

## Levels of brominated flame retardants in milk from the Norwegian human milk study: HUMIS

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### Introduction

Brominated chemicals are used as flame retardants (BFRs) to protect a wide variety of products from catching fire. Several BFRs have been shown to be potential persistent organic pollutants (POPs) and are found widespread in the environment, in wildlife and in humans<sup>1-4</sup>. At the Norwegian Institute of Public Health we are presently establishing a prospective birth cohort (HUMIS)<sup>5</sup>, which aims to recruit 6000 mother/child pairs, provided sufficient funding. Breast milk samples are collected in infancy and information on health outcomes are collected throughout the child's first seven years of life.

We present here the levels of polybrominateddiphenyl ethers (PBDEs) and hexabromo-cyclododecane (HBCD) in the 151 breast milk samples analysed so far, and show how they vary with the mothers' age, parity, education and place of residence.

### Materials and methods

#### *Chemicals*

The HBCD and PBDE standards (BDE-18, 28, 37, 47, 51, 77, 85, 99, 100, 103, 119, 138, 153, 154, 181 and 183) were obtained from Wellington Laboratories (Guelph, Ontario, Canada), CIL (Andover, MA) or AccuStandard (New Haven, CT). All solvents used were of pesticide grade from sds (Peypin, France) and sulphuric acid, silica gel and sodium sulphate were from Merck (Darmstadt, Germany).

#### *Breast milk samples*

Mother and child pairs living in five selected counties were consecutively recruited after birth. The five counties represent northern, southwestern, and eastern parts of Norway, and include both coastal and inland areas. Breast milk samples were collected in the period two weeks to two months after the child's birth, preferably by manual expression.

#### *Sample preparation and quantitative determination*

Five grams of milk was extracted using 15 ml of methanol/diethyl ether/heptane (1/1/1), and the extracts subsequently cleaned up on sulphuric acid-silica columns with a layer of silica on top using an automated solid phase extractor (ASPEC XL4, Gilson, Middleton, WI). Separation and quantitative determination of the BFRs were performed by GC-MS in the electron capture mode with methane as buffer gas as previously described<sup>6</sup>. The brominated compounds were monitored at  $m/z$  79 and 81 and identified based on retention time and isotope abundance ratio. The total uncertainty of the analytical method was found to be about 20 %.

#### *Statistical methods*

Statistical analysis was performed using SPSS 12 software. For bivariate analysis, means are given, but non-parametric tests were used to test for significance (Table 1). Linear regression was used in the adjusted analyses. The adequacy of the final linear regression model was tested by checking whether the assumptions of the model: linear effects and constant variance (homoscedastic), were met by plotting residuals versus predicted values. Furthermore, the model was checked for co-linearity and for points with high influence.

## Results

The median concentrations of the 11 measured PBDE congeners and HBCD in these 151 breast milk samples are shown in Figure 1. BDE-28, 47, 99, 100, 153 and 154 were quantified in almost all of the samples, BDE-183 was found in about half of the samples, while BDE-37, 85, 119 and 138 were only occasionally detected. BDE-47 was the dominating compound in all but 15 samples, where BDE-153 was found at the highest concentration. The mean contribution of BDE-47 to the sum 7 PBDE (see below) was 47 % (range 15-68 %). HBCD has so far been investigated in 85 samples and was observed in 49 (range 0.4 to 20 ng/g lipids). The detection limit for HBCD was about 0.3 ng/g lipids.

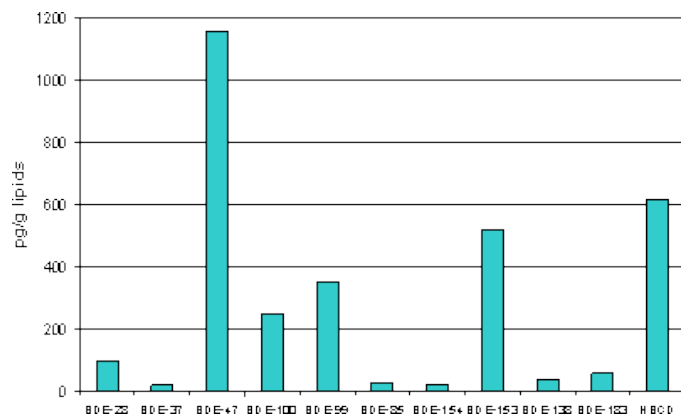


Figure 1. The median concentration in pg/g lipids of the PBDEs and HBCD in the 151 breast milk samples.

The sum of the seven most abundant PBDE congeners (28, 47, 99, 100, 153, 154 and 183) ranged from 0.95 to 21.05 ng/g lipids. The median was 2.34 ng/g lipids, which is in accordance with current levels reported in other European countries as e.g. Sweden (2.80 ng/g lipids, 2000)<sup>7</sup>, The Netherlands (3.31 ng/g lipids, 2003)<sup>8</sup> and the Czech Republic (1.68 ng/g lipids, 2003)<sup>8</sup>. The distribution was quite skewed (Figure 2), i.e. 5 % of the samples had a Sum 7 PBDE concentration exceeding 5 times the median.

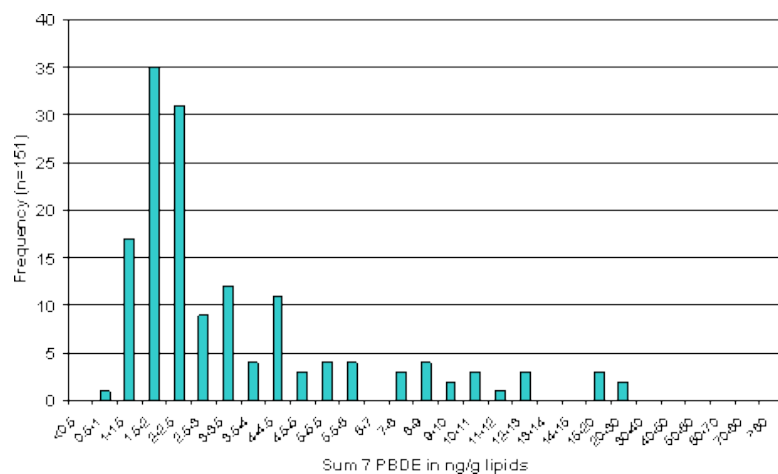


Figure 2. The frequency distribution of Sum 7 PBDE in ng/g lipids in the 151 breast milk samples.

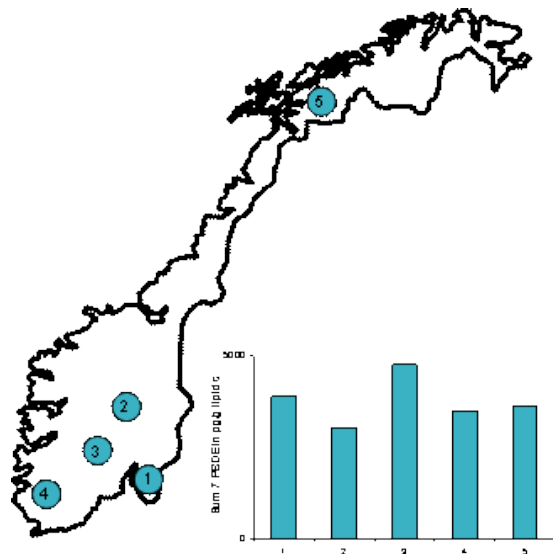


Figure 3. The sum 7 PBDEs (mean) in breast milk from mothers living in different regions of Norway.

The mean sum 7 PBDE in the breast milk of mothers living in different regions of Norway is presented in Figure 3. Small, but not significant differences were observed between the different geographical areas.

In the bivariate statistical analyses the levels in breast milk seemed to increase with the age of the mothers, and to decrease with parity and education, but the differences were not significant (Table 1). By use of a multiple linear regression model including these factors however, the trends were confirmed and found to be significant, even though the confidence intervals (CI) were quite wide (the adjusted analysis, Table 1). To our knowledge, this is the first time PBDE levels in breast milk have been shown to increase with the age of the mothers and to decrease with the number of children. In addition, Sum 7 PBDE levels were lower in breast milk from mothers with more than 12 years of education.

To summarise briefly, the median concentration of Sum 7 PBDE based on 151 Norwegian breast milk samples was 2.34 ng/g lipids and no significant geographical variation was found.

Adjusted statistical analyses reveal a positive significant association for Sum 7 PBDE with the mothers' age and significant negative associations with parity and education.

### Acknowledgement

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Table 1. Bivariate (unadjusted) and adjusted statistical analyses of the Sum 7 PBDE levels with respect to maternal age, parity and education. (N: number of subjects, B: estimated coefficient, CI: confidence interval)

	Bivariate (unadjusted)		Adjusted coefficients		
	N	Mean	B	CI	p-value
		(pg/g lipids)		(95%)	
<b>Maternal age</b>					0.123
< 28 years	52	3243			
28-31 years	56	3390			
>32 years	43	5173			
+ for 1 year of age			279	124 - 435	0.001
<b>Maternal parity</b>					
first child	46	4331			
more than one child	105	3635	-1862	-3310 - - 415	0.012
<b>Maternal education</b>					
< 12 years	56	4428			
> 12 years	86	3486	-1838	-3589 - -87	0.040

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