PBDE and HBCDD levels in blood from Dutch mothers and infants
- Analysis of a Dutch Groningen Infant Cohort

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Introduction

Brominated flame retardants (BFRs) are chemicals with a large industrial production and ubiquitous global use. The three major classes of BFRs produced worldwide are; tetrabromobisphenol A (TBBPA), three commercial mixtures of polybrominated diphenyl ethers (PBDEs), Deca-, Octa- and PentaBDE and hexabromocyclododecane (HBCDD)[1]. Several BFRs are, due to their lipophilicity and bioaccumulation properties, both expected and in reality found in wildlife and humans[2]. The aim of this study was to establish a clean up method for HBCDD analysis in human serum, as well as to investigate levels of PBDEs and HBCDD in serum from mothers and infants from a Dutch cohort. A total of 90 human serum samples were analyzed. To the best of our knowledge, HBCDD has not been reported previously in humans, and PBDEs have not been reported in human serum from The Netherlands[2].

Material and Method

Synthesized reference compounds used as external standard for the identification and calculation of the substances are listed in Table 1. All solvents are pesticide analysis reagent grade. Methyl-*tert*-butyl ether (MTBE) and isopropanol were glass-distilled prior to use. Silica gel (0.063-0.200 mm) was activated in an oven at 300 ºC over night. Gas chromatography (GC) was performed on a Varian 3400 (J&W Scientific), coupled with a transfer line (290 ºC) to a mass spectrometer (MS) (Finnigan MAT SSQ 710 from Thermoquest). A DB-5 HT capillary column (15 m, 0.25 i.d., 0.1 µm film; J&W Scientific) with helium as carrier gas was used. The samples were injected on column with a septum equipped programmable injector that was programmed from 60 ºC (1 min) to 300 ºC (150 ºC/min, 22 min). The column oven temperature was programmed from 80 ºC, (1 min) to 300 ºC (15 ºC/min, 10 min). The ion source temperature was 200 ºC. Electron capture negative ionization (ECNI) mode was carried out for both bromide isotopes m/z 79 and 81. Methane gas was used as electron thermalization buffer gas and the electron energy was 40 eV. Data were collected and processed using a PC-based ICIS 8.3.0 service pack 2 system.

The method applied for the analysis is a modified version of the method published by Hovander et al.[3]. To the serum sample (5 g), two internal surrogate standards (IS), BDE-138 (0.1

ng) and BDE-77 (0.1 ng) were added, followed by vibromix blending. Extraction was performed as described elsewhere[3]. No partitioning with potassium hydroxide was performed since HBCDD is not stable in an alkaline solution. The solvent was evaporated and lipid content was determined gravimetrically for each sample. Hexane (Hx) (4 ml) was added and the sample was treated with concentrated sulfuric acid (2 ml). Another clean up step was performed on a sulfuric acid (H_2SO_4) treated silica gel (SiO_2) (1:2 w.w.) column (0.1 g SiO_2, 0.9 g H_2SO_4/ SiO_2) with Hx/Dichloromethane (DCM) (1:1, 6ml) as the mobile phase. To exclude the majority of the PCBs, a fractionation was carried out on a SiO_2 (0.7 g) column, where the first fraction of Hx (3ml) was discarded, and the next fraction of DCM (5 ml) was collected. This step does not remove all PCBs but enough to prevent disturbance of the chromatogram for the PBDE and HBCDD analysis by high amounts of PCBs. Solvents were evaporated to dryness and the samples were transferred to vials with Hx. *p*-MeO-BDE121 (0.1 ng) was added as an internal volumetric standard (VS).

Serum samples were obtained from a Dutch Groningen PCB Infant Cohort, and contained 8 samples from mothers at the 20th week of pregnancy, 70 samples from mothers at the 35th week of pregnancy and 12 cord blood samples. The samples were kept frozen at -20 ºC until analyzed.

Results and Discussion

A recovery study was performed to establish the quality of the method. It was designed as a standard addition method, where 2 x 5 samples were added with low (0.1 ng), and high (1.0 ng) standard concentration (8 congeners) before clean up, and 2 x 5 samples were added the same amount after clean up. The recovery in the pre-added samples were calculated based on that the samples, with added standard solution after clean up, was considered to contain 100 % of the compound concentration. The results are given in Table 1.

To each batch of samples (n = 10) cleaned up, one solvent blank and one control sample were included. This was done to test the background contamination and the reproducibility of the method. The results are given in Table 2, where also the limit of detection (LOD) and the limit of quantification (LOQ) are given. The reproducibility is expressed as the coefficient of variation between nine control samples. Blank levels are not removed from the sample concentrations, but no levels are reported which are lower than the solvent blank level, which is cleaned up within the corresponding batch. Mean, median and min-max levels of the samples are given in Table 3. In all samples BDE-47 was detected. But in two samples were BDE-47 not quantified due to relevant background level in the solvent blank at the clean-up occasion.

Table 1. Structure of the PBDEs used for method development. Recovery results (%) and methods for the syntheses of the PBDE congeners are given.

		Low dose		High dose		
BDE No [a]	**Structure**	**Mean**	**CV**	**Mean**	**CV**	**Reference [b]**
BDE-47	2,2',4,4'	**94%**	6.9	**83%**	4.4	[4]
BDE-77 (IS)	3,3',4,4'	**90%**	5.9	**85%**	3.4	[5]
BDE-100	2,2'4,4',6	**90%**	4.6	**84%**	5.1	[5]
BDE-99	2,2',4,4',5	**88%**	4.6	**83%**	4.3	[4]
BDE-154	2,2',4,4',5,6'	**84%**	5.4	**81%**	4.7	[5]
BDE-153	2,2',4,4',5,5'	**81%**	5.5	**79%**	4.7	[4]
HBCDD	Hexabromocyclododecane	**70%**	7.5	**70%**	10.2	CIL [c]
BDE-138 (IS)	2,2',3,4,4',5'	**79%**	3.5	**83%**	8.2	[4]
p-MeO-BDE-121 (VS)	4'-methoxy-2,3',4,5',6					[5]

CV = coefficient of variation (%)

[a] *The PBDEs have been given numbers according the numbering of polychlorinated biphenyl*[6]

[b] *To method for synthesis* [c] *Cambridge Isotope laboratories*

Table 2. LOD (s/n = 5) and LOQ (s/n = 10) expressed as true pg detected and quantifiable with GC-MS analysis. Reproducibility (n = 9) expressed as the coefficient of variation (%). Solvent blank (n=9) levels, calculated on a 5 gram sample with 0.5% fat (ng/g l.w.).

	BDE-47	**BDE-100**	**BDE-99**	**BDE-154**	**BDE-153**	**HBCDD**
LOD (pg)	1.5	2.0	1.5	1.0	2.0	4.0
LOQ (pg)	3.0	4.0	3.0	2.0	4.0	8.0
			Reproducibility			
CV	8%	5%	16%	8%	9%	32%
			Solvent Blanks (ng/g l.w.)			
Mean	**0.96**	**0.17[a]**	**0.37**	**n.d.**	**n.d.**	**<0.32**
Range	0.6-1.5	n.d.-0.4	0.2-0.8	--	--	n.d.-n.q.

LOD = limit of detection (s/n = 5) *n.d. = not detected*

LOQ = limit of quantification (s/n = 10) *n.q. = not quantified*

[a] *Below LOQ is calculated as half of that value*

The serum levels of BDE-47 ranged from <LOQ up to 13 ng/g l.w., with mean concentrations of 3.2 and 3.6 ng/g l.w., in maternal serum and cord blood, respectively. This is lower than reported mean levels in the U.S.[7] and comparable to levels found in Swedish blood reported 2001[8]. It should be pointed out that the mean BDE-153 concentration represented the highest concentration of all PBDEs in maternal serum. BDE-47 has previously been the most abundant congener, representing approx. 50 % of the ΣPBDE[9,7]. In the present study the relative level of BDE-47 in maternal serum was around 30% of ΣPBDE (11 ng/g l.w.) and BDE-153 around 40% of ΣPBDE concentration. Further studies are needed to determine if this is a general shift to higher levels of higher brominated diphenyl ethers in human blood. However, a similar result is

shown for the PBDEs reported in breast milk from the Faeroe Islands[10]. In cord blood on the other hand BDE-47 levels were higher than BDE-153 levels. HBCDD was detected in almost all samples, with concentrations up to 7.0 ng/g l.w. The HBCDD concentrations were similar to serum sample concentrations in Mexican and Swedish women[11].

Table 3. Mean, median, min and max concentrations (ng/g l.w. and pmol/g l.w.) found in cord blood (n = 12) and mothers serum at pregnancy week 20 (n = 8) and week 35 (n = 70).

	Fat %	BDE-47	BDE-100	BDE-99	BDE-154	BDE-153	HBCDD
		Cord blood (n=12)					
Mean (ng/g lw)	**0.22**	**3.6**	**0.69**	**0.84**	**0.93**	**2.4**	**1.7**
Mean (pmol/g lw)		**7.4**	**1.2**	**1.5**	**1.5**	**3.4**	**2.7**
Median (ng/g lw)	0.22	3.80	0.67	0.87	0.72	1.80	0.32
min-max (ng/g lw)	0.17-0.32	<0.12-10	<0.08-2.3	<0.12-2.6	0.2-1.9	0.7-5.0	<0.16-4.2
		Week 35 and 20 of pregnancy (n=78)					
Mean (ng/g lw)	**0.77**	**3.2**	**0.69**	**0.92**	**1.4**	**4.5**	**1.1**
Mean (pmol/g lw)		**6.5**	**1.2**	**1.6**	**2.2**	**7.0**	**1.7**
Median (ng/g lw)	0.74	2.4	0.54	0.76	1.1	4.5	1.3
min-max (ng/g lw)	0.4-1.8	0.6-13	0.11-2.9	<0.12-4.3	0.26-6.8	0.79-39	<0.16-7.0

PBDE and HBCDD concentrations ranges, on a lipid weight basis were similar in maternal and cord blood (infant level). Similar relations have been reported previously[12,7]. But if the relative fat content is considered, i.e. 0.22 % lipids in cord blood and 0.77 % in maternal serum, the total exposure of the BFRs is lower for an infant than for the mother (Figure 1a and 1b).

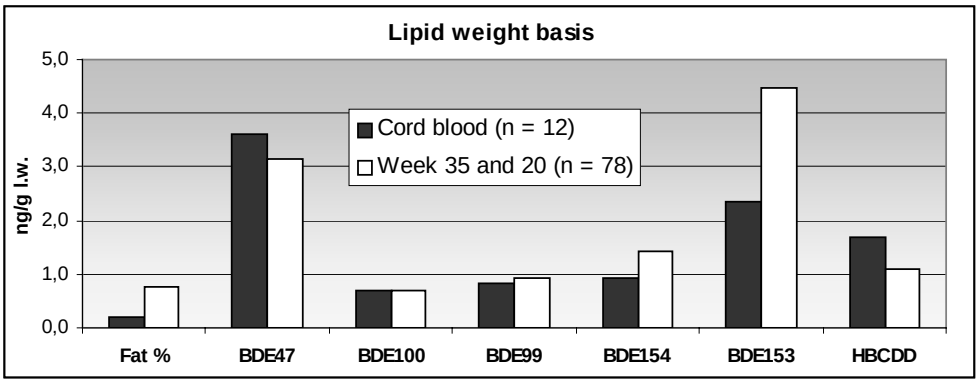

Figure 1a. Mean PBDE and HBCDD concentrations on a lipid weight basis (ng/g l.w.) in Dutch mothers and infants (cord blood).

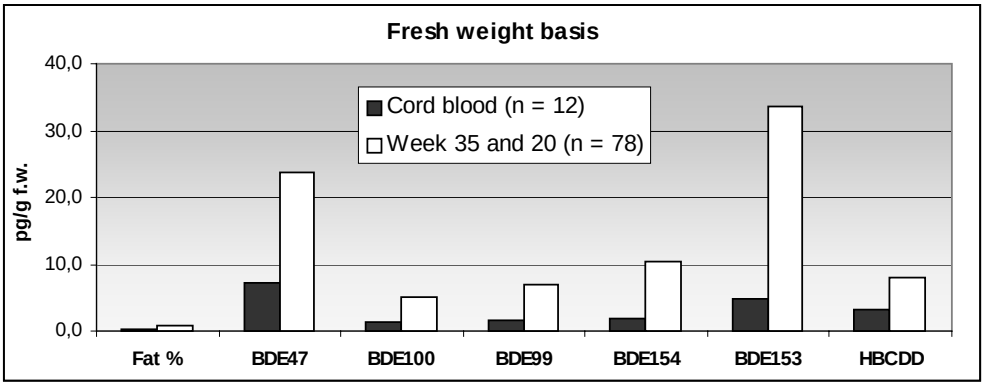

Figure 1b. Mean PBDE and HBCDD concentrations on a fresh weight basis (ng/g l.w.) in Dutch mothers and infants (cord blood).

Conclusion

It is notable that the HBCDD concentration in the cord blood is almost the same as for BDE-153 and half of BDE-47. This shows that HBCDD is a BFR contaminant of similar potency as the dominating PBDE congeners. Further, the higher BDE-153 concentrations, reported in this study, compared to BDE-47 is indicating a change in the PBDE profile in humans that need to be further investigated. The clean up procedure was successful for analysis of both HBCDD and PBDEs.

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Reference List

1. Birnbaum, L. S. and Staskal, D. F. (2004) *Environmental Health Perspectives* , **Vol. 112**, 9-17.
2. Hites, Ronald A. (2004) *Environmental Science and Technology*, **Vol. 38**, 945-956.
3. Hovander, L., Athanasiadou, M., Asplund, L., Jensen, S., and Klasson Wehler, E. (2000) *Journal of Analytical Toxicology*, **Vol. 24**, 696-703.
4. Örn, U., Eriksson, L., Jakobsson, E., and Bergman, Å. (1996) *Acta Chemica Scandinavica*, **Vol. 50**, 802-807.
5. Marsh, G., Hu, J., Jakobsson, E., Rahm, S., and Bergman, Å. (1999) *Environmental Science and Technology*, **Vol. 33**, 3033-3037.
6. Ballschmiter, K., Mennel, A., and Buyten, J. (1993) *Fresenius' Journal of Analytical Chemistry*, **Vol. 346**, 396-402.
7. Mazdai, A., Dodder, N. G., Abernathy, M. P., Hites, R. A., and Bigsby, R. M. (2003) *Environmental Health Perspectives*, **Vol. 111**, 1249-1252.
8. van Bavel, B., Hardell, L., Kitti, A., Liljedahl, M., Karlsson, M., and Pettersson, A. (2002) *Organohalogen compounds*, **Vol. 58**, 161-164.
9. Petreas, M., She, J., Brown, F. R., Winkler, J., Windham, G., Rogan, E., Zhao, G., Bhatia, R., and Charles, M. J. (2003) *Environmental Health Perspectives*, **Vol. 111**, 1175-1179.
10. Fängström, B., Athanassiadis, I., Strid, A., Grandjean, P., Weihe, P., and Bergman, A. (2004) *The Third International Workshop on Brominated Flame Retardants, BFR 2004*,
11. Lopez, D, Athanasiadou, M., Athanassiadis, I., Estrade, L. Y., Diaz-Barriga, F., and Bergman, A. (2004) *The Third International Workshop on Brominated Flame Retardants, BFR 2004*,
12. Meironyté Guvenius, D., Aronsson, A., Ekman-Ordeberg, G., Bergman, Å., and Norén, K. (2003) *Environmental Health Perspectives*, **Vol. 111**, 1235-1241.