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# Time trends of polybrominated diphenylether (PBDE) congeners in serum of Swedish mothers and comparisons to breast milk data

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ABSTRACT

In the present study our main focus was blood serum levels and time trends of the fully brominated diphenyl ether (PBDE) BDE-209 in Swedish first-time mothers, as relatively a few human data on this congener are currently available. Also, levels and temporal trends in serum of other more commonly reported PBDE congeners and HBCD were studied. In an ongoing study on POPs in Uppsala Primiparas (POPUP), serum samples ($N=413$) from first-time mothers from 1996 to 2010 were used. Pooling of individual samples (5–25 individuals/pool, approx. 3 pools/year) resulted in 36 pooled samples used for PBDE/HBCD analysis on GC-LRMS. In addition, serum/breast milk correlations for PBDE and HBCD levels in 30 paired samples from individual mothers sampled 2010 were studied.

The mean serum level of BDE-209 (1.3 ng/g lipid wt.) was highest of all studied PBDE congeners, followed by BDE-47 and BDE-153. There was no significant temporal trend for BDE-209 during the study period, whereas the levels of BDE-47, BDE-99, BDE-100 and of HBCD decreased significantly in pooled serum 1996–2010. After omission of one outlier, a significant increasing trend was observed for BDE-153. The serum/milk PBDE quotients in paired individual samples from 2010 ranged from 0.83 to 17, with the highest quotient for BDE-209. Differences in PBDE transfer from blood to milk are probably related to molecular weight or size. The correlations between serum and milk levels of tetra- to hexa-brominated congeners were generally strong ($r=0.83$–$0.97$), but weaker for BDE-183 ($r=0.57$) and BDE-209 ($r=0.38$). Regarding HBCD, serum levels in 2010 were mostly beneath LOQ which made serum/milk quotients impossible. The decreasing levels of some BFR compounds in serum over time show that exposures have decreased after the production and use of some of these substances have been restricted. The lack of temporal trend of BDE-209 suggests that the human exposure to this congener in Sweden has been stable for more than a decade.



## 1. Introduction

Polybrominated diphenyl ethers (PBDEs) and hexabromo cyclododecane (HBCD) are examples of brominated flame retardants that, due to extensive industrial use, have been globally found in the environment and also in human tissues (e.g. Darnerud et al., 2001; de Wit, 2002). Human exposure to PBDEs and HBCD occur via ingestion of food and dust, inhalation and dermal absorption (Frederiksen et al., 2009; Trudel et al., 2011). Measurable PBDE levels are found in food, in household dust, and in human matrices including breast milk (e.g. Domingo, 2004; Wu et al., 2007; Fromme et al., 2009; Frederiksen et al., 2009). In Sweden, breast milk from Swedish first-time mothers has been analysed for several POPs, including PBDEs and HBCD (Lignell et al. 2009a; Glynn et al., 2011; Fangstrom et al., 2008). In contrast to clear decreased temporal trends for organo-chlorinated compounds simultaneously studied, temporal trends for the PBDE group did not follow a consistent pattern during the years 1996–2006/08 (Lignell et al., 2009a). BDE-47, BDE-99 and BDE-100 decreased significantly during this time period, whereas BDE-153 levels significantly increased in breast milk. Only recently, this increase in BDE-153 levels may have ceased in Swedish milk samples (Lignell et al., 2012). The mentioned Swedish PBDE analyses in breast milk did not include higher brominated BDE congeners such as the decabrominated BDE-209, a limitation as this PBDE congener could be of importance for the total PBDE exposure.

The industrial decaBDE preparation, mainly containing the fully-brominated BDE-209, has been used as an industrial flame retardant with similar field of application as the lower brominated PBDEs, i.e. for use in electric, electronic and textile products (for

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the pentaBDE preparations specifically, a large use as a component in polyurethane foams (PUF) could also be noted). However, biological properties of BDE-209 are different from the lower brominated PBDEs. We know from literature that terminal half-lives of low and intermediately brominated BDEs in rats are between 20 and 60 days (Geyer et al., 2004), which will lead to considerably longer half-lives in man. Calculations of $t½$ in man for BDE-47 are, depending on data origin and calculation method, 1.8–3 years, and for BDE-153 even longer, 6.6–11.7 years (Geyer et al., 2004). On the contrary, BDE-209 is less persistent and calculations give decaBDE an average half-life of only 15 days (!) in occupationally exposed workers (Thuresson et al., 2006). Although low persistence would seem to be a beneficial property for an industrial chemical, the breakdown of the BDE-209 molecule may result in lower brominated PBDEs, or other transformation products, with increased toxic potential. The issue of BDE-209 degradation, including formation of brominated degradation products with potentially increased toxicity, has been discussed by e.g. U.S. Environmental Protection Agency (US-EPA, 2008).

From 2003, strict bans were put on the use of commercial penta- and octa-BDE mixtures in the EU (OJ, 2003) and at this time prohibitory and replacement actions against PBDEs started to appear also in the USA (EPA, 2006). This made decaBDE the remaining PBDE preparation for commercial use and may have resulted in an increased global demand for the decaBDE mixture for some years thereafter, although also non-PBDE preparations were likely used in replacement. However, since 2008 the decaBDE is also under ban by the EU for use in electrical and electronic applications as decided by the European Court of Justice (OJ, 2008), and large-scale producers of decaBDE have discontinued their production and sale by the end of 2013 (http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/deccadbe.html). The production and use of HBCD has been proposed for regulation and phasing out, but today HBCD is still in use.

The number of studies on BDE-209 in human samples has increased considerably during recent years, due to improved analytical techniques. BDE-209 data has been reported from various countries and regions with focus on serum analyses, although some data on breast milk are also reported (see e.g. Frederiksen et al., 2009). BDE-209 levels may be increased in subjects occupationally exposed to PBDEs, or living in areas where PBDE production is ongoing; this has been shown in several Chinese studies (e.g. Jin et al., 2009; Wang et al., 2012). BDE-209 could also be detected in serum from children in a German study where levels were reported to increase during the years 2002/3–2008/9 (Link et al., 2012).

The aim of this study was to investigate whether there is a temporal trend of BDE-209 in first-time mothers from Uppsala, Sweden, sampled 1996–2010, and, if present, how it relates to other BDE congeners. Tri- to hepta-brominated PBDE congeners, i.e. BDE-28, BDE-47, BDE-66, BDE-99, BDE-100, BDE-138, BDE-153, BDE-154 and BDE-183, as well as HBCD were also included in the study. In addition, in order to increase the knowledge on PBDE body distribution, serum and breast milk levels of the more prominent BDE congeners were compared in matched samples from 2010.

## 2. Material and methods

### 2.1. Recruitment and sampling

In the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas), first-time mothers from the general population living in Uppsala County were recruited between 1996 and 2010 ($N=454$) (Lignell et al., 2009a, 2011). Although the methods for recruitment differed slightly between 1996–1999 and 2000–2010 (e.g. Lignell et al., 2009a), the important steps in recruitment process were similar, randomly sending recruitment requests to first-time mothers based on delivery journals and at the same time securing an even recruitment rate over the calendar year. The recruitment success was generally 50–60%. Recruitment was done yearly except for 2001, 2003 and 2005 (in the present study, the nine 2001 samples belong to the year 2000 recruitment). The participants donated breast milk and a blood sample 3 weeks after delivery. Blood sampling was done using 9 ml Vacutainer® or Vacuette® serum tubes. Milk and serum were stored at −20 °C. The study was approved by the local ethics committee of Uppsala University, and the participating women gave informed consent prior to their inclusion in the study.

In the time trend study we used pooled serum samples from the participants for analysis of PBDEs and HBCD. Samples from mothers born in non-Nordic countries ($N=10$) were not included in the pools. As these samples have been used in earlier time trend studies of chlorinated and perfluorinated compounds (Glynn et al., 2007, 2012) sample volumes were sometimes too low. Thus, from about 30 women, there was no serum left or the volume was too small to allow inclusion in the pools. The total number of individual samples included in the pools was 413, and equal volumes from all individual samples were used to create the pools. An effort was made to produce 3 pooled serum samples for each sampling year. Five to 25 individual samples were included in each pool, resulting in 36 pooled samples for PBDE and HBCD analyses (Table 1).

In the study on serum/breast milk comparisons of BDE levels, individual samples from 2010 ($N=30$) were used.

### 2.2. Analytical method for determination of brominated flame retardants in human blood serum and milk

#### 2.2.1. Extraction and clean-up

*2.2.1.1. Pooled serum samples.* Thawed serum (4 g) was mixed with methanol by vortexing in a test tube. A mixture of diethyl ether/*n*-hexane (1+1, v/v) and internal surrogate standards, BDE-85 and $^{13}$C-BDE-209 was added. The sample was extracted on a rotary mixer and then centrifuged. The top, organic, layer was transferred to a pre-weighed test tube. The extraction step was repeated twice and the organic layers were combined. The solvent was evaporated using a gentle stream of nitrogen and the lipid weight was determined gravimetrically.

In order to remove the lipids and other polar materials the lipid extract was re-dissolved in *n*-hexane using ultra-sonication and then treated with concentrated sulphuric acid. After centrifugation and collection of the organic layer the procedure was repeated once by adding *n*-hexane to the acidic layer. The organic phases were combined and the volume was reduced by using a gentle stream of nitrogen.

The lipid-free extract was transferred to a pre-washed open silica gel column (8 mm id, 4.5 g of 3% deactivated silica gel). Most of the PCBs were separated from the PBDEs by elution with *n*-hexane (waste). A second fraction, containing the brominated flame retardants, was eluted with dichloromethane. The second fraction was reduced and transfer to a test tube where the solvent was changed to *n*-hexane. The final volume of the sample was adjusted to 100 μl using a gentle stream of nitrogen and then kept in an amber GC vial until analysis.

*2.2.1.2. Individual serum samples.* The extraction and clean-up were carried out as mentioned above with a few modifications. After extraction the organic layer was transferred to a test tube containing aqueous potassium chloride (1%, w/w). The denatured serum was re-extracted with diethyl ether/*n*-hexane (1+1, v/v)