Serum PBDE levels in exposed rats in relation to effects on thyroxine homeostasis

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Introduction

Brominated flame retardants (BFRs) is a group of environmental chemicals for which lately both interest and knowledge have increased considerably. Among the BFRs, the polybrominated diphenyl ethers (PBDEs) have attained special interest. Much data on environmental and human levels have been presented and several toxicological reviews are now published. Among interesting results is the difference in human PBDE levels that seem to exist between U.S.A. and Europe, results that suggest differences in exposure but without being able to pin-point the exact sources.

In experimental studies PBDEs alter serum thyroxin levels, an effect seen both in rats and in mice [1-5]. The mechanism(s) are still not completely clarified, but are thought to include alterations in serum transport, induced enzymatic degradation and possibly also direct effects on the thyroid gland. As perinatal alterations in thyroid homeostasis could affect brain development, early effects on thyroid hormones may be of special concern. Indeed, PBDEs have been shown to affect behaviour and learning in mice, when given neonatally [6].

The aim of the present study was to relate the serum levels of PBDEs in rats to effects of these compounds on thyroxine homeostasis in these animals. Specifically, the relation between serum PBDE levels and effects on serum thyroxine levels was investigated, after two weeks of daily oral exposure. The result may have consequences for the future risk assessment activities on PBDE and specifically in finding the critical serum PBDE concentration at which the effect on thyroid hormone levels begin to occur.

Materials and methods

The experimental part of the study has earlier been reported in two separate articles [2,3]. In short, mice and rats were given (among other substances) daily gastric intubations of PBDEs, namely the pure congener BDE-47 and the technical mixture Bromkal 70-5 DE (doses in selected groups of rats, see Table 1). After 14 days the animals were killed and selected organ were collected. Blood plasma was primarily used for analyses of thyroid hormones. The measurement of thyroid hormones was performed by RIA technique using standard kits for free and total thyroxine (FT4, TT4). Also TSH (thyroid-stimulating hormone) was monitored but data no significant alterations from controls were noted, and these data were not used in this presentation.

In some of the rat groups from the above-mentioned studies, the plasma not used for hormone measurements was pooled from animals within the group (Table 1). The pooled plasma was later analysed for the congeners BDE-28, BDE-47, BDE-66, BDE-99, BDE-100, BDE-138, BDE-153, and BDE-154. The analytical method used is based on liquid extraction, purification by

column chromatography and quantification on GC/ECD by use of a double column system. The method for liquid extraction was earlier described [7], and so were the purification and PBDE quantification methods [8]. PBDE congeners used for quantification were obtained as a gift from Dr. Åke Bergman, Stockholm University.

Results and discussion

The obtained analytical results of plasma PBDE levels, presented as BDE-47 and sumPBDE, are given in Table 1. In all cases BDE 47 levels are highest among the BDE congeners analysed, but in addition also BDE 99, 100, and 153 levels are substantial after exposure to Bromkal 70-5 DE (data not shown). The plasma levels are generally related to the doses given to the animals. Indeed, when comparing equal doses of BDE 47 and Bromkal (18 mg/kgbw/day; groups I:2 and II:4) the BDE 47 and sumPBDE levels in plasma are about similar. However, in case of rats dosed with 6 mg/kgbw/day of BDE 47 (II:3) the plasma levels of BDE 47 (590 ng/g fat) are much higher than expected, and substantially higher than in plasma from the groups receiving a higher dose, 18 mg/kgbw/day (421 ng/g fat.; II:4). The reason to this is unknown but could have several explanations, including errors in sample handling or analysis. This erroneous plasma value has been replaced by plasma data from a group of rats receiving the same dose of BDE 47 but in coadministration with Aroclor 1254 (inserted value 200 ng BDE 47/g fat).

Using the data from two separate rat studies, Figure 1 show the effect on plasma levels of FT4 (a) and TT4 (b) in relation to the pooled plasma sumPBDE or BDE 47 levels. The figures show that the FT4 levels begin to drop somewhere below 400 µg BDE 47/g plasma lipid, whereas TT4 may be affected at a somewhat higher plasma concentration of sumPBDE/BDE 47. In interpreting the figures it should be noted that the animals have been exposed to two different PBDE compounds, and we cannot say for sure that the same doses of BDE 47 and Bromkal 70-5 give an equal response on T4 levels in rats. In this study on T4 effects in rats, Bromkal seems to have a stronger potency than BDE 47 at similar plasma PBDE concentrations. On the other hand, in mice earlier results have shown that the effect of Bromkal 70-5 and of BDE-47 on T4 levels were about the same if the same given dose of the two compounds was administered [2].

The presented data could be used in a human risk assessment exercise, if some assumptions are made. First, the above model is suggested to be useful in spite of the fact that two separate experiments, with different PBDE compounds, were used (as mentioned above). Second, the levels of BDE or sumPBDE in plasma two weeks after daily exposure to rats are assumed to relate to the effects observed on plasma T4. Third, the effects seen of T4 in rats are suggested to occur also in humans, exposed to the same PBDE concentrations in blood (the differences in blood lipid content between man and rat have not been corrected for). Fourth, a no-effect level in plasma of 200 µg sumPBDE/g lipid is suggested on basis of effects on FT4 as shown in Fig. 1a and discussed earlier [3]. On these assumptions a NOEL level for T4 effects in rat plasma could be compared to the PBDE plasma levels found in human studies (Table 2). In Europe, reported background plasma levels are generally around 1-4 ng BDE-47/g blood lipid [9] but increased levels could found, due to occupational exposure [10,11]. In U.S.A., considerably higher levels have been found in blood [12] and even higher in breast milk. If the rat NOEL levels related to PBDE effects on plasma FT4 (200 µg/g) are compared to the reported levels in plasma from Europe and U.S.A. (Table 2) we obtain a margin of safety of about 4 000 (200 000/45). However, we should note that these results are valid for adult individuals whereas children may be more susceptible to the observed effects. Moreover, effects on neurobehavioural development are suggested to be a more sensitive endpoint regarding PBDEs than are TH effects [13]. These factors, and the possible finding of higher human plasma

levels, may decrease the margin of safety for the PBDEs between potential health effects and measured human tissue levels.

Acknowledgement
The skilful work of Ingalill Gadhasson and Elvy Netzel is acknowledged. Thanks are due to Dr Åke Bergman and colleagues for the gift of PBDE congeners. Economic support was obtained from the Swedish Environment Protection Agency.

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Table 1. Relation between PBDE dose, plasma BDE 47 or sumPBDE concentration, and thyroid hormone effects in rats (Study I= ref. 2; Study II= ref. 3)

Study/group	Substance	Dose	Plasma PBDE (µg/g lipid)		TH levels (% of control)	
no.		(mg/kgbw/day)	BDE 47	SumPBDE [1]	FT4	TT4
I:1	vehicle	-	0.03	0.07	100 [2]	100 [3]
I:2	Bromkal	18	179	463	30	58
I:3	Bromkal	36	652	1 495	23	52
II:1	vehicle	-	0.03	-	100 [2]	100 [3]
II:2	BDE 47	1	28	-	99	110
II:3	BDE 47	6	200 [4]	-	96	104
II:4	BDE 47	18	421	-	66	102

[1] Sum of BDE 28, BDE 47, BDE 66, BDE 99, BDE 100, BDE 138, BDE 153, BDE 154

[2] Absolute values of FT4 (medians): I:1= 19.6; II:1= 15.2 (pmol/l)

[3] Absolute values of TT4 (medians): I:1= 34.9; II:1= 27.9 (nmol/l)

[4] Due to an incongruous value, the original result on plasma BDE 47 has been replaced by the measured plasma level from a group exposed to the same dose of BDE 47, and to Aroclor 1254 (group 7 in study II)

Table 2. Human serum PBDE levels reported in the literature

Country, yr.	Exposure	No. of ind.	Compound	Value (ng/g lipid)	Reference
Germany, 1999	background	20 (M/F)	BDE 47	3.9 (3.4-4.7)	14
Norway, 1999	background	29 (M)	BDE 47	1.5	15
- " -	occup. exp.	5	BDE 47	0.4-14.6	11
Sweden, 1999?	background	20 (F)	sumPBDE	3.3	10
- " -	occup. exp.	19 (M/F)	sumPBDE	26	10
U.S.A.	background	5 pools	BDE 47	27-45	12

Figure 1. Graphs showing the effects of PBDEs on FT4 (a) or on TT4 (b), in percent of control values. The data are taken from two studies, exposing rats to Bromkal (study I; boxes in the figures) or to BDE 47 (study II; triangles).