Polybrominated Biphenyls and Diphenylethers in Sperm Whales and Other Marine Mammals - a New Threat to Ocean Life?

Jacob de Boer, Peter G. Wester, Dolors Pastor i Rodriguez*, Wilma E. Lewis** and Jan P. Boon**

DLO-Netherlands Institute for Fisheries Research, IJmuiden, The Netherlands
*National Institute for Coastal and Marine Management/RIKZ, Haren, The Netherlands
**Netherlands Institute for Sea Research, Den Burg, Texel, The Netherlands (NIOZ publ. no. 3287)

Introduction

Brominated flame retardants are used intensively in modern life. Many materials which can catch fire easily such as television sets, computers, clothing, etc., are impregnated with brominated flame retardants for protection (1). The most frequently used brominated flame retardants are tetrabromobisphenol-A, hexabromocyclododecane, polybrominated biphenyls (PBBs) and polybrominated diphenylethers (PBDEs), having together an estimated world production of 150,000 tons a year (1-3). PBBs and PBDEs are used as additive flame retardants (1), which are incorporated into the matrix of various plastic materials such as polystyrenes (2). Heating of PBBs and PBDEs may lead to the formation of brominated dioxins (PBDDs) and furans (PBDFs) (4). Apart from a direct absorption in humans by emittance from electronic circuit boards and plastic computer and TV cabinets, there is also an environmental problem. Due to their high lipophilicity (log K_{ow}>6) and resistance to degradative processes PBBs and PBDEs are expected to bioaccumulate easily (5). Only higher brominated compounds may show a reduced bioaccumulation because these larger molecules would have more difficulties to pass the membranes of organisms. However, bioaccumulation in fish has been demonstrated for decabrominated diphenylether in a laboratory experiment (6). A substantial part of these compounds will eventually reach the marine environment. Despite the similarities in behaviour and toxicity with well-known environmental contaminants as PCBs and DDT , no bans have been enacted and the production of these flame retardants has continuously increased (4,7).
The present study was focused on the determination of PBBs and PBDEs in marine animals from coastal seas and the Atlantic Ocean. The persistence of PBDEs and PBBs against oxidative metabolism was studied for the P450 enzyme system in an *in vitro* bioassay with hepatic microsomes of different marine mammals. Genotoxicity was tested in a mutatox® assay.

Materials

PBB and PBDE concentrations were determined in 13 samples belonging to 3 species of lung-breathing marine mammals feeding on invertebrates and/or fish: three sperm whales (*Physeter macrocephalus*; main food source squid and fish from deep water), one whitebeaked dolphin (*Lagenorhynchus albirostris*; main food source fish), one minke whale (*Balaenoptera acutorostrata*, a representative of the baleen whales (*mysticeti*), main food source fish), and four harbour seals (*Phoca vitulina*; main food source fish), and in one fish sample: mackerel (*Scomber scombrus*, southern North Sea). The sperm whales, dolphin and minke whale stranded alive at the Dutch coast, the seals were found shortly after they died. The three male sperm whales, all

with a length between 15 and 15.5 m, stranded alive on the Dutch coast near The Hague on 12 January 1995. The animals stranded together and were most probably adult males of about 35 years old, stemming from a bachelor school that got trapped in the shallow waters of the North Sea on their migration to waters around the Azores (8).
Hepatic microsomes of a whitebeaked dolphin, a sperm whale, and a harbour seal were used for the *in vitro* assays, which were carried out for the same congeners as were determined in the wildlife samples.

Methods
The PBBs and PBDEs were extracted from the samples by means of a Soxhlet extraction using hexane/acetone (3:1, v/v). The extracts were shaken with sulphuric acid to remove the lipids. After fractionation over silica columns the PBBs and the deca-BDE were found in the first (iso-octane) fraction and the remaining PBDEs in the second (diethylether/iso-octane) fraction. Recoveries were >85% for PBBs and >70% for PBDEs. The final analysis was carried out by gas chromatography/mass spectrometry using negative chemical ionisation (GC/NCI-MS) (4,9).
The basic methodology of the *in vitro* bioassays was reported before (10). The assays consisted of nine samples: 4 reference samples without NADPH, four samples with NADPH and one blank without incubation mixture and NADPH. The incubations took place at 37 °C for 90 min. The concentrations of the dosed compounds were: Bromkal 70-5DE 1.7 µg/ml, BDE 209 31 µg/ml, BB 15 25 µg/ml and other BBs 1-4 µg/ml (3 µl added to 2.1 ml). The samples were extracted with hexane and shaken with sulphuric acid to remove the lipids. The final analysis was carried out by GC with electron capture detection (ECD).

Results and discussion
The results of the chemical analyses are given in Table 1. The concentrations found in the cetaceans and pinnepeds are given for the individual animals because large mutual differences occur. The results show that most of the PBBs and PBDEs analysed were found in the sperm whales and also in the other samples. The total PBDE concentrations in sperm whale blubber (around 100 µg/kg) are ca. 50-fold higher than the total PBB concentrations in the blubber of the same animals (around 2 µg/kg), but in the liver total PBB concentrations are ca. 5-fold higher than the PBDE concentrations (Table 1). The presence of these xenobiotic compounds in sperm whales suggests that these compounds have reached deep ocean waters, since sperm whales do usually not occur in shelf seas. Female specimens do not migrate north of a latitude of 45° N (northern Spain), males occur north as far as northern Norway, Iceland and Greenland. Here, sperm whales hunt in waters with depths of 400-1200 m or more. Postmortem investigations of the same animals showed a complete absence of food in the alimentary tracts, in combination with evidence of weight loss and blubber reduction [13]. It is therefore likely that PBB and PBDE concentrations found in the blubber and liver of the sperm whales studied have been accumulated from deep water organisms of the oceanic food chain. Knap et al. (14) reported a flux of PCBs of 1.6 µg/m^2/yr to a depth of 3,200 m in the North Atlantic Ocean (Sargasso Sea), which shows that organic contaminants can be transported to deeper waters fairly soon. Relatively high PBDE concentrations were found in whitebeaked dolphins (> 7 mg/kg) and harbour seals (> 1 mg/kg) (Table 1), which had been feeding in the North Sea and the Wadden Sea. The levels of PBDEs in dolphins and seals indicate that an ongoing production of PBDEs may create an environmental problem similar to that of PCBs of which concentrations up to 128 mg/kg in marine mammals have been observed (15).
The results of the *in vitro* biotransformation tests with hepatic microsomes (Figure 1 - seal and sperm whale) confirmed that the two classes of brominated flame retardants studied are very persistent. In both cases some PCB congeners were added as a control of the assay since information on the behaviour of hepatic microsomes with regard to PCBs was obtained before (10). Apart from a ca. 80% decrease in BB 15 (4,4'-dibromobiphenyl) concentrations in the three animals tested, none of the other PBBs and PBDEs showed any indication of biotransformation. This indicates an even higher persistency than for PCBs, of which in some

animals and for some congeners (e.g. CBs 26, 28, 101) biotransformation was observed (Figure 1).

Table 1. Concentrations of bromobiphenyls[a] and bromodiphenylethers[a] in marine wildlife samples in µg/kg wet weight

Species	Tissue	Fat[b] (g/kg)	PBB concentrations						
			15	49	52	101	153	169	209
Sperm whale 1	Blubber	722	0.06	0.24	0.40	0.91	1.9	<0.1	<0.5
Sperm whale 2	Blubber	234	0.04	0.13	0.21	0.40	0.73	0.05	<0.3
Sperm whale 3	Blubber	317	0.07	0.20	0.36	0.70	1.1	<0.1	<0.4
Sperm whale 2	Liver	23	<0.01	<0.01	<0.01	0.63	18	<0.04	<0.3
Whiteb. dolphin	Blubber	990	0.2	7.5	4.1	8.3	13	<0.2	<0.9
Whiteb. dolphin	Liver	27	<0.01	0.06	0.03	0.74	19	<0.02	<0.1
Minke whale	Blubber	140	0.11	0.27	0.24	0.54	0.82	<0.02	<0.1
Harbour seal 1	Blubber	244	<0.05	34	5.7	9.3	61	12	<1
Harbour seal 2	Blubber	963	<0.05	3.1	2.3	1.4	18	<0.2	<1
Harbour seal 3	Blubber	722	<0.05	3.0	0.52	1.1	13	<0.1	<1
Harbour seal 2	Liver	35	<0.01	0.10	0.05	0.62	1.5	<0.02	<0.1
Harbour seal 3	Liver	51	<0.01	0.10	0.03	0.04	0.82	<0.01	<0.1
Harbour seal 4	Liver	30	<0.01	0.90	0.14	0.44	13	<0.02	<0.1
Mackerel	Muscle	152	0.01	0.01	0.01	<0.01	0.04	<0.03	<0.2

[a] Numbering of PBB and PBDE congeners is identical to PCB numbering system of Ballschmiter *et al.* (1992) (11); xy-PBDE is pentabrominated BDE with unknown structure; [b] Total lipid contents according to Bligh and Dyer (1959) (12), except seal 1 blubber and seal 3 blubber and liver, which are extractable lipid contents.

Species	Tissue	Fat content[b] (g/kg)	PBDE concentrations			
			47	xy	99	209
Sperm whale 1	Blubber	722	95	15	26	<6
Sperm whale 2	Blubber	234	58	8.1	15	<3
Sperm whale 3	Blubber	317	61	7.5	10	<5
Sperm whale 2	Liver	23	2.7	0.54	0.91	<3
Whiteb. dolphin	Blubber	990	5500	1200	1000	<10
Whiteb. dolphin	Liver	27	22	5.8	3.0	<1
Minke whale	Blubber	140	88	11	23	<1
Harbour seal 1	Blubber	244	1200	110	160	<15
Harbour seal 2	Blubber	963	1200	100	40	<10
Harbour seal 3	Blubber	722	280	18	140	<10
Harbour seal 2	Liver	35	21	0.93	0.85	<2
Harbour seal 3	Liver	51	12	0.33	5.1	<1
Harbour seal 4	Liver	30	20	0.07	0.53	<2
Mackerel	Muscle	152	5.4	1.8	1.9	<2

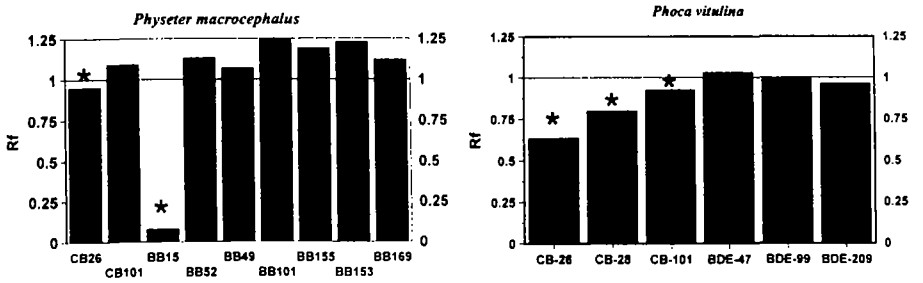

Figure 1. Results of *in vitro* tests for sperm whale (PBBs) and harbour seal (PBDEs).

Both classes of compounds did not show a genotoxic response in the mutatox® assay at concentration ranges of 0.07 - 900 ng/ml (PBDEs) and 0.002 - 9 µg/ml (PBBs). However, PBBs are able to potentiate the carcinogenicity of other compounds (16).
There may be different reasons for the absence of the decabrominated congeners BB 209 and BDE 209 in the wildlife samples: i) they may disappear (and possibly degrade to lower brominated congeners) due to chemical and microbial degradation (weathering), or ii) they may not or only slowly enter cells of organisms because their relatively large molecules have difficulties in passing biomembranes. Clearly this topic needs further research, particularly because there is a tendency with producers of flame retardants to increase the production of higher brominated compounds.

Conclusions

The presence of PBBs and PBDEs in sperm whales suggests that these classes of compounds have reached deep ocean waters (17). Their high persistence to biotransformation by the cytochrome P450 system of marine mammals, which was shown in *in vitro* bioassays, may, in combination with an ongoing production, create an environmental problem which has many similarities to that of PCBs. High levels of particularly PBDEs in seals and dolphins show that such a problem may be coming up fairly soon. More research is required to understand the environmental behaviour of the decabrominated compounds, which, in spite of an increasing production, have not been found in aquatic organisms yet.

References

1. Sellström U (*Licentiate thesis*), Polybrominated diphenylethers in the Swedish environment, Stockholm University, **1996**, ITM-report 1996 45, Solna, Sweden.
2. Zitko V; *Mar. Pollut. Bull.* **1993**, 26, 584.
3. Sellström U, Jansson B; *Chemosphere* **1995**, 31, 3085.
4. Pijnenburg AMCM, Everts JW, de Boer J, Boon JP; *Rev. Environ. Contam. Toxicol.* **1995**, 141, 1.
5. Anon. Brominated diphenylethers. IPCS, **1994**, Environmental Health Criteria nr. 162, World Health Organisation, Geneva, Switzerland.
6. Kierkegaard A, Balk L, Sellström U, Tjärnlund U, Ørn U, de Wit C, Jansson B; Uptake of decabromodiphenylether (DeBDE) in rainbow trout via administration in the diet. Proceed. SETAC Conference, June **1995**, Copenhagen, Denmark.
7. Anon. Polybrominate biphenyls. IPCS, **1994**, Environmental Health Criteria nr. 152. World Health Organisation, Geneva, Switzerland.
8. Kompanje EJO, Reumer JWF; *Deinsea* **1995**, 2, 89.
9. de Boer J; *Chemosphere* **1989**, 18, 2131.
10. Boon JP, Sleiderink HM, Helle MS, Dekker M, van Schanke A, Roex E, Hillebrand TJ, Klamer, JC, Govers B, Pastor D, Morse D, Wester PG and de Boer J. The use of a microsomal in vitro assay to study phase I biotransformation of chlorobornanes (Toxaphene®) in marine mammals and birds. Possible consequences of biotransformation for bioaccumulation and genotoxicity. *Compar. Biochem. Physiol.* **1998**, in press.
11. Ballschmiter K, Bacher R, Mennel A, Fischer R, Riehle U., Swerev M; *J. High Resolut. Chromatogr.* **1992**, 15, 260.
12. Bligh, EG, Dyer, WJ; *Can . J. Biochem. Physiol.* **1959**, 37, 911.
13. Jauniaux T, Brosens L, Jacquinet E, Lambrigts D, Addink M, Smeenk C, Coignoul F; *J. Wildlife Diseases* **1998**, 34 , 99.
14. Knap, AH, Binkley, KS, Deuser WG; . *Nature* **1986**, 319, 572.
15. Boon JP, van der Meer J, Allchin CR, Law RJ, Klungsøyr J, Leonards PEG, McKenzie C, Wells DE ; *Arch. Contam. Toxicol.* **1997**, 33, 298.
16. Silberhorn EM, Glauert HP, Robertson LW; *Critical Rev. Toxicol.* **1990**, 20, 440.
17. de Boer J, Wester PG, Klamer HC, Lewis WE, Boon JP; Do flame retardants threaten ocean life? *Nature* **1998**, in press.