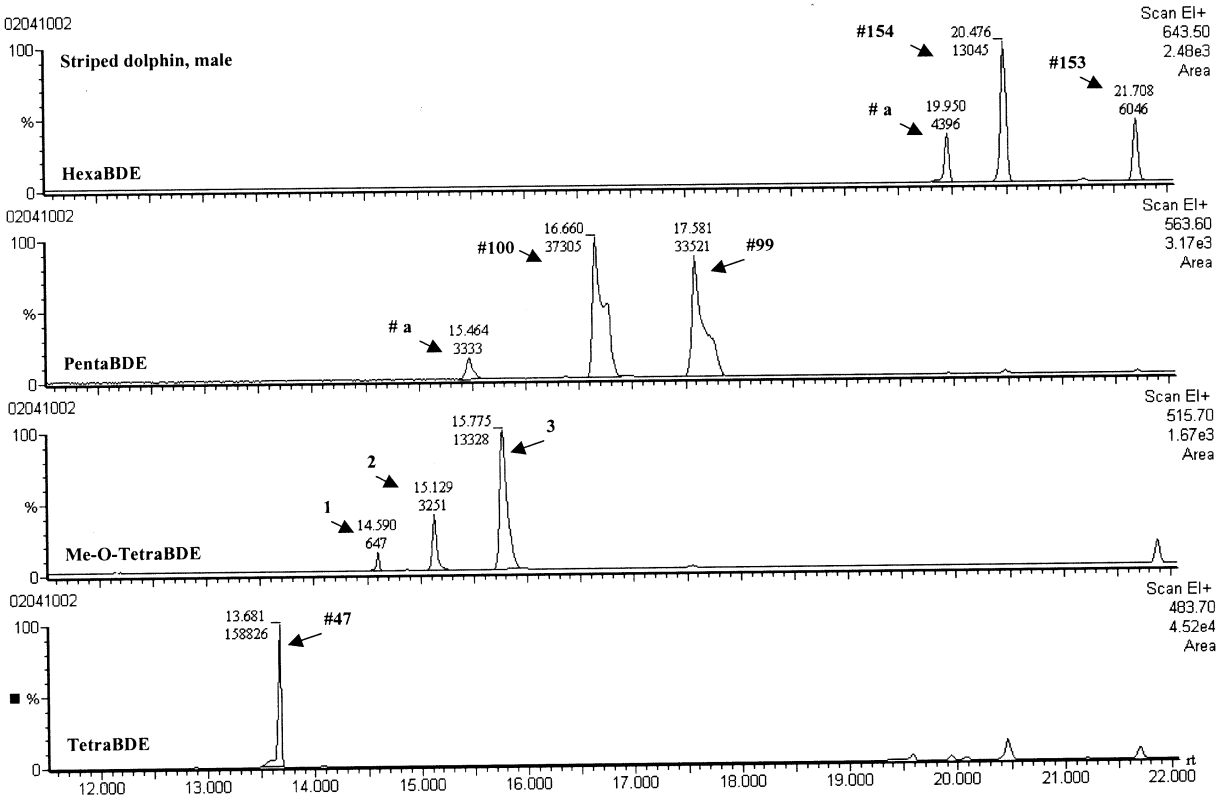

Fig. 3. Chromatogram from the GC-MS EI run of sample L6 (striped dolphin). Channels 483.7 (TeBDE), 515.7 (MeO-TeBDE), 563.6 (PeBDE), and 643.5 (HxBDE) are shown from 11 to 22 minutes. Confirmation ions 485.7 (TeBDE), 517.7 (MeO-TeBDE), 565.6 (PeBDE), and 645.5 (HxBDE) were measured but are not shown here. EI = electron impact ionization; GC/MS = gas chromatography/mass spectrometry; HxBDE = hexabromodiphenylether; MeO-TeBDE = methoxylated tetrabromodiphenylether; PeBDE = pentabromodiphenylether; TeBDE = tetrabromodiphenylether

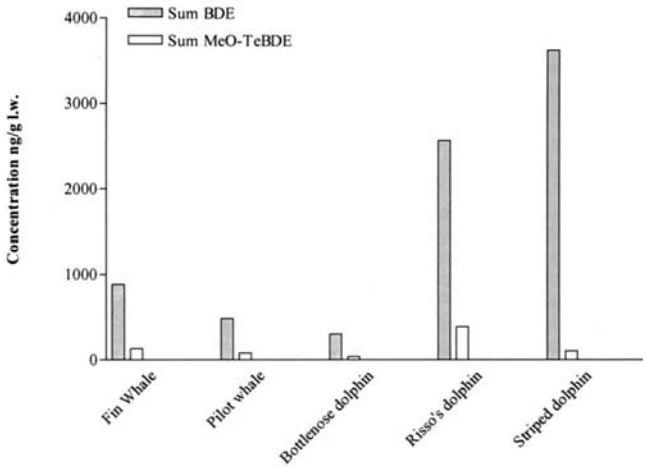

Fig. 4. Comparison of the total amounts of PBDEs and MeO-TeBDEs (ng/g l.w.) in the different cetacean samples. MeO-TeBDEs = methoxylated tetrabromodiphenylethers; PBDEs = polybrominated diphenylethers

reported concentrations of MeO-PBDEs in the range of 1 to 3.8 mg/kg in marine mammals from Queensland, Australia, in which anthropogenic PBDEs were below detection limits, which strengthens the suggestion that these compounds are natural products.

Other Bromine Compounds

Three of the samples were run on GC/MS NCI—scanning m/z 79 and 81—to screen for other bromine-containing organic compounds. As can be seen from the NCI chromatograms in Figure 5, several organic bromine compounds were detected. The PBDE internal standards are marked with arrows in Figure 5, and it should be noted that when using NCI and monitoring only m/z 79 and 81, no distinction can be made between the ^{13}C-labeled internal standards and the "native" PBDEs in the samples. Although not perfect, and with some interference (probably lipid carryover) around RT 16 to 19 minutes, several other organic bromine compounds were found. The lipid content in the fin whale sample was high (64%) and could have been a result of a metabolism disorder in the animal, but this cannot be confirmed because the samples were taken from stranded dead animals.

One interesting peak was found at RT 23.376 in the fin whale sample (Fig. 5). Around this retention time the HxBDE congener # 138 usually appears, but in this sample no such congener could be detected when running GC/MS EI (SIM).

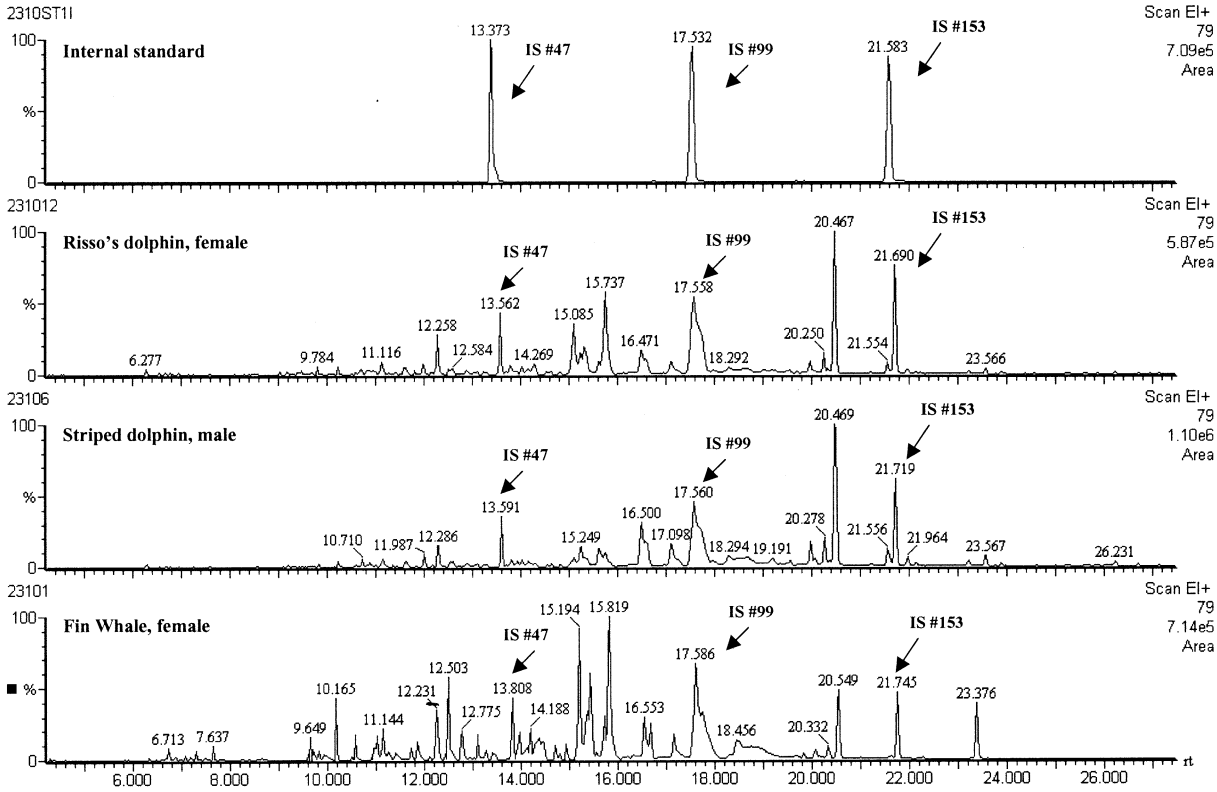

Fig. 5. Chromatogram from GC-MS NCI runs of three different samples (M1, L6, and M10) and the internal standard. Several unidentified bromine compounds in the cetacean samples can be seen. Of special interest are the peaks at RT 20.549 and 23.376 from the fin whale, which are not BDE congener # 154 and # 138 but coelute with these isomers. Only m/z 79 is shown; however, m/z 81 was also measured but is not shown here. BDE = brominated diphenylether; GC/MS = gas chromotography/mass spectrometry; NCI = negative-chemical ionization; RT = retention time

Therefore, this peak was not HxBDE # 138 and—in addition—the peak area was much larger than expected for HxBDE # 138. This peak most probably represented an organic bromine compound, present at high concentration and of unknown structure, that interfered with the determination of HxBDE # 138 when only monitoring m/z 79 and 81 running NCI. A weak trace of this peak was also present in the dolphin samples at RTs 23.567 and 23.566, respectively. Other peaks that did not seem to correlate to known PBDE congeners were the ones at R 20.549, 20.469, and 20.467 in the fin whale, striped dolphin, and Risso's dolphin samples, respectively. Around this time, HxBDE # 154 normally elutes on the used HP-5MS column, which was shown by GC/MS EI analysis to be present in the samples. However, considering the detected peak area, it was too large to only represent # 154. The peak area of this unknown was much larger than the internal standard peaks of HxBDE # 153 and also larger than for TeBDE # 47, which was the most abundant congener in all of the cetacean samples. This peak represented both the ^{13}C-labeled internal standard # 47 and the "native" occurring TeBDE # 47. This indicated that another bromine compound coeluted with HxBDE # 154. These two examples of peak detection clearly displayed the need for running both EI and NCI on environmental samples. EI is more specific for monitoring the molecular ion than NCI, which might overestimate the concentration of certain PBDE congeners. Other organic bromine compounds that could give rise to these unidentified peaks are polybrominated biphenyls, hexabromocyclododecane, or tetrabromobisphenol A (Athanasiadou 2003).

The samples from the NCI run showed a clear difference in the chromatograms between the fin whale sample compared with the dolphin samples. A completely different pattern for total organic bromine compounds was seen, which was probably related to the fact that the fin whale is of the order Mysticeti and the dolphins are Odontoceti, e.g., baleen (= Mysticeti) compared with toothed whales (= Odontoceti). The baleen whales usually eat Euphausiids (i.e., krill), copepods, and sometimes fish compared with toothed whales, which often feed on predatory fish and/or squid (Evans 1987). The baleen whale's brominated compound pattern was more complex than that of toothed whales, for which many of the compounds can be metabolized throughout the food chain. Furthermore, in this study the levels of MeO-TeBDEs were not much higher in the toothed whale samples compared with the baleen whale samples, thus indicating compounds present in the marine environment at different trophic levels.

In conclusion, cetacean samples from the Mediterranean Sea display the same PBDE congener pattern seen in cetaceans from the Atlantic Ocean and the North Sea (Boon *et al.* 2002; de Boer *et al.* 1998a; Lindström *et al.* 1999a; Lindström *et al.* 1999b; van Bavel *et al.* 1999). Several unidentified bromine compounds were found in the liver samples. Three isomers of

MeO-TeBDE were found in the samples, and the origin of these compounds is still unknown although natural sources cannot be ruled out.

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