Levels of PBDEs in serum of Dutch adolescents

M.M. Leijs[1,2] , K. Olie[1], J.G. Koppe[3] , G.W. ten Tusscher[5], W.M.C. van Aalderen[2], P. de Voogt[1,4]

[1] IBED/ESPM, University of Amsterdam, The Netherlands.
[2] Department of Paediatrics and Neonatology, Emma Children's Hospital Academic Medical Centre, Amsterdam, The Netherlands.
[3] Ecobaby Foundation, Loenersloot, Netherlands.
[4] KIWA Water Research, P.O. Box 1072, 3430 BB Nieuwegein, Netherlands.
[5] Department of Paediatrics and Neonatology, Westfriesgasthuis, Maelsonstraat 3, 1624 NP Hoorn, Netherlands.

Abstract:
An increase in levels of PBDEs in humans has been seen over the last decades. This is disconcerting because of possible negative health effects. The present study is part of a longitudinal study on the influence of exposure to background levels of dioxins and PBDEs on average, healthy children from The Netherlands.

Blood samples were spiked with 17 ^{13}C-PCDD/PCDF, 9 ^{13}C-PCBs and 6 ^{13}C-BDEs. After spiking the lipid fraction was isolated by liquid-liquid extractions. Planar compounds were separated from non-planar compounds using an activated carbon column. For the clean-up an acid-base column and an aluminium oxide column was used. PBDEs were separated on a 30 m DB-1 column and identified and quantified using HR-GC/MS.

Mean current PCDD/F levels measured in the same samples were 2.20 pg/g lipid and the mean current serum p-PCBs were 2.17 pg/g lipid .
PBDE-153 was the dominant congener (mean 2.81 pg/g lipid). The mean concentration of the sum PBDEs (28, 47, 85, 99, 100, 153, 154, 183) was 13.96 ng/g lipid. There was one outlier in the samples.

Introduction:
Polybrominated diphenylethers (PBDEs) have, over the last decades, become an integral part of daily life, being used in electronic equipment, plastics, carpet liners and textiles. Since the early 1990s PBDEs have been found in human serum. Background levels of these compounds are infrequently measured in human populations all over the world. The highest levels of PBDEs are found in the United States [1]. Current serum levels of the sum of 13 PBDEs in the US were reported as 61.7 and 79.7 ng/g lipid in 2 pooled samples [2]. Human and environmental background levels of these compounds have increased over the last decades [2,3]. An almost four-fold increase of 4 serum PBDE levels, from 0.5 to 1.8 ng/g lipid, has been reported for the 15 years s from 1980 till the mid 1990s [3]. The increase of these compounds in humans is disconcerting because of their possible negative health effects through endocrine disruption [4].

Children are exposed to higher serum PBDE levels than are adults [5]. A study of serum levels of New Zeelanders found mean ∑PBDE concentrations of congeners 47, 99, 100, 153, 154, and 183 of on average of 7.17 ng/g lipid [6]. A Belgian study found serum ∑PBDEs in adolescents varying from 3.57-4.53 ng/g lipid in different regions in Flanders [7].

The present study is part of a longitudinal study on the influence of exposure to background levels of dioxins and PBDEs on average, healthy children from The Netherlands [8]. The subjects, now aged 14-19 years, were previously studied during their neonatal (n=60) [9], toddler [10] and pre-pubertal period (n=44) [11,12]. All 32 children, consenting to the current follow-up study, were born in the Amsterdam/Zaandam region of The Netherlands.

Methods and materials

Venous blood samples were obtained and were spiked with 17 ^{13}C-PCDD/PCDF and 9 ^{13}C-PCBs and 6 ^{13}C-BDEs. After spiking the lipid fraction was isolated by liquid-liquid extraction using diethyl ether and petroleum ether, respectively. The lipids were re-dissolved in 10 ml dichloromethane and brought to the top of an activated carbon column. The solvent fraction eluting from the column was fraction 1 (see figure 1). Hereafter, the activated carbon column was placed in a reflux unit and refluxed for 2h with 20 ml dichloromethane (fraction 2). The column was then rinsed with 20 ml toluene (fraction 3) and thereafter refluxed with 20 ml toluene for 1 h (fraction 4). Following this the Carbosphere column was inverted and refluxed for 16 h with 40 ml toluene (fraction 5). Fractions 1-3 were combined. They contained the mono and diortho substituted PCBs together with the BDEs. Fraction 4 contained the planar (dioxinlike) PCBs and fraction 5 contained the PCDD/PCDF fraction. The fractions were evaporated to dryness.

The PBDE fractions were cleaned-up and transferred to an acid-base column (12 mm i.d.) containing 5 cm 33% sulphuric acid on silica on top of 0.5 cm 6% potassium hydroxide on silica repeated thrice in one column. The column was pre-washed with 30 ml of dichloromethane: hexane (1:1) and thereafter the PCBs and BDEs were eluted with 50 ml of dichloromethane: hexane (1:1). The eluted fraction was evaporated to dryness and dissolved in 2 ml hexane. Hereafter, the fractions were brought into a high aspect column (6mm i.d.) filled with 19 cm activated aluminium oxide. Before using the aluminium oxide its activity was tested using the Brockmann test. The PCBs and BDEs were recovered by elution with 30 ml of dichloromethane. The resulting elute is evaporated to dryness and re-dissolved in 50 µl of nonane.
PBDEs were separated on a 30 m DB-1 column and identified and quantified using low resolution MS (single quadrupole, Trace/ Thermoquest).

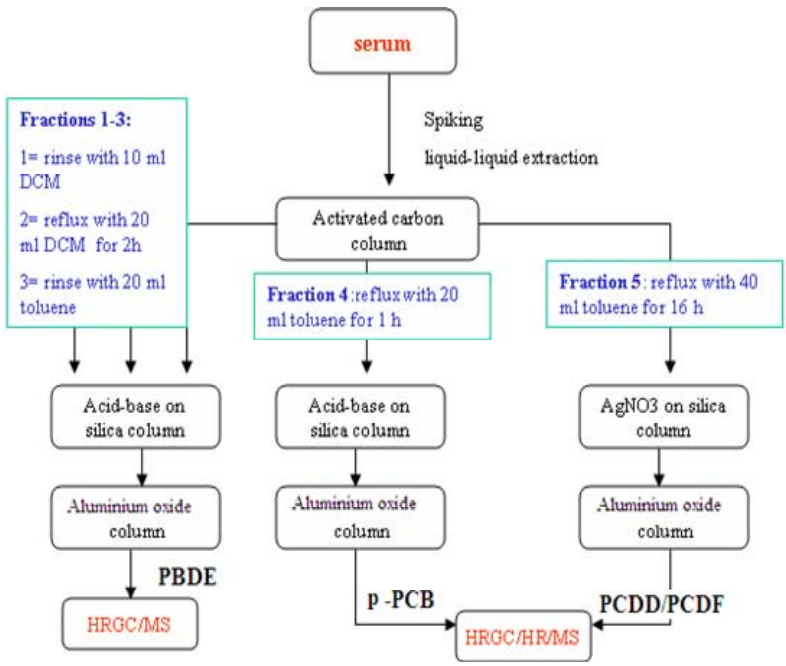

Figure 1: Sample extraction and clean-up methods used

Results

Recovery experiments showed that tetra to hepta-BDE congeners were mainly found in fractions 1 and 2 of the Carbosphere column reflux/extraction procedure (see figure 1). The recoveries from this procedure ranged between 80 and 97 %. Some minor recoveries were found in fraction 3. Recoveries of PBDEs from the aluminia columns varied between 98 and 120 %.

Tetra- to hepta-BDE congeners could be identified in the serum (see table 1). Deca-BDE could not be quantified due to insufficient recovery of the internal standard.

Mean current PCDD/F levels measured in the same samples were 2.20 pg/g lipid (0.36-6.06) and the mean current serum p-PCBs were 2.17 pg/g lipid (0.04-7.78). The mean prenatal PCDD/F exposure was 36.4 pg/g lipid milk fat and mean lactational exposure was 75.4 ng, in the same cohort of children.

The PBDE levels found in the serum are shown in table 1. PBDE-153 was the dominant congener (mean 2.81 pg/g lipid). The mean concentration of the sum of PBDEs was 13.96 ng/g lipid. This is higher than levels found in adolescents in Belgium [7].

There was one outlier (sample 30) which contained large amounts of BDE-28, 85, 99, 100, 153 and 154. The outlier was not an analytical outlier, but a sample that contained considerably more PBDE 85, 99 and 100 than the other samples. Outliers have also been documented in other studies and are alarming due to the unknown manner of exposure and the possible health effects.

Table 1: PBDE levels in the serum of adolescents (ng/g lipid)

Sample	BDE 28	BDE 47	BDE 85	BDE 99	BDE 100	BDE 153	BDE 154	BDE 183
S1	0.20	4.31	0.51	2.07	1.56	4.00	0.38	0.98
S2	0.13	2.78	0.57	1.45	0.61	2.18	0.37	0.80
S3	0.00	1.74	0.12	0.90	0.67	2.23	0.22	0.82
S7	0.15	2.76	0.77	1.99	1.13	3.57	0.52	3.24
S8	0.44	2.01	1.29	3.03	1.47	3.81	0.93	2.98
S9	0.21	1.64	0.84	2.52	1.11	4.47	1.07	2.32
S10	0.00	1.70	2.49	0.84	0.96	4.36	0.80	3.83
S11	0.12	1.11	0.90	1.23	0.94	1.71	0.56	1.62
S12	0.09	2.66	0.52	4.21	1.30	1.77	0.63	1.29
S15	0.00	1.35	0.24	0.43	1.24	1.30	0.00	0.88
S16	0.11	1.41	0.00	0.90	1.58	1.64	0.26	0.41
S19	0.13	5.05	4.25	3.19	2.87	4.82	0.92	0.83
S20	0.00	1.53	0.42	0.97	0.67	1.51	0.85	1.76
S21	0.09	2.19	0.29	0.36	1.08	1.34	0.25	0.77
S24	0.07	1.74	2.41	2.27	2.14	1.51	0.00	0.72
S26	0.08	2.19	1.84	2.40	2.19	2.03	0.00	1.17
S28	0.13	1,.3	1.78	0.55	1.92	1.06	0.64	0.77
S29	0.11	1.45	0.60	0.49	0.34	1.02	0.29	0.56
S30	1.73	3.82	25.8	14.9	15.0	9.09	2.21	0.96
Mean	0.20	2.16	2.41	2.36	2.04	2.81	0.57	1.40
std	0.38	1.05	5.78	3.24	3.20	1.98	0.52	0.98

Acknowledgements
Financial support for this study was provided by:
- the Netherlands Ministery of Housing, Spatial Planning and Environment
- Stichting Natuur en Milieu
- Ecobaby Foundation

References:

(1) Petreas M, She J, Brown FR, Winkler J, Windham G, Rogers E et al. High body burdens of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in California women. *Environ Health Perspec*t 2003; 111:1175.
(2) Schecter A, Papke O, Tung KC, Joseph J, Harris TR, Dahlgren J. Polybrominated diphenyl ether flame retardants in the U.S. population: current levels, temporal trends, and comparison with dioxins, dibenzofurans, and polychlorinated biphenyls. *J Occup Environ Med* 2005; 47:199.
(3) Koizumi A, Yoshinaga T, Harada K, Inoue K, Morikawa A, Muroi J et al. Assessment of human exposure to polychlorinated biphenyls and polybrominated diphenyl ethers in Japan using archived samples from the early 1980s and mid-1990s. *Environ Res* 2005; 99:31.
(4) Zhou T, Taylor MM, DeVito MJ, Crofton KM. Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicol Sci* 2002; 66:105.
(5) Fischer D, Hooper K, Athanasiadou M, Athanassiadis I, Bergman A. Children show highest levels of polybrominated diphenyl ethers in a California family of four: a case study. *Environ Health Perspect* 2006 ;114:1581.
(6) Harrad S, Porter L. Concentrations of polybrominated diphenyl ethers in blood serum from New Zealand. Chemosphere 2007; 66:2019.
(7) Roosens L, Neels H, Koppen G, Schoeters, G, Nelen V et al. PBDE levels in pooled serum samles of newborns, adolescents and adults from Flanders, Belgium.*BFR congres 2007,* Amsterdam 2007.
(8) Leijs M, Koppe J, Olie K, de Voogt P, Vulsma T, van Aalderen et al. Delayed initiation of breast development in girls with higher prenatal dioxin exposure.*Organohalogen Compounds* 2006;68: 968.
(9) Pluim HJ, Koppe JG, Olie K, van der Slikke JW, Slot PC, van Boxtel CJ. Clinical laboratory manifestations of exposure to background levels of dioxins in the perinatal period. *Acta Paediatr* 1994; 83:583.
(10) Ilsen A, Briet JM, Koppe JG, Pluim HJ, Oosting J. Signs of enhanced neuromotor maturation in children due to perinatal load with background levels of dioxins. Follow-up until age 2 years and 7 months. *Chemosphere* 1996; 33:1317.
(11) ten Tusscher GW, de Weerdt J, Roos CM, Griffioen RW, De Jongh FH, Westra M et al. Decreased lung function associated with perinatal exposure to Dutch background levels of dioxins. *Acta Paediatr* 2001 November; 90:1292.
(12) ten Tusscher GW, Steerenberg PA, van Loveren H, Vos JG, dem Borne AE, Westra M et al. Persistent hematologic and immunologic disturbances in 8-year-old Dutch children associated with perinatal dioxin exposure. *Environ Health Perspect* 2003; 111:1519.