LEVELS OF POLYBROMINATED DIPENYL ETHERS IN MARINE MAMMALS

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Introduction

Polybrominated diphenyl ethers (PBDEs) have been found in the environment since the beginning of the 1980s[1,2]. Until recently this class of persistent compounds has not attracted major attention as a global pollutant. However recent reports on rising PBDE levels in human milk[3], and the relatively high levels discovered in the sperm whales from the North Sea[4] have caused concern on the usage and environmental distribution of these compounds. Relatively high levels are found in the marine environment, especially in marine mammals. Levels of PBDE have been reported in the literature for different marine mammals including seal[4,5,6,7,8,9], dolphin[9,10,11] and whale[4,12,13,14]. Within the presented study a large number samples from marine mammals were analysed for PBDEs and their derivatives. The marine species include Long Finned Pilot Whale, Beluga Whale, Killer Whale and Polar Bears. For the chemical analysis supercritical carbon dioxide was use as extraction medium. By coupling to liquid chromatography (SFE-LC) a large number of samples was analysed both environmentally friendly and cost/time effectively. Results for the pilot whale will be discussed in more detail, in relation to the large individual variation in the levels.

Material and Methods

Samples of long finned pilot whales were taken in connection with the traditional drive kills in Torshavn, Faroe Islands, from 1994 to 2000. Polar bear and beluga samples were taken at Svalbard, in 1998, by collecting small amounts of fat through skin biopsy. The samples were kept frozen at -20°C until homogenised with sodium sulphate (1:5). About 3 g of the homogenised tissue was packed in a Supprex standard extraction vessels (10 ml). An internal standard consisting of ^{13}C-labeled PBDEs (Wellington Laboratories, Canada) was added before the SFE extraction. On the top of the sample around 4.5 g basic aluminium oxide (AlOx) was added as a fat retainer. The extractions were carried out on a Supprex A44 SFE using CO_2 as the supercritical fluid. The chamber temperature was 40°C and the pressure 281 bar during extraction, resulting in a

supercritical fluid density of 0,9 g/ml and a flow rate of 2 ml/min. During SFE extraction the target compound were trapped on a solid phase trap containing C18 absorbent (ODS, Octadecylsilica). The nozzle and trap temperatures were kept at 45°C and 40°C respectively. After completion of the extraction the trap was rinsed with 2 ml hexane and 2 ml methylene chloride at a rate of 2 ml/min. After addition of the recovery standard containing in tetradecane the sample volume was reduced to 30 µl, producing an extract ready for GC/MS analysis. Separate lipid determination was performed by applying a part (~1g) of the homogenate on a small column and quantitatively extract with methylene chloride and hexane (1:1). The weight of the extracted lipids was determined gravimetrically.

Selected ion SIR HRGC/MS spectra were recorded using a Fisons GC 8000 gas chromatograph coupled to a MD800 mass spectrometer. Chromatographic separation was achieved by splitless injection of 2 µl on a non-polar DB-5 column using helium as the carrier gas. The GC oven was programmed as follows: 180°C initial hold for 2 min. increase at a rate of 15°C/min to 205°C, followed by an increase of 3,7°C/min to 300°C, final hold at 300°C for 15 minutes. The two most intense ions of the molecular ion cluster were monitored for TeBDE (*m/z* 483.7, 485.7), PeBDE (*m/z* 563.6, 565.6) and HxBDE (*m/z* 641.5, 643.5) in addition to masses for the ^{13}C-labeled internal standard (*m/z* 495.71, 497.68). The quantification standard consisted of TeBDE, PeBDE and HxBDE, and detected PBDEs were quantified against a PBDE of the same bromination level closest to its retention time. The Methoxylated derivative of TeBDE, Me-O-TeBDE was quantified assuming the same MS response as TeBDE, for this compound also the two most abundant molecular masses were monitored (*m/z* 515.7, 517.7).

Results and discussion

In all samples analysed PBDEs were found at low ppm and medium ppb level. The results are summarised in Table 1. Highest levels were measured for the pilot whale caught at the Faroe Islands, somewhat lower levels were measured in the beluga whales and the polar bears. Pilot whales are known to migrate through the Atlantic and are thus less stationary than beluga whales, which are distributed through the arctic or sub arctic. Because of this behaviour pilot whales migrate to more industrialised area's which might be the cause of the higher levels. The levels are in agreement with the levels in sperm whales (187-348 ng/g lw.) and minke whale (869 ng/g lw.) collected at the Dutch coast[4]. The levels for the pilot whales caught in 1992-94 were somewhat higher in our study. The levels in the beluga whales from Svalbard were somewhat higher than the levels in beluga whales from Cumberland Sound, Canada (2-15 ng/g lw.) collected over a period from 1982 to 1997[13]. The levels in polar bear were somewhat lower than expected when compared to the very high levels of PCBs found in the same samples. However in the GC/MS run of the SFE-LC extract several other unidentified brominated compounds were seen. Both the low levels of PBDEs and the presence of unknown bromine containing organic compounds might

suggest that the polar bear is able to metabolise the PBDEs. No data on PBDEs in the polar bear could be found in the literature for comparison.

Table 1. Concentrations (ng/g lipid) of the sum of PBDEs and Me-O-PBDEs in pilot whale blubber from the Faroe Islands, Beluga Whale from Svalbard and Polar Bear from Svalbard.

species	PBDEs	sampling year	number	sample type
Pilot Whale	82-3160 (ng/g)	1994-2000	n =20	pooled and individual samples
Beluga	41-332 (ng/g)	1998	n =10	individual samples
Polar Bear	14-144 (ng/g)	1998	n =20	individual samples

In both the beluga and the pilot whales methoxylated PBDE (Me-O-PBDE) were found to be present. Although no standard compounds were available an estimation of the concentration of this compounds was made using the mass response of TeBDE. These way semi-quantitative levels of Me-O-PBDE were calculated. When doing this, surprisingly high levels of Me-O-TeBDE were found in both the pilot whale and the beluga whale. The levels of these compounds seem almost comparable to the most abundant TeBDE #47. No Me-O-TeBDE was found in the polar bear. This might be an indication of a different metabolism in the polar bear, however the origin of Me-O-TeBDE is not yet known[15].

Over the period 1992-2000 a large variation of the level of PBDEs was seen in the pilot whale. The highest levels were seen in 1994 and have since decreased. This might indicate lower levels in the environment but this trend is confounded by a large individual variation. Large differences were seen between adult and juvenile whales and between adult male and females in the samples taken in 1997[16]. The highest levels were found in the juvenile animals, somewhat lower values in the adult males and the lowest values in the adult females. This observation indicates transfer of the PBDEs from mother/cow to the calf by lactation. This was confirmed, at the same time indicating that there is a transplacental barrier, by the samples taken in 2000. Here both the mother and foetus were analysed for PBDEs. The level in the foetus was lower for two samples as shown in Table 2. Unfortunately this could not be confirmed by the third sample, the result of this analysis were biased by an extremely low fat content of the sample.

Table 2. Concentrations (ng/g lipid) of PBDEs in pilot whale blubber from the Faroe Island, in 2000. ND = Not detected. Unidentified PBDEs are denoted as Lindström et al.[12]

	Pilot Whale 1		Pilot Whale2		Pilot Whale 3	
	Female	Foetus	Female	Foetus	Female	Foetus
Lipids %	64%	71%	83%	65%	76%	2%[1)]
TeBDE (a)	1.7	1.4	ND	1.2	0.5	ND
TeBDE (b)	0.2	0.2	ND	0.3	0.1	ND
TeBDE (c)	0.7	0.6	ND	0.3	0.1	ND
TeBDE (d)	2.5	1.9	ND	2.1	1.6	ND
TeBDE #47	234	141	121	120	37	*146*
TeBDE (e)	ND	ND	ND	ND	ND	ND
TeBDE (f)	6.0	4.0	3.5	3.8	1.4	ND
PeBDE (a)	2.9	1.2	ND	ND	ND	ND
PeBDE (c)	ND	ND	ND	ND	ND	ND
PeBDE (d)	23	7.4	26	5.7	3.3	*39*
PeBDE #99	44	14	30	12	10	*60*
PeBDE (g)	1.4	ND	ND	ND	ND	ND
HxBDE (a)	2.6	ND	4.5	ND	1.4	ND
HxBDE (b)	9.3	ND	19	ND	6.1	ND
HxBDE (c)	ND	ND	ND	ND	ND	ND
HxBDE #153	3.0	ND	2.8	ND	ND	ND
TeBDE-O-Me (a)	2.1	1.5	1.9	1.7	0.5	ND
TeBDE-O-Me (b)	16	8.5	11	7.5	5.8	*18*
TeBDE-O-Me (c)	83	24	95	16	14	*121*
Total PBDE	**433**	**206**	**314**	**171**	**82**	*385*

ND Not detected
[1)] invalid for comparrison

Conclusion

Using supercritical carbon dioxide for both extraction and clean up a large number of marine samples were analysed for PBDEs and their derivatives. By coupling supercritical fluid extraction to liquid chromatography (SFE-LC) nearly solvent free extraction and clean up is achieved resulting in an environmental acceptable analytical method, in contrast to traditional methods based on liquid extraction.

The results showed the presence of PBDEs in all samples (pilot, beluga and killer whale and polar bear) at relatively high concentrations (14-3160 ng/g lw.). In the pilot whale and the beluga whale Me-O-PBDE was found at comparable levels as the most abundant TeBDE #47. Transfer of PBDEs from mother to calf through mother's milk is observed. The levels of PBDEs in the foetus were lower than in the mothers (pilot whales), indicating that also for marine mammals there is a transplacental barrier, and the perinatal exposure (in quantitative terms) is mainly due to lactational transfer.

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