CONCENTRATION OF POLYBROMINATED DIPHENYLETHERS AND THYROID HORMONES IN HUMAN PLASMA FROM EXPOSED WORKERS

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Introduction

The extensive usage of brominated flame retardants (BFRs) in electronic equipment has lead to elevated air levels, particularly within the electronic dismantling industry. BFRs are used in different materials to prevent or retard the initial stages of fire development[1]. Work-related exposure to these substances has been studied in two different electronic dismantling plants[2,3]. The air concentrations were found to be elevated in both studies. For workers in these settings occupational uptake of polybrominated diphenyl ethers (PBDEs) has been demonstrated by blood analysis[4,5]. Concerns have been risen lately about the uptake and accumulation of certain congeners in humans. No complete evaluation of the toxicity of PBDEs is available[6]. PBDEs have a close structural resemblance, in particular their hydroxylated metabolites, to the thyroid hormones triiodothyronine (T_3), having the strongest metabolic effect in man, and to thyroxine (T_4)[7]. All of the technical PBDE mixtures disrupt the thyroid hormone balance, although the decaBDE mixture seems to exhibit a much lower potency than the penta- and octa BDE mixtures[6,8].

The aim of this study was to evaluate the association between potential occupational exposure to PBDE and alterations of thyroid hormone status, at an electronic dismantling plant in Örebro, Sweden. 14 workers from the electronic dismantling plant have, on a voluntary basis, donated blood at various occasions from the start of their employment, during vacation and after their employment terminated. In this abstract, plasma analyses from three of the workers are reported.

Material and Methods

Plasma samples

The blood samples were collected in BD vacutatiner tubes with heparin (Becton, Dickinson and Company). The samples were centrifuged and the plasma was collected into glass bottles pre-washed with acetone and immediately frozen and stored in -20°C until analysis. At the day of sampling additional samples were drawn for analysis of the thyroid hormones, total T_3 and free T_4.

Plasma preparation and determination

The method used for extraction has been reported elsewhere[9]. In short, the serum sample was applied to a column packed with the adsorbent hydromatrix and eluated with a mixture of isopropanol/ hexane. Excess water was removed with sodium sulphate and the lipid weight was determined gravimetrically. The sample was cleaned-up using multilayer silica columns. All solvents used for the analysis were from Labscan, Sweden (grade enviroscan).

Hormone assay

Plasma T_3 and free T_4 were analysed with time-resolved fluoroimmunoassay (Wallac Oy, Turku, Finland). The intraassay coefficient of variation (CV) for T_3 was 3.2 %, and for free T_4, 2.0 %. The normal range was 1.3-2.5 nM (n=195) and 10.2-17.0 pM (=202) for T_3 and free T_4, respectively for the method, when tested on apparently healthy men and women by Wallac Oy.

GC/MS

Single ion GC/MS spectra were recorded using a HP 6890 gas chromatograph coupled to a HP 5973 mass spectrometer. Chromatographic separation was achieved by splitless injection of 2ml on a HP-5MS 5% phenyl methyl siloxane capillary column, using helium as carrier gas at a constant flow of 1.1 ml/minute. The GC oven temperature was programmed as follows: initial temperature 180 °C, hold for 2 min. increase to 205 °C at a rate of 15 °C/min., followed by an increase of 4 °C/min. to 325 °C, final hold at 325 °C for 15 minutes. The MS operated in negative chemical ionisation mode with methane as reagent gas (flow 3.5 ml/min.). The interface, the ion source and the quadrupole were held at 180, 230 and 106°C, respectively. PBDEs were monitored at *m/z 79;81* and ^{13}C-PBDE#77 was used as the internal standard. As recovery standard ^{13}C-PCB#138 was used and measured at *m/z 372*. All detected PBDEs were identified and quantified using a quantification standard containing IS ^{13}C--PBDE#77, RS ^{13}C-PCB#138 and a mix containing PBDEs #28, 47, 66, 100, 99, 85, 154, 153, 138 and 183 with a concentration of 62 pg/ml, (except for #66 and #183 for which a concentration of 60 pg/ml was used). Relative response factors of the standard solutions with a RSD >20% were rerun or the second standard of the batch of samples was used. Samples with lower recovery values than 50% were omitted from the data set. Blood samples were reanalysed if the blank levels were more than 20% of the levels in the sample.

Results and Discussion

Plasma concentrations of the eight analysed PBDE congeners (tetra- to heptaBDE) are presented in Table 1. Each individual is reported separately to facilitate the interpretation over time, since the start of their employment. The sum of PBDE presented in table 1 represents the tetra through hexa PBDE isomers; PBDE #183, a hepta isomer is presented separately. All concentrations reported in this paper are in the same order of magnitude as previously reported in the literature[4,5]. Three isomers increased (PBDE #47, #99 and #100) in plasma from day 0 to the second sampling occasion. In Figure 1, the concentrations of T_3 and free T_4 in blood from the three workers are plotted against their work days since employment started. A decrease in T_3 levels from the start of employment until the first day of vacation can be seen for individuals 2 and 3 (from day 170-198 and 146-177, respectively). Furthermore, we were unable to find any trends between the levels of the thyroid hormones and those of the analysed tetra to hexa PBDEs. The correlation reported for PBDEs and thyroid hormones for rats and mice occurred at higher blood concentrations of PBDE than in this study[10,11]. In conclusion, PBDE levels fluctuated during the sampling period of 8 months. The levels of one higher brominated diphenylether (#183) varied during this period. One reason for the large fluctuations in PBDE #183 levels might be the larger analytical uncertainty because of the usage of a tetra PBDE as an internal standard for this hepta congener. One reason for the overall inconsistent pattern and the modest increase in three congeners (#47, #99, #100) over time may be low exposure of PBDE, due to a smaller than usual inflow and handling of waste during the studied period.

Table 1. Plasma concentrations (pmol/g lipids and *ng/g lipids* given in italics) of selected PBDE congeners from three individuals working with electronic dismantling. Each individual is reported separately to show variation in concentration related to work days since the employment started.

Individual 1	**#28**		**#47**		**#100**		**#99**		**#85**		**#154**		**#153**		**S BDE***		**#183**	
Day 0	0.61	*0.25*	5.0	*2.4*	0.73	*0.41*	2.9	*1.6*	n.d	*n.d*	n.d	*n.d*	4.0	*2.6*	13	*7.3*	8.9	*6.4*
Day 74	0.34	*0.14*	8.2	*4.0*	1.4	*0.80*	5.6	*3.1*	0.29	*0.16*	n.d	*n.d*	3.3	*2.1*	19	*10*	5.5	*4.0*
Day 117	0.32	*0.13*	8.2	*4.0*	1.3	*0.71*	5.7	*3.2*	n.d	*n.d*	n.d	*n.d*	3.8	*2.5*	19	*11*	8.3	*6.0*
Day 152	0.46	*0.19*	11	*5.3*	1.6	*0.90*	10	*5.8*	n.d	*n.d*	n.d	*n.d*	3.5	*2.2*	27	*14*	6.8	*4.9*
Day 163	0.43	*0.18*	8.1	*3.9*	1.3	*0.75*	6.1	*3.4*	0.29	*0.17*	n.d	*n.d*	2.8	*1.8*	19	*10*	1.9	*1.4*
Day 170	0.56	*0.23*	9.7	*4.7*	1.4	*0.78*	7.2	*4.1*	n.d	*n.d*	0.47	*0.30*	2.2	*1.4*	22	*11*	1.1	*0.8*
Day 198	0.58	*0.24*	8.6	*4.2*	1.6	*0.93*	7.0	*3.9*	n.d	*n.d*	n.d	*n.d*	2.5	*1.6*	20	*11*	1.1	*0.8*
Mean	**0.47**	***0.19***	**8.4**	***4.1***	**1.3**	***0.75***	**6.4**	***3.6***	**0.29**	***0.16***	**0.47**	***0.30***	**3.2**	***2.0***	**20**	***11***	**4.8**	***3.5***
Individual 2	**#28**		**#47**		**#100**		**#99**		**#85**		**#154**		**#153**		**S BDE***		**#183**	
Day 0	0.35	*0.14*	4.2	*2.0*	0.65	*0.37*	2.0	*1.1*	0.19	*0.11*	n.d	*n.d*	2.7	*1.8*	10	*5.6*	7.0	*5.0*
Day 118	0.26	*0.11*	7.4	*3.6*	1.3	*0.75*	1.4	*0.8*	0.26	*0.15*	1.7	*1.1*	7.3	*4.7*	20	*11*	13	*9.2*
Day 167	0.34	*0.14*	5.5	*2.7*	1.2	*0.66*	5.1	*2.9*	0.22	*0.13*	1.4	*0.88*	3.6	*2.3*	17	*9.7*	6.8	*4.9*
Day 170	0.48	*0.20*	5.4	*2.6*	1.1	*0.62*	4.8	*2.7*	n.d	*n.d*	1.2	*0.78*	3.2	*2.0*	16	*9.0*	5.0	*3.6*
Day 177	0.40	*0.16*	6.5	*3.1*	1.5	*0.87*	7.2	*4.1*	n.d	*n.d*	1.3	*0.86*	3.5	*2.2*	20	*11*	4.2	*3.0*
Day 198	0.65	*0.26*	8.3	*4.0*	1.6	*0.90*	7.2	*4.0*	n.d	*n.d*	n.d	*n.d*	3.2	*2.1*	21	*11*	3.0	*2.2*
Day 216	0.36	*0.15*	6.8	*3.3*	1.3	*0.74*	6.6	*3.7*	0.23	*0.13*	n.d	*n.d*	3.9	*2.5*	19	*11*	4.6	*3.3*
Day 244	0.51	*0.21*	7.9	*3.8*	1.5	*0.84*	8.6	*4.8*	0.17	*0.10*	n.d	*n.d*	3.4	*2.2*	22	*12*	3.8	*2.8*
Mean	**0.42**	***0.19***	**6.5**	***3.2***	**1.3**	***0.72***	**5.4**	***3.1***	**0.22**	***0.12***	**1.4**	***0.90***	**3.9**	***2.5***	**18**	***10***	**5.9**	***4.3***
Individual 3	#28		#47		#100		#99		#85		#154		#153		S BDE*		#183	
Day 0	0.79	*0.32*	9.9	*4.8*	1.1	*0.63*	4.5	*2.6*	0.50	*0.28*	n.d	*n.d*	1.9	*1.2*	19	*9.9*	3.1	*2.2*
Day 74	0.86	*0.35*	17	*8.2*	1.5	*0.82*	12	*6.9*	0.24	*0.14*	n.d	*n.d*	2.0	*1.3*	34	*18*	1.8	*1.3*
Day 118	0.46	*0.19*	7.5	*3.6*	0.49	*0.28*	4.2	*2.4*	n.d	*n.d*	0.33	*0.21*	n.d	*n.d*	13	*6.7*	1.2	*0.84*
Day 146	0.43	*0.17*	8.6	*4.2*	1.5	*0.84*	7.7	*4.4*	0.25	*0.14*	0.19	*0.12*	2.1	*1.4*	21	*11*	2.4	*1.8*
Day 177	0.60	*0.24*	8.2	*4.0*	1.4	*0.77*	6.7	*3.8*	0.26	*0.14*	n.d	*n.d*	1.6	*1.0*	19	*10*	1.2	*0.89*
Day 195	0.76	*0.31*	15	*7.5*	2.5	*1.43*	14	*7.7*	0.41	*0.23*	0.54	*0.35*	2.1	*1.4*	36	*19*	1.6	*1.2*
Day 221	0.39	*0.16*	9.3	*4.5*	1.4	*0.80*	6.5	*3.7*	0.23	*0.13*	0.38	*0.24*	1.4	*0.9*	20	*10*	1.2	*0.89*
Mean	**0.61**	***0.25***	**11**	***5.3***	**1.4**	***0.80***	**7.9**	***4.5***	**0.27**	***0.18***	**0.21**	***0.23***	**1.6**	***1.2***	**23**	***12***	**1.8**	***1.3***

*Sum of PBDE - #183 not included

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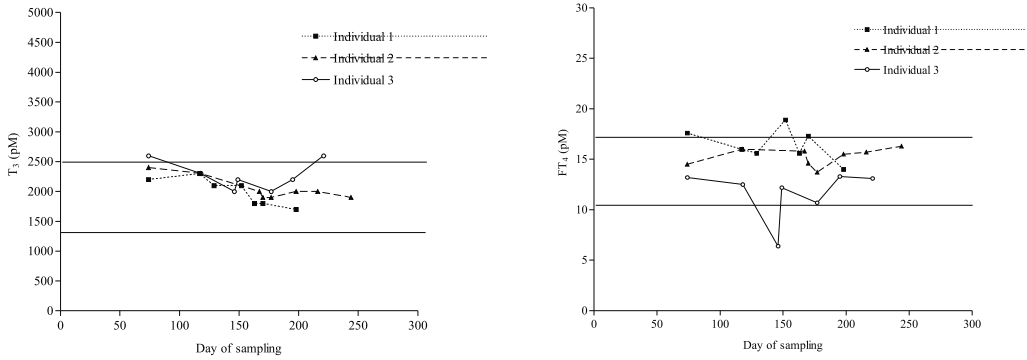

Figure 1. Concentration of T_3 and free T_4 (pM) in blood from 3 workers. The intraassay coefficient of variance (CV) is 3.2 % and 2.0 % for T_3 and free T_4, respectively. The horizontal lines represent the normal range (n=159 and n= 202, for T_3 and freeT_4).

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