Studies on immunological effects of polybrominated diphenyl ether (PBDE) and polychlorinated biphenyl (PCB) exposure in rats and mice

Per Ola Darnerud and Ann Thuvander

National Food Administration, P.O. Box 622, SE-751 26 Uppsala, Sweden

Introduction

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in various materials such as plastics, rubber and paints. As result from the global and ubiquitous use of PBDEs, environmental residues have been detected both in sediment and biota. Apart from measured PBDE levels in fish, very little is known about levels in other types of human food. Time trend data on this substance group indicate increasing levels with time, unlike the trend for many other environmental pollutants (e.g. PCBs) where the levels gradually become lower (reviews:1-4).

Structural similarity to other environmental chemicals with known toxic effects (PCBs, PBBs, dioxins) could indicate than also PBDEs could be harmful to health. In experimental animals, the sensitive endpoints seem to be liver cell alterations, thyroid hormone effects and the reproductive process. Few studies have dealt with immunologic parameters, and those studies were essentially negative (5, 6). In order to improve the basis for assessment of immunotoxic effects of PBDEs, the present study was performed. In this study the immunotoxic potential of a commercial PBDE preparation (Bromkal 70-5 DE) was compared with Aroclor 1254 in rats and mice, but the mice were also exposed to pure congeners of PBDEs and PCBs (BDE-47 and CB-105, respectively).

Materials and Methods

Female rats (Sprague-Dawley) and mice (C57BL; both Charles River strains) were used. From start of life to termination of the experiment, the animals were given pelleted feed and tap water ad libitum. The animal room temperature was 22-23 °C, and a 12 h light-12 h dark schedule was used. The animals were 7 weeks old at the start of experiment. The test substances were 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), 2,3,3',4,4'-pentachlorobiphenyl (CB-105), and two technical mixtures, Bromkal 70-5 DE and Aroclor 1254. The substances were given by gavage to the animals (n=6-8, controls 10-12), dissolved in corn oil, in daily oral doses described in Table 1. The does of 10 mg PCBs/kg body wt. and the 18 mg PBDEs/kg body wt. will approximately give the same molar dose (ca 30 μmol/kg body wt.)

Table 1. Treatments, daily doses and group numbers of the animals used in the study

treatment	control	PBDE			PCB	
		Bromkal 70		BDE-47	Aroclor	CB-105
dose (mg/kg/day)	-	18	36	18	10	10
rats (n)	10	6	6	-	6	-
mice (n)	12	8	8	8	8	8

Two weeks from start of exposure, the animals were killed and livers, spleens and tymi were removed and weighed. Lymphoid cells were counted, and lymphocyte subpopulations (CD4, CD8, CD45R/RA) were labelled with fluorescent monoclonal antibodies and quantified by flow cytometry. Immunoglobulin production *in vitro* was measured in supernatants from Pokeweed-stimulated splenocyte cultures by ELISA. Group means were compared by the Mann Whitney unpaired u-test. Differences between experimental and control groups were considered to be statistically significant at $p<0.05$.

Results and Discussion

No signs of clinical toxicity were seen in the study. In case of liver weights, all substances, except Bromkal 18 mg/kg in rats, gave significantly higher values in comparison to control values. This indicates an induction of liver enzymes, which has subsequently been confirmed by microsomal enzyme activity experiments (unpublished).

The weight and cellularity of lymphoid organs were studied: The most pronounced effects were seen after BDE-47 exposure in mice, where the splenocyte number was markedly reduced. Reduced splenocyte numbers were also seen after Aroclor 1254 and CB-105 exposure. In rats, the spleen was not affected by any treatment. Instead, significant reductions in thymus weight and thymocyte numbers were seen after Aroclor 1254 exposure.

In mice, the significant decrease in splenocyte numbers in animals exposed to BDE-47, Aroclor 1254 or CB-105 was reflected in decreased numbers of CD45R+, CD4+ and CD8+ cells in spleens from animals in these groups (Table 2). In addition, the absolute numbers of double negative thymocytes were significantly lower in mice exposed to Bromkal as compared to control mice (data not shown). In rats, only Aroclor 1254 resulted in significant effects on lymphocyte subsets, i.e. an increase in CD8+ cells from thymi, and a corresponding decrease in the proportion of immature, double negative (CD4-CD8-) cells. As regards immune function, decreased *in vitro* production of IgG was seen in splenocyte cultures from mice exposed to Bromkal 18 mg/kg or Aroclor 1254 (Table 2), while *in vitro* Ig production was not affected in exposed rats.

Table 2. *Flow cytometric analysis of CD45R+, CD4+ and CD8+ splenocytes (expressed as the number of positive lymphocytes per spleen), and IgG concentrations in supernatants from Pokeweed-stimulated splenocyte cultures from C57BL mice exposed to PCB or PBDE by gavage for 14 days.*

	CD45R+ (x 10^6/spleen)	CD4+ (x 10^6/spleen)	CD8+ (x 10^6/spleen)	IgG ng/ml
Control	9.4±2.0	14±2.2	6.4±1.2	1695±315
Bromkal 18 mg/kg	9.4±1.2	13±1.3	6.3±0.6	1471±380
Bromkal 36 mg/kg	8.5±2.3	13±3.0	5.8±1.2	1175±205 **
BDE-47 18 mg/kg	6.4±1.2**	9.0±1.2***	4.5±0.6***	1668±689
Aroclor 1254 10 mg/kg	9.2±2.0	11±0.4**	5.0±0.6**	1381±17 *
CB-105 10 mg/kg	8.7±2.4	11±0.9***	4.9±0.5**	1774±405

Values are expressed as mean ±SD of 8 female mice. * P <0.05,** P <0.01, *** P <0.001; Mann-Whitney unpaired u-test

Generally, the C57BL/6 mice were more susceptible to the immunotoxic action of both PBDEs and PCBs than were the Sprague-Dawley rats in the present study, although PCB-induced alterations in the thymus were only observed in rats (Table 3). The decrease in mouse splenocyte weight and cellularity was most pronounced after BDE-47 exposure, whereas the effects of Aroclor 1254 and CB-105 were less marked although significant. Notably, the technical PBDE preparation Bromkal 70 did not result in any significant effects on the spleen, which could seem strange as the Bromkal preparation consists of ca 40% BDE-47. A possible explanation to these dissimilarities in effects is that other substances in the Bromkal

Table 3. *Overview of effects on studied immunological parameters as a consequence of PBDE or PCB gavage to rats or mice*

parameter	rat		mouse	
	PBDE	PCB	PBDE	PCB
thymus wt./cell no.		+		
spleen wt./cell no.			++	+
thymocyte subsets		++	+	
splenocyte subsets			++	++
IgG synthesis			++	+

+, ++ = significant effect (moderate; strong)

preparation might modify the resulting splenocyte effect. The effect on the thymocyte on the other hand seems to follow Ah-receptor binding criteria at least in rats: Liver size enlargement and microsomal enzyme induction (EROD, MROD; unpublished observations) was most pronounced in case of the Aroclor group, likely a result of the presence of coplanar, dioxin-like substanses. The immunotoxic effects of these substances include alteration of the normal thymocyte maturational process (7).

To conclude, the exposure to PBDEs induced certain immunological alterations in mice, but not in rats, while exposure to PCB induced immunotoxicity in both species. The finding in the present study suggest further experiment on the immunotoxic potential of PBDE congeners in different species.

Acknowledgements
Thanks are due to Ingalill Gadhasson, Elvy Netzel and Kerstin Pödra for their excellent technical assistance. Dr. Anders Bergendorff and Dr. Eva Jakobsson are gratefully acknowledged for the gifts of test substances.

References

1. IPCS (WHO) , Environmental Health Criteria 162, Brominated Diphenyl Ethers, **1994,** ISBN 92-4-157162-4.
2. KEMI (Swedish National Chemicals Inspectorate), report no. 9/94, Risk assessment of polybrominated diphenyl ethers, **1994**, ISSN 0284-1185
3. Pijnenburg AMCM, Everts JW, de Boer J and Boon JP; Rev. Environ. Contam. Toxicol. **1995**, 141, 1.
4. TemaNord, Polybrominated Diphenyl Ethers: Food Contamination and Potential Risks, no. 503, **1998**, ISBN 92-893-0150-3.
5. Fowles JR, Fairbrother A, Beacher-Steppan L, Kerkvliet NI; *Toxicology* **1994**, 86, 49.
6. Fernlöf G, Gadhasson IL, Pödra K, Darnerud PO, Thuvander A; *Toxicol. Lett.* **1997**, 90, 189.
7. Kremer J, Lai Z-W, Esser C; *Environ. Toxicol, Pharmacol. Section* **1995**, 293, 413.