Serum levels of brominated flame retardants (BFRs: PBDE, HBCD) and influence of dietary factors in a population-based study on Swedish adults

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HIGHLIGHTS

- PBDEs and HBCD analyzed in serum in a national population-based Swedish study.
- Main congeners (medians, ng/g fat): BDE-153 (1.2), BDE-209 (0.95), BDE-47 (0.49).
- Sex difference for BDE-153; and direct associations with fish and meat consumption.
- Swedish levels comparable with those in other European countries, but lower than in the US.
- Body burden MOEs suggest sufficient health protection (not for extreme values).

ABSTRACT

The aim of this study was to investigate associations between serum concentrations of brominated flame retardants and personal characteristics, including diet, in adults participating in a population-based study in Sweden 2010–11. Moreover, observed concentrations were used in a health risk assessment, using published health-based reference values. Serum samples of 170 adult individuals of both sexes were analyzed for 10 PBDE congeners and HBCD by GC-MS. When including concentrations between LOD and LOQ, highest median serum concentration was observed for BDE-153 (1.2 ng/g serum lipid), followed by BDE-209 (0.95 ng/g lipid), BDE-47 (0.49 ng/g lipid) and BDE-100 (0.21 ng/g lipid). Median concentration of HBCD was 0.10 ng/g lipid. A few markedly elevated concentrations of BDE-209, HBCD (77–78 ng/g lipid) and BDE-47 (44 ng/g lipid) were observed. The only statistical significant findings were higher BDE-153 concentrations in men than in women, and positive associations between serum BDE-153 concentrations and consumption of fish (total), beef, mutton and poultry. PBDE concentrations were in accordance with concentrations reported in other European countries but generally lower than those found in North America. Median PBDE serum concentrations observed in adults from Sweden suggest sufficient health protection, when compared with published health-based reference values, although some outliers with high serum concentrations had lower safety margins.

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1. Introduction

Brominated flame retardants (BFRs) have for a long time been used to decrease flammability of a variety of products in houses and homes, for instance electronic devices, electric cables and home textiles. An unwanted side effect of BFR use is environmental BFR pollution and human BFR exposure (de Wit, 2002; Darnerud et al., 2001). In experimental animals BFRs adversely affect liver and thyroid function, and causes developmental neurotoxicity (Darnerud, 2003; Birnbaum and Staskal, 2004; Legler, 2008). Neurodevelopment was used as a critical endpoint in the human health risk assessment of the BFRs polybrominated diphenyl ethers (PBDEs) in food performed by the European Food Safety Authority.
(EFSA) in 2011 (EFSA, 2011). However, food is not the only source for BFR exposure. For small children a large part of the total BFR exposure may come from ingestion of indoor dust (e.g. Harrad et al., 2010). An increasing number of epidemiological studies show associations between exposure to BFRs and adverse health effects, such as thyroid function disturbances, diabetes, and neuro-behavioral and developmental disorders (Kim et al., 2014), although causality of associations have not been firmly established. Human exposure to most of the studied PBDE increased up to the end of the 1990s in Sweden but has thereafter decreased (Fångström et al., 2008; Lignell et al., 2009).

The Swedish National Food Agency (NFA) recurrently performs population-based food consumption surveys and in the most recent food survey (Riksmaten 2010–11, adults) BFRs were bio-monitored in a subsample of the participants. The aim of the present study is to determine serum concentrations of BFRs in a representative sample of the adult Swedish population, and to investigate possible associations with diet or lifestyle factors. Observed concentrations were used in a health risk assessment, using health-based reference values published by EFSA (EFSA, 2011).

2. Materials and methods

2.1. Study design and population

Riksmaten 2010–11 is a Swedish national survey investigating dietary habits. The study was conducted between May 2010 and July 2011. The biomonitoring study was based on a subgroup of individuals donating blood samples. The subjects in the subgroup were randomly selected from the Swedish population registry, taking season and where the blood and urine collection would take place into account, but independent of the main study population. The blood sampling was performed at the Swedish Occupational and Environmental Medicine Centers (OEMCs). Based on the OEMC service areas, Sweden were divided into seven regions. From each region, the region capital (Linköping, Lund, Stockholm, Umeå, Uppsala, Gothenburg, Örebro) and two random additional counties were selected. Data were collected at four occasions (i.e. May/June 2010, August/September 2010, January/February 2011, and April/May 2011). An equal number of individuals were selected in each region independent of population size. Altogether, 1008 individuals between 18 and 80 years old were randomly selected and invited to participate in the blood sampling (12 individuals per county and occasion) by letter. The invitation was followed-up with a telephone call. Those accepting to participate were given further instructions on how to record their diet (see below) by additional phone calls. The relevant OEMC also contacted the participant for an appointment to draw blood and urine. Of the 300 individuals who choose to participate (30%), blood samples from 170 individuals were analyzed for BFR concentrations. The other 130 participants were excluded due to small serum volumes. Further exclusion of 24 participants due to incomplete food record and/or questionnaire data left 146 individuals in the sample population in which associations were studied. All participants gave oral informed consent before entering the study. The study was approved by the regional ethical committee in Uppsala.

2.2. Assessment of diet and lifestyle

Food consumption was registered by the participants using a web-based food record. The registration was performed during four consecutive days and the starting day for each participant was randomly selected (Tuesday, Wednesday, Saturday or Sunday) to cover all days of the week. Portion size was estimated by the participants using a portion guide with photographs. Additionally, a food frequency questionnaire was used to assess consumption of less frequently consumed food items (e.g. fish, meat). Information about physical activity and breastfeeding were collected by a questionnaire. Highest completed education was assessed using Swedish register data and participants were divided into three groups of education, having completed elementary school, high-school, or university education. Breastfeeding by women was categorized as not having breastfed, having breastfed for totally 1–6 months, 7–12 months, or more than 12 months. Weight and height were reported by the participants and body mass index (BMI) was calculated as weight [kg] divided by height [m] squared.

2.3. Sampling of serum

Non-fasting serum was sampled at the OEMC in each region or by district health care centers. Blood was drawn from an antecubital vein. Serum was separated and stored at −20 °C until analysis. Four ml serum was used for the BFR analyses.

2.4. BFR analyses

The analytical method used for determination of brominated flame retardants in human blood serum has been described earlier (Darnerud et al., 2015). Briefly, serum was extracted with methanol and a diethyl ether/n-hexane mixture. The organic phase was washed twice with aqueous potassium chloride and transferred to a pre-weighed test tube. The lipid weight was determined gravimetrically. In order to remove lipids and other polar materials the lipid extract was re-dissolved in n-hexane and treated with concentrated sulfuric acid and subsequently transferred to an impregnated silica/sulfuric acid gel column and eluted with a mixture of dichloromethane/n-hexane. The lipid-free extract was transferred to a pre-washed silica gel column and eluted with n-hexane (fraction 1) and dichloromethane/n-hexane (fraction 2). The second fraction was kept in an amber GC vial until analysis.

Quantification of the analytes (BDE-28, -47, -66, -99, -100, -138, -153, -154, -183, -209 and HBCD) was performed by GC/MS/ECNI-SIM (Agilent 6890 N GC/Agilent 5973 N MS). Six μl (2 × 3 μl) was injected in pulsed splitless mode using a programmable temperature vaporizing (PTV) injector. The analytes were separated on a DB-5MS column (J&W Scientific) and the oven temperature was programmed from 60 °C to 325 °C. The different analytes were identified by their retention times relative to the internal standards (BDE-85 and 13C-BDE-209). The GC and MS operating conditions are described in more detail elsewhere (Darnerud et al., 2015). The samples were quantified using calibration curves created from the calibration standards analyzed in the same run. Quadratic regression with the inverse square of concentration was used for the calibration curves. Since the analytical method do not separate brominated biphenyl 153 (BB-153) and BDE-154, the concentration of BDE-154 is reported as a sum of BDE-154 and BB-153.

2.5. Quality assurance

The glassware used was either heated in an oven or rinsed with acetone before use and all solvents were tested for trace amounts of analytes. A chemical blank and a spiked in-house control sample were included in each extraction series to monitor background levels and the methods accuracy. For each batch of samples, the corresponding blank sample levels were subtracted from the sample levels. The limit of detection (LOD) was determined as three times the standard deviation of the blanks analyzed together with the samples and the limit of quantification (LOQ) was determined as ten times the standard deviation of the blanks or the lowest
calibration level if no levels were detected on the blanks. The higher value of this two was selected as LOQ. The LOQ varied between 0.25 and 1.9 ng/g lipid weight. The expanded uncertainty in the measurements was 20–40% (calculated using a coverage factor of 2 which gives a level of confidence of approximately 95%). Levels between LOD and LOQ were reported in order to improve the power of statistical analyses, although such data are less accurate with a higher probable uncertainty.

2.6. Statistical analyses

Clustering of the BDE congeners was investigated by average linkage cluster analysis. The cluster analysis gives information on the degree of similarity in occurrence of the studied congeners in the study group, suggesting similar exposure sources. In statistical analyses results of cluster analyses may be used to group data for congeners clustering together. Distributions of the variables were investigated by Shapiro Wilk test. BDE-154/-brominated biphenyl (BB)-153, BDE-153 and BDE-183 were normally distributed after logarithmically transformation whereas all other BDEs were not. Non-parametric tests were used when investigating the associations between serum BDE concentrations and personal characteristics of the participants due to the non-linearity of most associations. Furthermore, non-parametric tests are less sensitive for interference of extreme values, which is common for some of the studied BFRs. Continuous variables were correlated with BDE concentrations by Spearman’s correlation. The relationship between BDE and categorical variables were examined using Wilcoxon rank sum test or Kruskal-Wallis test. Associations found to be significant in univariate analyses were further investigated by adjusting for potential confounders, i.e. age, sex, BMI and education. These personal characteristics have earlier been found to be significantly associated with serum concentrations of chemically related POPs (PCBs and chloropesticides) in the same study group (Bjermo et al., 2013). The residual method was used in these adjusted models (Willett and Stampfer, 1986). Continuous variables were categorized into quintiles when using the residual method. Means of BDE-153 adjusted for age, sex, education and BMI were calculated by linear models. The statistical analyses were performed using the software package STATA version 11 (STATA corporation). P < 0.05 was considered statistically significant.

When the serum concentrations were between LOD and LOQ, the actual registered concentrations from the analysis were used in the statistical analysis to improve the statistical power. Levels below LOD were assigned the value \(\frac{1}{2}\)LOD. Even if analytically registered values below LOQ are less accurate than levels above LOQ, the present procedure will probably result in less bias compared to replacement of concentrations below LOQ with zero or \(\frac{1}{2}\)LOQ (as the two latter will introduce a systematic error) (RSC, 2001; Bergstrand and Karlsson, 2009). In an earlier publication we have discussed the problems and benefits of the use of registered concentrations below LOQ in analyses of BFR in serum (Daranrud et al., 2015). In the present study, statistical analyses of associations between BFR concentrations and personal characteristics were limited to BFR congeners for which more than 60% of the values were above LOQ, i.e. BDE-153 and BDE-209. For the remaining BDE congeners, and HBCD, statistical analyses would mainly be based on concentrations below LOQ, making the results uncertain.

Average linkage cluster analysis was performed on the serum BFR concentration data (Fig. 1). Intermediately brominated (3–5 Br) BDE congeners showed relatively close relationship, whereas the hexabrominated BDE-153 as well as both the full-brominated BDE-209 and HBCD were well separated from each other, and from the 3–5 bromo-DEs. Based on these findings, we decided to treat the BDE congeners BDE-153 and BDE-209 separately and not to group them together in the statistical analyses of associations with personal characteristics.

3. Results

3.1. Personal characteristics

Table 1 presents population characteristics of the 170 participants in comparison with the whole Riksmaten biomonitoring group (n = 300), and with the adult Swedish population (at year 2010). There were no marked differences in characteristics between the two former groups. However, the study group had a higher proportion of individuals with a completed university education than the general Swedish population (Table 1).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study Group (n=170)</th>
<th>Riksmaten Biomonitoring (n=300)</th>
<th>Adult Swedish Population (n=10447)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male: 68 (40%)</td>
<td>Male: 176 (59%)</td>
<td>Male: 5119 (51%)</td>
</tr>
<tr>
<td></td>
<td>Female: 102 (60%)</td>
<td>Female: 124 (41%)</td>
<td>Female: 5328 (49%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Median: 50.7</td>
<td>Median: 46.3</td>
<td>Median: 44</td>
</tr>
<tr>
<td></td>
<td>Range: 19-86</td>
<td>Range: 20-85</td>
<td>Range: 20-85</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Median: 24.4</td>
<td>Median: 24.9</td>
<td>Median: 25.1</td>
</tr>
</tbody>
</table>

Table 1 presents population characteristics of the 170 participants in comparison with the whole Riksmaten biomonitoring group (n = 300), and with the adult Swedish population (at year 2010). There were no marked differences in characteristics between the two former groups. However, the study group had a higher proportion of individuals with a completed university education than the general Swedish population.

3.2. Serum concentrations of BFRs

Concentrations of PBDE congeners and HBCD are given in Table 2. The BDE congeners with the highest median levels were BDE-153 (1.2 ng/g serum lipid), followed by BDE-209 (0.95 ng/g lipid), BDE-47 (0.49 ng/g lipid) and BDE-100 (0.21 ng/g lipid). The median concentration of HBCD was 0.10 ng/g lipid. The median concentration of mixed BDE-154/BB-153 was estimated to 0.28 ng/g lipid, but with no possibility to separate the relative contribution of the two congeners. Notably, a few markedly elevated levels were observed for BDE-209, HBCD (both 77–78 ng/g lipid) and BDE-47 (44 ng/g lipid). All BDE congeners, except BDE-153 and BDE-209, had a substantial amount of concentrations below LOQ (76–99%) and were therefore excluded from further statistical analyses (see Materials and methods, Statistical analysis). BDE-154 was excluded due to the above mentioned chemical-analytical considerations.

3.3. Associations with personal characteristics

Serum concentrations of BDE-153 were higher among men than among women (median [Q1–Q3]: 1.6 [1.2–2.1] vs. 1.0 [0.8–1.3] ng/kg lipid), also after adjustment for age, education and BMI. No sex differences were observed for BDE-209 (0.9 [0.7–1.8] for men, 1.0 [0.7–1.6] for women). Among women, BDE-153 and BDE-209 concentrations did not differ depending on breast feeding time, and BDE-153 was still associated with sex after breast feeding of the women was taken into account. BDE-153 and BDE-209 concentrations were not correlated with age and BMI (BDE-153: \(r = 0.09\),...
p = 0.27 and r = −0.14, p = 0.08; BDE-209: r = −0.06, p = 0.42 and r = −0.06, p = 0.41).

In univariate analyses serum BDE-153 and BDE-209 concentrations were not related to consumption of different types of foods (results not shown), except in the cases of BDE-153 and fish and meats (Fig. 2). BDE-153 was positively associated with total fish consumption, including shellfish, when assessed by 4-days food records (r = 0.15, p = 0.04), but not when assessed by the frequency questionnaire (p = 0.11). BDE-153 concentrations were positively related with consumption of fatty fish (food frequency questionnaire) whereas no relationships were observed for lean fish (r = 0.16, p = 0.04; r = 0.12 and p = 0.13). Fig. 2a presents the association between BDE-153 levels and fatty fish intake (categorized as consumption less than once per month, at least once per month but less than once per week, and at least once per week). Associations between BDE-153 and fish consumption were not significant after adjustments for age, sex, education and BMI.

BDE-153 concentrations were positively associated with consumption of beef, mutton and poultry assessed by the food frequency questionnaire (Fig. 2b–d). The associations with consumption of mutton and fowl were significant also after adjustment for age, sex, education and BMI.

4. Discussion

4.1. Serum concentrations of BFRs

The results of our population-based biomonitoring study of BFRs in adults from Swedish show that the median concentration of BDE-153 was highest, followed by BDE-209, BDE-47 and BDE-100. During the most recent years these four congeners have generally been reported to be present at highest concentrations among BFRs in human blood samples from general populations in Europe, North America, Asia and Oceania (Frederiksen et al., 2009). Particularly in North America, BDE-47 concentrations in blood have been found especially high, and this BDE congener has been the main driver for the high North American PBDE levels as compared to other regions, including Europe (Fromme et al., 2016). The review by Frederiksen et al. (2009), including data from 1977 to 2006, in most cases reported higher concentrations of BDE-47 relative to BDE-153 also from regions other than North America. This is in line with results of earlier Swedish studies of non-occupationally exposed women (Sjödin et al., 1999; Guvenius et al., 2003; sampled 1997 and 2000–01, respectively). In a more recent Swedish study from 2010, a group of men and women (N = 31) with expected background PBDE exposure, had similar BDE-47 and BDE-153 serum concentrations (both around 1 ng/g lipid) (Strid et al., 2014). In the present study, representing adults in Sweden in 2010–11, concentrations of BDE-153 were even higher than BDE-47 concentrations. This gradual shift is most likely a consequence of a recent temporal decrease in BDE-47 and an increase, or steady state, in BDE-153.

The median BDE-209 concentration was the second highest among the BDE congeners analyzed in our population-based study, representing adults in Sweden in 2010–11, concentrations of the four main BDE congeners (BDE-47, -100, -153, -209) were lower (60–94%) than among the Swedish adults of both sexes in the present study. The lower BDE average serum concentrations of the four main BDE congeners (BDE-47, -100, -153, -209) were lower (60–94%) than among the Swedish adults of both sexes in the present study. The lower BDE concentrations in the young nursing women could reflect pregnancy/nursing-related changes in physiological and kinetic
factors affecting serum/plasma concentrations of persistent and lipid-soluble POPs (Glynn et al., 2011). For instance, the increase in lipid content of the blood during pregnancy could result in a “dilution effect” on serum lipid PBDE concentrations and this effect could persist at least during the early part of the nursing period. In addition, excretion of PBDEs via breast milk may contribute to the lower serum lipid PBDE concentrations in young nursing women (e.g. Sahlström et al., 2014). In our study serum lipid BDE-153 concentrations among women were not associated with breast-feeding time. A possible explanation could be that there was a relatively long time-span between the periods of nursing and the blood sampling of the participating women in our study. Moreover, a limited number of study participants may have weakened the statistical power for detecting an association between BDE-153 serum concentrations in the women and breast-feeding time.

4.2. Associations with personal characteristics

It is well known from several studies that fish and fish products are major dietary sources of PBDEs in Sweden (e.g. Törnvist et al., 2011). We found a weak positive association between serum concentrations of BDE-153 and fish consumption, as registered by food records. However, the association dissipated after adjustment for covariates (i.e. age, sex, education and BMI), showing that other personal characteristics than fish consumption explain the major part of the variation in BDE-153 concentrations observed by us. We observed positive associations between BDE-153 concentrations and consumption of beef, mutton and fowl meat. The presence of PBDEs in meat and meat products has been reported from both Europe and North America (Bocio et al., 2003; Spain; Schecter et al., 2006, 2010; Huwe and West, 2011; USA; Törnvist et al., 2011 (Sweden)). In the most recent Swedish market basket study, consumption of meat and meat products were estimated to contribute on average 24% of the total BDE-153 intake from food (NFA, 2012).

Certain study participants had much higher BDE serum concentrations than the other participants. For both BDE-153 and BDE-209 the maximum measured concentrations were approximately 100 times higher than the median concentrations. This shows that certain individuals have a high PBDE exposure from unknown sources.

4.3. Risk assessment

In the EFSA Scientific Opinion from 2011, effects on neurodevelopment were identified as the critical endpoint for PBDEs in the health risk assessment (EFSA, 2011). By using estimated body burden (BB) data obtained in our study, comparisons could be made with the BB data at BMDL10 from animal studies reported by EFSA. From these comparisons BB margin of exposures (BB MOE) were determined. The individual BFR BB in our study was estimated from the measured serum lipid concentrations of BFRs and the body weight of each participant, assuming 20% body fat. The comparison (Table 3) showed that BB MOEs based on median serum levels were large, above 250, in case of BDE-47 and BDE-153, and therefore of no apparent health concern as defined by EFSA (EFSA, 2011). Maximum serum concentrations resulted in much lower MOEs and thus also lower safety margins. Based on EU dietary BFR intake data, EFSA expressed concern about BDE-99. Notably, BDE-99 showed the lowest MOE in our assessment (MOE = 7.5) when using maximum serum concentrations. According to EFSA any MOE larger than 2.5 indicates that health concerns are unlikely to happen. The general temporal trends for PBDEs in Swedish human samples are showing decreasing concentrations, except for higher brominated PBDEs (Darnérud et al., 2015), which imply that the MOEs for BDE-99 will likely increase in the future.
4.4. Strengths and limitations

BDE-153 was the only congener with all serum samples having concentrations above LOQ. BDE-153 was also the congener showing concentrations above LOQ. BDE-153 was also the congener with lower body burden values achieved by assuming 20% body fat (fat adjusted serum levels \( \times 0.2 \)).

<table>
<thead>
<tr>
<th>Congener</th>
<th>Median</th>
<th>Median/Max</th>
<th>Serum level</th>
<th>BB MEDL10 (EFSA data)</th>
<th>MOE (BB MEDL10/BB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE-47</td>
<td>Median</td>
<td>0.49</td>
<td>0.098</td>
<td>232</td>
<td>2337</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>44</td>
<td>8.8</td>
<td>232</td>
<td>26</td>
</tr>
<tr>
<td>BDE-99</td>
<td>Median</td>
<td>1.2</td>
<td>0.24</td>
<td>9</td>
<td>7.5</td>
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<tr>
<td></td>
<td>Maximum</td>
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<td>258</td>
</tr>
<tr>
<td>BDE-153</td>
<td>Median</td>
<td>1.2</td>
<td>0.24</td>
<td>9</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>1.4</td>
<td>1.0</td>
<td>62</td>
<td>44</td>
</tr>
</tbody>
</table>

\( \text{a} \) BDE-209 not included due to lack of BB MEDL10 data.
\( \text{b} \) In ng/g lipid.
\( \text{c} \) In μg/kg body wt.
\( \text{d} \) Body burden values were achieved by assuming 20% body fat (fat adjusted serum levels \( \times 0.2 \)).
\( \text{e} \) Only maximum level of BDE-99 due to few levels above LOD, making median level uncertain.

5. Conclusions

In this Swedish population-based study median serum lipid concentrations of BDE-153 and BDE-209 were highest among the studied BFRs. The variation of serum concentrations were high and for BDE-47 and BDE-209 the maximum/median concentration quotient was around 100. The serum concentrations in this population with background BFR exposures are generally in accordance with those reported from other European countries, but generally lower than levels found in USA. A few associations were found between BDE-153 and meat consumption, but no significant associations with other personal characteristics were found. Based on our results, current BFR body burdens among adults give sufficient MOEs in relation to body burdens causing negative effects in animals.

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References


