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Serum levels of decabromodiphenyl ether (BDE-209) in women from different European
countries and possible relationships with lifestyle and diet.

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Abstract

To determine possible effects of lifestyle, diet, housing and professional activities on differences in individual levels of decabromodiphenyl ether (BDE-209) in serum of women, 20 to 40 years of age, in the Netherlands, the United Kingdom, Norway and Spain.

BDE-209 was measured in serum of 145 female volunteers with no known occupational exposure from Norway, United Kingdom, The Netherlands and Spain. Blood levels of BDE-209 in a subgroup of 40 Dutch women were determined twice at a six months’ interval. An extensive questionnaire was used to obtain detailed information about lifestyle factors that might contribute to BDE-209 exposure. Serum levels were used to determine margin of systemic exposure compared with a 28d rat toxicity study.

Median BDE-209 serum concentrations were highest in the Netherlands and United Kingdom, respectively 8.8 and 9.3 pg/g w.w. or 2.6 and 2.8 ng/g lipid. Median levels in Spain and Norway were lower, respectively 7.4 and 5.2 pg/g w.w. or 3.3 and 0.8 ng/g lipid. Maximum levels in individual women were higher by one order of magnitude than the mean or median. The country of residence was the only variable significantly associated with BDE-209 levels; we found that the differences between countries could not be explained by any of the investigated exposure variables, and that these did not explain differences between individuals either. No consistent relationships were determined between diets, household, clothes, number and duration of use of electronics and occupational activities for the whole study group.

We could not identify which of the multiple sources of exposure accounted for individual differences in blood levels. Although small differences in mean BDE-209 serum levels were recognized between countries, these differences are unlikely to cause a differential result with respect to risk assessment.

KEY WORDS: DecaBDE, BDE-209, PBDEs, serum, lifestyle, diet, household, risk assessment
Introduction

In modern life, flame retardants have become part of efforts to protect society against injuries, death and economic damage due to fires. A wide range of chemicals have been developed as flame retardants, from which the polybrominated diphenyl ethers (PBDEs) have been commonly used for many decades. Various PBDE products have been in production and use for several decades, commercial PentaBDE, OctaBDE and DecaBDE mixtures comprising diphenyl ethers of varying bromination degree. Some PBDEs have physico-chemical properties that promote environmental persistence and accumulation in food chains and humans (Darnerud et al. 2001; de Wit 2002; Frederiksen et al. 2009; Tanabe et al. 2008; Zhu et al. 2009). Certain lower brominated congeners have been reported to have long half-lives in humans, wild life and experimental animals, indicating a distinct role of bromine atoms in reducing metabolic rates of these compounds (Geyer et al. 2004; Gill et al. 2004; Toms et al. 2009b). As a result, levels of tetra- to heptabrominated BDEs in environmental biota and humans can equal those for PCBs in many industrialized countries (Haraguchi et al. 2009; Hites 2004; Schec ter et al. 2005).

In the European Union, the commercial Penta- and OctaBDE mixtures were taken off the market in 2005 because of adverse effects observed in experimental animals (Directive 2003/11/EC). In North America these commercial formulations were voluntarily withdrawn from the market by industry in 2004 (BSEF 2009). Further, since May 2009, tetra- to heptabrominated diphenyl ethers have been listed in the UN Stockholm Convention on Persistent Organic Pollutants (http://www.pops.int). In contrast, commercial DecaBDE is still use as flame retardant for plastics and textiles when our study was done (BSEF 2009; Harrad et al. 2008; Public Health England 2009; 2013). The commercial mixture consists primarily of the fully brominated diphenyl ether (BDE-209) and smaller amounts of nonabrominated BDE (0.3-21.8%) and octabrominated BDE (0-0.04%). Although its use in electrical and electronic equipment had been banned in the EU in 2008 (BSEF 2009) and the production and sales of commercial DecaBDE (c-DecaBDE) has been phased out in North America (BSEF 2016), there is ongoing human exposure from dust in indoor environments (Harrad et al. 2006, Law et al., 2014) and from diet, particularly seafood (Shaw et al. 2009). Presently c-DecaBDE is still under consideration for restriction and elimination under EU’s REACH regulation and UN’s Stockholm Convention, respectively (http://chm.pops.int/Default.aspx?tabid=5171). In order to evaluate the result of these regulations in reducing human exposure, it is of great importance to establish good biomonitoring data for DecaBDE in particular.
Because of the adverse properties and effects of the lower brominated BDEs, commercial Penta- and OctaBDE have been phased out in European countries in the early 2000s and were globally banned by the UN Stockholm Convention in 2009. As a result, the increasing temporal trends of levels of tetra- to heptabrominated BDEs in human blood and milk have leveled off in the late 1990s in Europe and have declined since, this (Fängström et al. 2008; Thomsen et al. 2007), but is less distinct for North America (Law et al. 2014). Furthermore, an upward trend for decaBDE has been observed in the same time period (Law et al. 2014). It is well established that levels of lower brominated PBDEs in humans may vary strongly among geographical regions, e.g., mean total PBDE levels in North America are about one order of magnitude higher than in Europe (Frederiksen et al. 2009; Fromme et al. 2016; Hites 2004). In non-occupational situations the relative contribution of decabromodiphenyl ether (BDE-209) in humans constitute a variable part of the total amount of PBDE body burden (Antignac et al. 2009; Frederiksen et al. 2009; Gomara et al. 2007; Thuresson et al. 2005). The geographical differences might partly be explained by different regional fire safety regulations and use of decaBDE containing flame-retardants in consumer products (Harrad et al. 2008). Also, within countries individual differences in PBDE levels can be quite substantial and may easily exceed more than one order of magnitude in human blood and milk (Frederiksen et al. 2009; Hites 2004).

At present, the cause for this strong variability in human levels is unclear, but lifestyle factors have been suggested as a contributing factor. Although, food is an important pathway for human exposure to PBDEs (Fromme et al. 2009; Meng et al. 2007; Schecter et al. 2006; Schecter et al. 2008; Voorspoels et al. 2007; Wu et al. 2007), the ingestion of house dust is also considered to be an important exposure pathway, especially for BDE-209 (Harrad et al. 2006; Harrad et al. 2008; Jones-Otazo et al. 2005; Sjodin et al. 2008; Toms et al. 2009a).

Many in vivo toxicokinetic and toxicological studies with PBDEs with different degrees of bromination were done over the last decade to support risk assessment for humans and wildlife (Birnbaum and Cohen Hubal 2006; Darnerud 2003; Staskal et al. 2008). As a result, multiple toxic and biological effects have been identified (Darnerud 2003; He et al. 2009; Kuriyama et al. 2005), which show similarities between the lower brominated PBDEs and decaBDE (Dingemans et al. 2016). These include interactions with the pregnane X (PXR) and sex steroid receptors (Dang et al. 2007; Fery et al. 2009; Mercado-Feliciano and Bigsby 2008; Pacyniak et al. 2007), steroidogenesis (Canton et al. 2006; Canton et al. 2008) and thyroid hormone homeostasis (Lema et al. 2008; Talsness et al. 2008). In addition, effects on neurodevelopment and behavior in mammalian test systems have been observed for these compounds, including
BDE-209 (Viberg et al. 2003; Viberg et al. 2006; Viberg et al. 2008; Viberg 2009a, b); these effects bear similarity with non-dioxin-like PCBs (Eriksson et al. 2006; He et al. 2009). With respect to mechanism of action involvement of metabolites has also been determined for various endpoints such as sex steroid hormone receptors, steroidogenesis (Canton et al. 2006; Canton et al. 2008; He et al. 2008) and regulation and interference with calcium homeostasis in neuronal cells (Alm et al. 2006; Bocio et al. 2003; Dingemans et al. 2008). There is also emerging evidence that exposure to PBDEs in early human life stage can influence endocrine and neurobehavioral development (Sagiv et al. 2015; Harley et al. 2017; Zota et al. 2011).

Recently, it has been argued that risk assessment for PBDEs and non-dioxin PCBs should be combined (Dingemans et al. 2016) Earlier studies suggested that PBDEs can have a dioxin-like mechanism of action, but this is now attributed to contamination of commercial PBDE mixtures with brominated dibenzo-p-dioxins and dibenzofurans (Luthe et al. 2008; Peters et al. 2004; Peters et al. 2006; Van den Berg et al. 2006).

Many lower brominated PBDEs bioaccumulate in the aquatic and human food chain and in the past, bioaccumulation of BDE-209 was assumed to be low due to the large molecular size, extreme hydrophobicity and low bioavailability (Darnerud et al. 2001; Debruyn et al. 2009; Drouillard et al. 2007; Hardy et al. 2009; Huwe et al. 2008b; Kelly et al. 2008; Shaw et al. 2008). However, recent results from both aquatic and terrestrial food web studies demonstrate that BDE-209 bioaccumulates, i.e., bioaccumulation factors and trophic magnification factors above 1 (Chen et al. 2007; Chen et al. 2008; Law et al. 2006; UNEP 2015). Further, environmental levels of BDE-209 can be up to lower ppm levels in abiotic compartments like sediment and house dust (Harrad et al. 2008; Song et al. 2005a; Song et al. 2005b; Xiang et al. 2007; Zegers et al. 2003). Thus, risk assessment of PBDEs is complicated by significant differences among congeners with respect to toxicokinetics, toxicology as well as differences between species, including humans (Birnbaum and Cohen Hubal 2006). Our present study was conducted to determine systemic exposure via blood of BDE-209 in women in four different European countries (the Netherlands, United Kingdom, Norway and Spain) and to study factors influencing those levels, for example differences in lifestyle and fire safety regulations. So far, there are few systematic studies that have focused on systemic exposure of DecaBDE in residents and their households. Blood samples were collected from a group of volunteers, women 20 to 40 years of age. In view of the uncertainties in human exposure to c-DecaBDE, a questionnaire was designed to obtain broad and specific information regarding possible sources of exposure, including lifestyle, use of electrical and electronic devices, diet and country of residence. This questionnaire was compiled based on the information by the
BSEF or EU on the (possible) use of DecaBDE in household products (cf EFSA 2011). The combined information might explain any individual differences in levels of BDE-209 in serum and elicit the possible manner in which human exposure to c-DecaBDE occurs in non-occupational situations. The present report describes the results of a first study of an originally planned 10-year human monitoring program in Europe that would provide the authorities with insight into the long term serum levels of BDE-209 in humans and possible causal relationships with specific exposure scenarios.
Materials and Methods

Blood sampling and data collection

In view of different European dietary and lifestyle factors Norway, Spain and The Netherlands were selected from the Nordic, Mediterranean and West European Regions. In addition, the United Kingdom was included because of more stringent fire safety regulations compared with the rest of the European Union. A total of 145 women, age 20 to 40 years were recruited to participate in the study. This particular population was chosen in order to determine the range in systemic exposure of women around the (theoretical) age of first pregnancy and the initial first sampling round focused on blood of these women. A requirement for the first round was that no breastfeeding had occurred in the six months prior to sampling, so as to avoid a possible depletion of the body burden due to lactation. Later, it turned out that 15 women did not fulfill this criterion and statistical analyses were done with and without this subgroup.

All volunteers completed a questionnaire that contained questions related to lifestyle, work and diet that might have been related to the exposure to c-DecaBDE. Topics were selected based on the knowledge available at the time of the first sampling with respect to use, exposure and occurrence of c-DecaBDE in the environment and products. The questionnaire is included in the supplementary information.

In all four participating countries, the Netherlands (NL), Norway (NO), United Kingdom (UK) and Spain (ES) the approval of a medical ethical committee was obtained as well as informed consents from the women, before sampling of the blood. By December 2007, the sampling in NO and the NL (first and second round) was completed, while those in ES and the UK were finalized in May and June 2008, respectively. The Institute for Risk Assessment Sciences (IRAS) of Utrecht University, the Netherlands, coordinated this study and was also responsible for the collection of two rounds of serum samples from the same volunteers (n=40) in the Netherlands with a six-month interval. The interval analyses were done to collect information on variation in time for non-occupational exposed individuals. Collection of the blood samples in the UK (n=40), Norway (n=40) and Spain (n=25) was done respectively by the Institute of Occupational Medicine, (Edinburgh, UK), Division of Environmental Medicine, Norwegian Institute of Public Health (Oslo, Norway) and Municipal Institute for Medical Research (Barcelona, Spain). The Institute for Environmental Studies (VU University, Amsterdam, the Netherlands) analyzed the serum samples for BDE-209 as described below. A detailed protocol of the collection, storage and handling of the blood samples is included in the supplementary information.
**Sample preparation and chemical analysis**

BDE-209 was extracted from 5 g of serum using an automated solid phase extraction (SPE) technique, followed by an acid silica column clean up step. Extracts were analyzed by gas chromatography with electron capture negative ionization mass spectrometry (GC/ECNI-MS) using a short DB-5 column (15 m, internal diameter 0.2 mm, film thickness 0.01 mm).

Before starting the analysis of samples, multiple blank analyses were performed to ensure a minimal background contamination with BDE-209. For each series of 10 serum samples, several blank analyses (at least three) were performed using calf serum that contained no detectable BDE-209. In each series of 10 samples, one sample was performed in duplicate, demonstrating low variability. Duplicate analyses included samples at very low levels such as 4.1 (s.d. 0.7) and 3.9 (s.d. 0.43) pg/g. 13C-labelled BDE-209 was used as internal standard and serum samples were analyzed at least in duplicate. BDE-209 was quantified using 13C-BDE-209 as internal standard. **The recoveries of the 13C decaBDE internal standard were on average 79% (median 76%), with an relative standard deviation of 32%**.

Concentrations in serum samples reported have all been corrected for the mean blank value in the control chart at the time the series was analyzed.

**The limit of detection (LOD) was calculated as 3 times the standard deviation of the average of blank values. In all sample series, the LOD varied between 3.8 and 4.2 pg/g ww serum.**

The limit of quantification (LOQ) was defined as 10 times the standard deviation of the blanks. The LOQ in all series varied between 12.6 and 13.8 pg/g ww serum.

To determine the lipids in the serum samples, cholesterol and triglycerides were measured enzymatically by the Clinical Chemistry Laboratory at the VU Medical Centre. Total lipids (TL) were calculated based on the formula TL (g/l) = 1.12 × CHOL + 1.33 × TG + 1.48 used by Covaci et al. (2006).

**Data analysis:**

Questionnaire data from all four participating countries were checked intensively, converted to a uniform format where necessary, and merged with serum BDE-209 concentrations into one single dataset. Following the method proposed by Baccarelli and co-workers all samples below the LOD were assigned $1/\sqrt{2}$ of the value of the LOD (approx. 2/3 of LOD) (Baccarelli et al. 2005). BDE-209 levels were strongly skewed and therefore log-transformed for statistical analyses to better satisfy the assumptions of normality. Statistical analyses were performed using concentrations on wet weight basis as in vitro and in vivo studies.
have shown that BDE-209 binds to serum albumin and accumulates primary in plasma and liver
and not in fat tissue (Huwe et al. 2008a; Wang et al. 2014).

Statistical analyses were performed using SAS software (SAS System for Windows version
9.1, SAS Institute, Cary, NC). First, BDE-209 levels were tabulated in different categories of
the potential explanatory variables. Second, BDE-209 levels were log-transformed before
further analysis in view of the skewness of their distribution. Finally, multivariate regression
analyses (PROC REG) were done with all potential explanatory variables summarized in
groups and in tertiles. The log transformed data in the five data sets were not normally
distributed. A Kruskal-Wallis test indicated significant differences. Making a parameter-free
comparison using Anova of ranked data and significant difference between Norway and UK
(<0.000), and Norway and NL 2nd round (p<0.01) data sets was detected. Graphpad Prism 6
was used to calculate the Spearman correlation coefficients between the DecaBDE serum levels
in the first and the second round from The Netherlands and to construct the figure 1.
Results

Serum concentrations of BDE-209.

In Table 1 the mean and median serum concentrations based on either on wet weight (ww) or lipid weight (lw) are presented. Only minor differences were observed in median concentrations on a wet weight basis (all within a factor of 2), with UK having the highest (9.8 pg/g ww) and Norway having the lowest (5.2 pg/g ww). The UK and Dutch second round data sets were both statistically significantly higher than the Norway data set (p<0.000 and p<0.01 resp.). No differences among any other data sets were statistically significant. Based on the difference observed between the mean concentrations and the medians, it can be concluded that for all countries positively skewed distributions occur. A noticeable aspect of the individual serum samples was the extreme variation expressed by very high maximum levels, which could easily be one order of magnitude above the mean concentrations.

Although mean levels of BDE-209 for UK and the Netherlands were almost similar, respectively 12.6 vs. 12.4 pg/g ww, the highest individual levels of BDE-209 were observed from the Netherlands. This was consistent for both rounds of sampling within a six months’ interval in this country. With the limit of detection being around 4 pg/g ww, the highest number of non-detects (38%) was found in samples from Norway (See table 1 for details). Of all the serum samples analyzed, 75% had BDE-209 concentrations above 5 pg/g ww. In deviation from the original study design, 15 women had ceased breastfeeding less than six months prior sampling. When these women were excluded from the study population the overall pattern between countries did not change significantly, with the UK still having the highest mean levels of BDE-209, closely followed by the Netherlands and with Spain and Norway having the lowest levels (data not shown).

Variables explaining individual variability of BDE-209 levels

There were no meaningful correlations between BDE-209 levels and any of the dietary variables (Table 2), use of electronic equipment (Table 3) or use of synthetic clothing (Table 4). The type of diet was examined for its influence on the BDE-209 serum levels (Table 2). Most women (n=128) reported having a normal diet including meat, with only few individuals being vegetarian. The type of diet (normal, vegetarian, vegan) did not show any statistically significant association with BDE-209 levels. However, mean serum concentrations of vegetarians with or without fish consumption (n=7) were somewhat higher than those with a
normal diet (See table 2 for details), but this was not statistically significant. The possible influence of specific dietary components was also taken into account, but no statistically significant contribution of eggs, dairy products, fish or meat could be established (Table 2).

We also investigated if the presence or use of electronic equipment in the household was related to the individual serum concentrations (See table 3 for more details). No correlations were found with any type of equipment, except for coffeemakers and electric water kettles. With respect to the duration electronic equipment was used some positive trends were observed, e.g. hours of use of TV’s, radio’s and CD/DVD/video player, but none of these were statistically significant (Table 3).

The type of materials used in the living and bedroom and in clothes (natural vs. synthetic) was also included as covariate to explain individual differences in serum levels. While the abundance of synthetic material on floors or in furniture did not explain any variability in BDE-209 levels, it was observed that the use of synthetic fabric in clothes appeared to be associated with approximately 25% lower serum levels of BDE-209 compared to natural clothes. For these comparisons the mean serum levels in Norwegian women, being the lowest in the study, were used as a base line (See table 4 for details)

The multivariate regression analyses results are shown in table 5 which contains the variables that have been studied in relation to clothing, electronic appliances, diet and parity. Compared to low exposed Norwegian women, BDE-209 levels were significantly higher in the UK when expressed in pg/g wet weight, and significantly higher in the UK and Spain when expressed in ng/g lipid weight. Use of synthetic clothes was associated with lower BDE-209 levels when expressed in pg/g wet weight but not when expressed in ng/g lipid weight.

For the group of Dutch participants, serum concentrations from two rounds of blood sampling were included in the study to examine individual differences within a six months’ time interval (Table 1). The serum concentrations (wet and lipid weight) were plotted against each other on a logarithmic scale in figure 1. These relationships were only (weakly) statistically significant (p < 0.02) for the wet weight analyses, which indicates that serum levels of DecaBDE within an individual can vary significantly over a six months time period.
Discussion

Results in global context.

Of the three commercial PBDE mixtures only one, c-DecaBDE, is still applied as a flame retardant. This study describes BDE-209 serum levels in women from four different European countries, aged 20-40 years, who were non-occupationally exposed. Samples were collected between mid 2007 and mid 2008. Median serum concentrations were in the same range in all four countries, between 5 and 10 pg/g ww. Nevertheless, subtle differences were observed and the country of residence contributed most to the variation in BDE-209 serum concentrations of all factors examined. The highest concentrations were found in the UK and the Netherlands with mean serum levels around 12.5 pg/g ww or 2.2 to 3.5 ng/g lw. The apparent lack of a real difference in serum levels between both countries is notable, as the UK has stricter national fire safety regulations, comparable with that in some US states (Harrad et al. 2008). UK and Dutch BDE-209 serum levels in the present study are slightly higher than mean or median concentrations in other studies from Europe, e.g. France, Spain, Norway and Sweden (Antignac et al. 2009; Frederiksen et al. 2009; Gomara et al. 2007; Thuresson et al. 2005). Median BDE-209 levels from Spain determined in this study are three fold higher than those reported earlier from this country (Gomara et al. 2007), but still in the very low ng/g lw range. Median serum levels of BDE-209 from Norway were around 1.5 ng/g lw (Thomsen et al. 2008), which is almost one order of magnitude lower than those determined in pooled serum samples from the period 1998 to 2003 (Thomsen et al. 2007), but rather similar as those reported from Sweden (Hites 2004). In a French study, median concentrations of BDE-209 in women were in the same range as in our study, respectively 5.8 versus 1 to 4 ng/g lw. (Antignac et al. 2009). BDE-209 blood levels reported in one study for the U.S. are in the same range as those observed in our study (Schecter et al. 2005). This observation is in contrast with mean blood levels of the lower brominated PBDEs, which can be one order of magnitude higher as those in Europe (Hites 2004; Schecter et al. 2005). This noticeable difference between both continents has been explained by the significantly higher use of commercial PentaBDE and OctaBDE mixtures in North America in the past, while the use of c-DecaBDE is in the same range for both continents (Harrad et al. 2004; Hites 2004). Several studies from Japan and China have reported BDE-209 levels in blood from non-occupational exposed individuals. In Japan the mean or median blood levels were similar to our study, around 1 to 7 ng/g lw. (Inoue et al. 2006; Kawashiro et al. 2008). However, two studies from China reported serum levels that were on average one to two orders of magnitude higher than in our study (Jin et al. 2009; Zhu et al. 2009). One of these studies reported the levels in residents living close to a BDE-209 production
area (Jin et al. 2008) and further investigations are needed to clarify whether these results are indeed representative for general background exposure in China. Mean serum levels of BDE-209 in Australia are in the same range as those observed in our study (Toms et al. 2009b). For a more detailed recent analysis of global temporal and spatial time trends see Law et al. (2014).

Possible DecaBDE sources.

In our study, consumption frequencies of major food groups were included as a possible contributing factor to differences in serum levels of BDE-209, but no association with any type of food was found. A vegetarian diet with or without fish was associated with some higher, but not statistically significant, serum levels in our study (Table 2). However, as our study included only 7 participants on a vegetarian diet and therefore it may well be that this observation is underpowered to detect a statistical association. Possibly, the consumption of eggs could have contributed slightly to the observed levels in our study, but a consistent relationship was not observed (Table 3). Studies from Belgium and the US indicate that BDE-209 can be a common contaminant in eggs (Covaci et al. 2009; Schecter et al. 2006), but our study did not unequivocally confirm this as a major contribution to exposure of our volunteers.

BDE-209 may easily enter the home as an additive frequently used in household electronic equipment, carpets and furniture upholstery. As house dust has been established as a major source for human exposure to PBDEs, including DecaBDE, it may very likely originate from these household articles (Harrad et al. 2008; Jones-Otazo et al. 2005; Sjodin et al. 2008; Stapleton et al. 2005). We did not detect any statistically significant correlation between serum BDE-209 levels and the number of electronic devices nor duration of their use in the households of the participants (Table 3). Serum levels of BDE-209 in our multi-country study are much lower than those of workers in electronic waste dismantling facilities, who have serum levels that are one to two orders of magnitude higher than observed in individuals with only background exposure (Bi et al. 2007; Jakobsson et al. 2002; Thuresson et al. 2005). The contact with electronic equipment for our study population should also be considered as common.

The abundance of textiles in households, e.g. carpets and upholstery, was also used in the analysis of variance for the whole study population, but again no relationship with serum levels could be found in our study. The use of synthetic clothing was included as an exploratory parameter in our study, because the use of flame retardants in their production process is unknown (pers. comm. BSEF). However, no relationship was found between the use of synthetic clothing and DecaBDE blood levels (See table 4). A study from California reported high levels of lower brominated PBDEs in house dust suggesting this as an explanation for the
observed high serum levels in Californian populations. However, it should be noted that a limitation of this study is the lack of direct comparison of individual serum levels and dust from the same household at the time of sampling (Zota et al. 2008).

For the Dutch participants, BDE-209 concentrations were measured in two separate serum samples collected with a six months’ time interval. A low, but statistically significant correlation between both results was observed. A possible explanation for this could be the relative short half-life (~two weeks) found in humans for BDE-209 (Thuresson et al. 2005; Thuresson et al. 2006) where fluctuations in exposure may change body burdens within the six-months period considerably. With such relatively short half-life fluctuations in exposure to specific (unknown) point sources could easily and significantly change body burdens within the six months’ time period.

Implications for human health.

The question can be raised to which extent our observed serum levels of BDE-209 in these female volunteers can be compared with results from experimental toxicity studies. In a review of the toxicology and human health risks of BDE-209, a significant number of toxicity studies were evaluated (Hardy et al. 2009). Three studies with endpoints on liver, spleen, maternal and developmental toxicity were used to determine a reference dose (RfD) of 4 mg/kg-day (Hardy 2002; NTP 1986; Silberberg et al. 2009). This RfD value is similar to the one determined by the National Academy of Sciences (NAS) using the NTP study (NTP 1986). In contrast, the US EPA derived a much lower RfD of 0.007 mg/kg-day (EPA 2008), but its derivation has been the subject of scientific debate (Goodman 2009; Hardy et al. 2009). If these RfD values are used in combination with a recent estimate for background dietary intake of 1–5 ng/kg-day in Europe (EFSA 2011; Frederiksen et al. 2009; Fromme et al. 2009; Knutsen et al. 2008; Lorber 2008), a margin of exposure (MOE) between 1.4 and $800 \times 10^3$ would be calculated. However, this range of MOE does not take into account other routes of exposure e.g. ingestion of house dust, which might be especially relevant for children due to their increased hand-to-mouth activity (Stapleton et al. 2012). Recently, adult exposure to PBDEs, including BDE-209, in the US was summarized and the daily uptake was estimated to be about 2 ng/kg/day for an adult of 70 kg. Daily intake for US children aged 1-5, 6-11 and 12-19 years was estimated significantly higher, respectively 49, 14 and 9 ng/kg/day (Lorber 2008). Even with the higher infant exposure levels the MOE with the RfD still remains above a factor 100. However, if the maximum serum levels reported in our study are considered, this MOE could be approximately a factor of 10 lower.
Our study did not focus on uptake but blood levels of BDE-209, which would be a better proxy for systemic concentrations observed in experimental studies. The only animal study so far which has calculated systemic concentrations of BDE-209 in plasma on lipid weight basis in relation to observed effects, is a subchronic 28-days study with rats (Van der Ven et al. 2008). The hepatic benchmark dose levels (BMDLs) for the most sensitive effect was converted to a plasma level of 1.5 – 3.2 μg/g lw. Assuming comparable levels in serum and plasma on a lipid basis for BDE-209 (Frederiksen et al. 2009), this can be used for a comparison with our study. Using a mean and median serum concentration of 1 – 5 ng/g lw it provides a MOE for these women of approximately 300 to 3000 compared with the benchmark dose derived from the rat study. However, our study also indicates that in this non-occupationally exposed group of women maximum serum concentrations could be up to one order of magnitude above the mean or median serum concentrations in which case the MOE with the BMDL from the rat study would be lower than 100. Such a situation can also occur for occupational exposed individuals who may have BDE-209 blood levels one to two orders of magnitude higher than those found in background exposed populations. Theoretically, a comparison between human blood levels and experimental rat data should provide fewer uncertainties for risk assessment, but it has to be recognized that the bench mark dose derived from this 28-day rat study for BDE-209 has been criticized from a methodological point of view with respect to the computational model used (Hardy et al. 2008; Van der Ven et al. 2008)

With respect to risk assessment and an MOE derived solely for BDE-209 it should also be recognized that human exposure is not confined solely to BDE-209, but also to other PBDE congeners. It has been estimated that BDE-209 exposure in adults may contribute only 20 to 30% of total PBDE exposure (Fromme et al. 2009; Lorber 2008). It can therefore be discussed whether or not risk assessment should be based on single congeners only, such as BDE-209, or on the whole mixture of congeners. A number of experimental studies indicate similar mechanisms of action for different PBDE congeners (Canton et al. 2006; Fery et al. 2009; Pacyniak et al. 2007; Sanders et al. 2005; Viberg et al. 2006), which could provide an argument for using the complete PBDE mixture in the risk assessment proposing additivity as default method. Clearly such a mixture approach would have influence on the risk assessment compared with that based on individual congeners.

Finally, the question can be asked to which extent the observed serum concentrations in the female participants of our study would be related to the levels in their breast milk, as all these women were in childbearing age. Several studies have shown that there is a discrepancy between lipid adjusted BDE-209 concentrations in serum and milk, with breast milk levels
being about one order of magnitude lower (Darnerud et al. 2015; Frederiksen et al. 2009). To
perform a risk assessment of BDE-209 exposure for breastfed infants, concentrations in breast
milk as well as maternal blood should be investigated in future studies. In view of the usually
higher sensitivity of the fetus, neonate and infants to organohalogen compounds, breastfeeding
women should be given priority in future studies with BDE-209 to determine the MOE in
relation to experimental studies.

Conclusions

The present study determined concentrations of BDE-209 in serum from women aged
20-40 years in the Netherlands, UK, Norway and Spain. Samples were collected in 2007-2008
prior to major restrictions on used of commercial DecaBDE. Mean and median serum levels
were within the same range for all four groups, around 5 – 15 pg/g ww or 1 – 10 ng/g lw.
Maximum serum levels were one order of magnitude higher. Lifestyle, type of clothes, diet and
professional activities could not explain the individual differences observed in the total study
group, but country of residence was a significant predictor. In view of the virtual absence of the
role of lifestyle factors, type of household, use of electronic devices, diet and occupational
activities in individual serum levels, it is likely that multifactorial exposures from different
sources may account for individual differences in blood levels. In view of the established role
of house dust for PBDE exposure, future studies should focus on this matrix and blood levels
of residents in the same household. If mean serum levels are compared with the most sensitive
endpoints in an experimental study the MOE for average exposure is still two to three orders of
magnitude. However, this MOE would be one order of magnitude lower for women with the
highest serum level observed in our study.

Acknowledgements: The authors like to thank dr. Timo Hamers from the Department of
Environment and Health, Faculty of Earth and Life Sciences (FALW), Vrije Universiteit in
Amsterdam for assistance with the statistical analysis.
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DecaBDE in serum of European Women
Manuscript version R2 13-06-2017


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Table 1  BDE-209 concentrations in human serum samples (pg/g wet weight and ng/g lipid).

<table>
<thead>
<tr>
<th></th>
<th>The Netherlands Round 1</th>
<th>Norway</th>
<th>Spain</th>
<th>UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>pg/g serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>11.6</td>
<td>12.4</td>
<td>7.3</td>
<td>8.5</td>
</tr>
<tr>
<td>s.d.</td>
<td>15.5</td>
<td>18.5</td>
<td>9.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Median</td>
<td>6.5</td>
<td>8.8</td>
<td>5.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Minimum</td>
<td>&lt; 3.9</td>
<td>&lt; 4.5</td>
<td>&lt; 3.8</td>
<td>&lt; 4.7</td>
</tr>
<tr>
<td>Maximum</td>
<td>79.0</td>
<td>120.0</td>
<td>62.0</td>
<td>19.0</td>
</tr>
<tr>
<td>n</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>n&lt;LOD</td>
<td>4</td>
<td>8</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

| ng/g lipid       |                         |        |       |    |
| Mean             | 2.2                     | 4.6    | 1.3   | 3.5| 3.5 |
| s.d.             | 3.0                     | 7.6    | 1.8   | 1.9| 4.7 |
| Median           | 1.2                     | 2.6    | 0.8   | 3.3| 2.8 |
| Minimum          | < 0.7                   | < 1.5  | < 0.5 | < 0.8| < 0.8|
| Maximum          | 16.0                    | 49.1   | 11.8  | 7.7 | 28.8|
| n                | 40                      | 40     | 40    | 25 | 39  |
| n<LOD            | 4                       | 8      | 15    | 5  | 2   |

1 Non-detects have been included in the calculation of the mean and s.d. as LOD/√2
### Table 2: Mean serum concentrations of BDE-209 in individuals in relation to different types of diets.

<table>
<thead>
<tr>
<th>Type of diet</th>
<th>Mean (sd) pg/g ww</th>
<th>Mean (sd) ng/g lw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet (N=128)</td>
<td>10.0 (10.8)</td>
<td>2.4 (2.5)</td>
</tr>
<tr>
<td>Vegetarian with fish (N=4)</td>
<td>20.2 (25.3)</td>
<td>8.1 (13.8)</td>
</tr>
<tr>
<td>Strictly vegetarian (N=3)</td>
<td>16.8 (7.9)</td>
<td>3.7 (1.0)</td>
</tr>
<tr>
<td>Veganist (N=1)</td>
<td>4.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Other (N=4)</td>
<td>6.8 (1.2)</td>
<td>1.8 (1.0)</td>
</tr>
</tbody>
</table>

**Egg consumption**

<table>
<thead>
<tr>
<th>Egg consumption</th>
<th>Mean (sd) pg/g ww</th>
<th>Mean (sd) ng/g lw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never (N=7)</td>
<td>17.9 (19.9)</td>
<td>7.0 (10.5)</td>
</tr>
<tr>
<td>&lt; 1 / week (N=51)</td>
<td>8.5 (5.6)</td>
<td>2.1 (1.8)</td>
</tr>
<tr>
<td>1-2 / week (N=75)</td>
<td>10.6 (11.7)</td>
<td>2.5 (2.4)</td>
</tr>
<tr>
<td>3-5 / week (N=7)</td>
<td>4.5 (2.3)</td>
<td>1.2 (1.1)</td>
</tr>
<tr>
<td>6-7 / week (N=4)</td>
<td>21.4 (27.3)</td>
<td>4.1 (5.1)</td>
</tr>
</tbody>
</table>

**Food consumption (milk, fish, shellfish, meat, eggs) in tertiles**

<table>
<thead>
<tr>
<th>Food consumption</th>
<th>Mean (sd) pg/g ww</th>
<th>Mean (sd) ng/g lw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (N=41)</td>
<td>11.4 (10.3)</td>
<td>3.1 (4.7)</td>
</tr>
<tr>
<td>Medium (N=55)</td>
<td>9.1 (9.3)</td>
<td>1.9 (1.8)</td>
</tr>
<tr>
<td>High (N=47)</td>
<td>10.5 (13.7)</td>
<td>2.9 (3.0)</td>
</tr>
</tbody>
</table>

N = Number of samples within each category
Table 3 Mean serum concentrations of BDE-209 in individuals in relation to the use of electronic equipment

<table>
<thead>
<tr>
<th></th>
<th>Mean (sd) pg/g ww</th>
<th>Mean (sd) ng/g lw</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of TV’s</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (N=6)</td>
<td>12.0 (11.9)</td>
<td>2.8 (2.1)</td>
</tr>
<tr>
<td>1 (N=90)</td>
<td>9.5 (9.7)</td>
<td>2.5 (3.6)</td>
</tr>
<tr>
<td>2 (N=27)</td>
<td>10.7 (14.4)</td>
<td>2.6 (3.1)</td>
</tr>
<tr>
<td>3 (N=13)</td>
<td>10.3 (4.9)</td>
<td>2.9 (2.2)</td>
</tr>
<tr>
<td>4 or more (N=8)</td>
<td>14.4 (19.9)</td>
<td>2.5 (2.5)</td>
</tr>
<tr>
<td><strong>Number of coffee makers &amp; water kettles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (N=20)</td>
<td>15.5 (16.7)</td>
<td>5.4 (6.2)</td>
</tr>
<tr>
<td>1 (N=77)</td>
<td>8.8 (5.5)</td>
<td>2.3 (2.0)</td>
</tr>
<tr>
<td>2 or more (N=47)</td>
<td>10.2 (14.3)</td>
<td>1.8 (2.5)</td>
</tr>
<tr>
<td><strong>Number of dishwashers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (N=74)</td>
<td>12.0 (13.3)</td>
<td>2.9 (4.2)</td>
</tr>
<tr>
<td>1 (N=70)</td>
<td>8.3 (7.9)</td>
<td>2.2 (1.9)</td>
</tr>
<tr>
<td><strong>Hours of use TV and computers in tertiles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (less than 120 hours; N=42)</td>
<td>11.4 (13.1)</td>
<td>3.1 (4.9)</td>
</tr>
<tr>
<td>Medium (120-210 hours; N=46)</td>
<td>10.6 (11.8)</td>
<td>2.5 (2.7)</td>
</tr>
<tr>
<td>High (&gt; 210 hours; N=47)</td>
<td>9.3 (9.6)</td>
<td>2.1 (1.9)</td>
</tr>
<tr>
<td><strong>Hours of use radio and CD/DVD/Video in tertiles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (less than 25 hours; N=47)</td>
<td>8.3 (4.6)</td>
<td>2.1 (1.8)</td>
</tr>
<tr>
<td>Medium (25-50 hours; N=45)</td>
<td>9.9 (10.3)</td>
<td>2.4 (2.8)</td>
</tr>
<tr>
<td>High (&gt; 50 hours; N=47)</td>
<td>12.2 (15.6)</td>
<td>3.1 (4.7)</td>
</tr>
<tr>
<td><strong>Number of other electrical apparatuses (microwave, coffee maker, electrical water kettle, blender, oven, dishwasher, washer, dryer, vacuum cleaner)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (1-5; N=39)</td>
<td>12.2 (13.2)</td>
<td>3.3 (4.8)</td>
</tr>
<tr>
<td>Medium (6-7; N=58)</td>
<td>9.4 (6.2)</td>
<td>2.4 (2.2)</td>
</tr>
<tr>
<td>High (8-11; N=48)</td>
<td>9.6 (13.7)</td>
<td>2.1 (2.7)</td>
</tr>
</tbody>
</table>

N = Number of samples within each category
Table 4  Mean serum concentrations of BDE in individuals in relation to the use of synthetic and natural clothes

<table>
<thead>
<tr>
<th>Use of synthetic blouses</th>
<th>Mean (sd) pg/g ww</th>
<th>Mean (sd) ng/g lw</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 100% natural materials (N=29)</td>
<td>15.1 (14.8)</td>
<td>4.1 (5.6)</td>
</tr>
<tr>
<td>2 (N=42)</td>
<td>7.5 (4.9)</td>
<td>2.0 (1.9)</td>
</tr>
<tr>
<td>3 (N=51)</td>
<td>10.5 (13.4)</td>
<td>2.5 (2.9)</td>
</tr>
<tr>
<td>4 (N=18)</td>
<td>8.6 (4.8)</td>
<td>1.9 (1.4)</td>
</tr>
<tr>
<td>5: 100% synthetics (N=4)</td>
<td>5.4 (2.2)</td>
<td>1.5 (1.0)</td>
</tr>
</tbody>
</table>

Use of synthetic clothes (underwear, pants, blouses, socks) in tertiles

| Low (sum score 4-7; N=38) | 13.7 (13.4) | 3.4 (5.0) |
| Medium (sum score 8-9; N=45) | 8.0 (5.5) | 2.1 (1.9) |
| High (sum score 10-17; N=61) | 9.6 (12.3) | 2.3 (2.7) |

N = Number of samples within each category
### Table 5: Multivariate analysis of geometric means of BDE-209 concentrations in A) pg/g wet weight and B) ng/g lipid weight

<table>
<thead>
<tr>
<th>A</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline = geometric mean serum BDE-209 pg/g wet weight in Norwegian women</td>
<td>7.7</td>
</tr>
<tr>
<td>Ratio to baseline</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>1.42</td>
</tr>
<tr>
<td>UK</td>
<td><strong>1.74</strong></td>
</tr>
<tr>
<td>Spain</td>
<td>1.45</td>
</tr>
<tr>
<td>Milk, fish, shellfish meat &amp; eggs, 2nd tertile</td>
<td>0.97</td>
</tr>
<tr>
<td>Milk, fish, shellfish meat &amp; eggs, 3d tertile</td>
<td>1.03</td>
</tr>
<tr>
<td>Television &amp; computer use, 2nd tertile</td>
<td>1.07</td>
</tr>
<tr>
<td>Television &amp; computer use, 3rd tertile</td>
<td>0.91</td>
</tr>
<tr>
<td>Radio, CD, DVD &amp; video use, 2nd tertile</td>
<td>0.93</td>
</tr>
<tr>
<td>Radio, CD, DVD &amp; video use, 3rd tertile</td>
<td>1.01</td>
</tr>
<tr>
<td>N of other electric appliances, 2nd tertile</td>
<td>0.93</td>
</tr>
<tr>
<td>N of other electric appliances, 3d tertile</td>
<td>0.93</td>
</tr>
<tr>
<td>Use of synthetic clothes, 2nd tertile</td>
<td><strong>0.72</strong></td>
</tr>
<tr>
<td>Use of synthetic clothes, 3rd tertile</td>
<td><strong>0.73</strong></td>
</tr>
<tr>
<td>Having children, yes vs. no</td>
<td>0.96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline = geometric mean serum BDE-209 ng/g lipid weight in unexposed Norwegian women</td>
<td>1.39</td>
</tr>
<tr>
<td>Ratio to baseline</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>1.41</td>
</tr>
<tr>
<td>UK</td>
<td><strong>2.47</strong></td>
</tr>
<tr>
<td>Spain</td>
<td><strong>3.68</strong></td>
</tr>
<tr>
<td>Milk, fish, shellfish meat &amp; eggs, 2nd tertile</td>
<td>0.89</td>
</tr>
<tr>
<td>Milk, fish, shellfish meat &amp; eggs, 3d tertile</td>
<td>1.14</td>
</tr>
<tr>
<td>Television &amp; computer use, 2nd tertile</td>
<td>1.02</td>
</tr>
<tr>
<td>Television &amp; computer use, 3rd tertile</td>
<td>0.90</td>
</tr>
<tr>
<td>Radio, CD, DVD &amp; video use, 2nd tertile</td>
<td>0.96</td>
</tr>
<tr>
<td>Radio, CD, DVD &amp; video use, 3rd tertile</td>
<td>1.03</td>
</tr>
<tr>
<td>N of other electric appliances, 2nd tertile</td>
<td>0.85</td>
</tr>
<tr>
<td>N of other electric appliances, 3d tertile</td>
<td>0.88</td>
</tr>
<tr>
<td>Use of synthetic clothes, 2nd tertile</td>
<td><strong>0.85</strong></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Use of synthetic clothes, 3\textsuperscript{4}terile</td>
<td>0.76</td>
</tr>
<tr>
<td>Having children, yes vs. no</td>
<td>0.81</td>
</tr>
</tbody>
</table>

**Bold:** statistically significant at $p<0.05$
Figure 1. Possible associations between serum concentrations of BDE-209 (upper panel: pg/g wet weight; lower panel: ng/g lipid weight) measured twice, 6 months apart, in Dutch participants.
Supplementary information:

Decabromodiphenylether Monitoring in Human Blood Project

Instructions for Whole Blood Sampling and Sample Transport

A sampling guide for partner laboratories in Spain, UK, Norway and the Netherlands

June 2007

by

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Institute for Environmental Studies (IVM)

VU University Amsterdam

and

Drs. R. Fernández Cantón

(Director of Sampling)

Institute for Risk Assessment Sciences (IRAS)

Utrecht University
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Decabromodiphenylether (also called BDE209 or decaBDE) is a brominated flame retardant used to reduce the flammability of various products in the EU and in countries around the world. It is currently the subject of a risk assessment in the EU. As part of the input for the risk assessment, the Bromine Science Environment Form, BSEF is sponsoring a 10-year human biomonitoring project in which the levels of BDE209 will be followed in human subjects. As part of the project, blood samples will be collected in 2007 from donors in Spain, the UK, Norway and the Netherlands (40 blood samples per country). The samples will be analysed to determine the concentration of BDE209. The blood samples will be collected by health care professionals at partner laboratories in each of these countries.

The concentrations of BDE209 currently being found in humans are very low, and are often reported in the picogram\(^1\) per gram range. The analysis of this compound in human blood therefore requires a high level of laboratory expertise. However, sampling of the blood is equally as important to successful determination of BDE209 levels in humans as the analytical part of the determination process. Particularly because of the ubiquitous nature of BDE209, (which is present at high levels in dust particles for instance), special care must be taken during the sampling process in order not to introduce BDE209 from outside the body into the sample during the sampling and sample handling process. This means working very “cleanly” during all steps of the process, starting with clean, dust-free sampling equipment. One particle of dust in a blood sample can significantly affect the concentration of BDE209 that we measure in a blood sample.

On the other hand, care must also be taken to prevent the BDE209 in the sample from degrading before it can be analysed. BDE209 will degrade if exposed to sunlight. Therefore, samples must be kept in the dark. In addition the samples should be preserved by freezing. These and other important aspects are covered in the sampling protocol provided in this document.

In addition, this document will provide you with useful information concerning the transport of the blood samples to the laboratory of the Institute for Environmental Studies (IVM) at the VU University in Amsterdam, the Netherlands. The IVM will be performing the chemical analysis of all samples taken. The role of the Institute for Risk Assessment Sciences (IRAS) at Utrecht University in this project is to coordinate the blood sampling and compile the epidemiological data. IVM and IRAS cooperate closely on the project. Drs. Rocio Fernández Cantón will be coordinating the sampling; Dr. Heather Leslie (IVM) will be coordinating the chemical analysis.

### Safety Considerations

This project involves working with human blood and risks of infection are always present. These must be minimized by strictly observing professional safety rules for working with human blood. The analytical methods used for the detection of BDE209 are not automated. In the interest of maximizing laboratory safety, the blood donors to be selected by partner laboratories should be generally healthy with low risk of infectious diseases (such as HIV or hepatitis B).

\(^1\) One picogram is 0.000000000001 g.
Blood Sampling Protocol

Selection of donors
The preferred target group is healthy women of (20-40 yr) without occupational exposure and with non-smoking habits. Subjects working in offices where some exposure may occur will not be excluded. Occupational exposures during production and further handling of BDE209 will not be accepted; however factors as lifestyle and living environment are likely to play a key role in the possible uptake of BDE209 by humans. These influencing parameters will be studied as accurately as possible by using detailed questionnaires about lifestyle and living environment for the human volunteers.

Each partner laboratory must supply blood samples of the prescribed volume from 40 different individual donors. (Keep in mind the possibility that the required volume may not be possible to draw from some of the donors for whatever reason. Therefore, it is advisable to keep a few qualified donors in reserve in order to be sure that a total of 40 blood samples will be available).

Privacy of Donors
Before starting volunteer donors will be fully informed of the sampling procedure by the scientists at the partner laboratories involved in the study, who will answer any questions they might have.

The identity of the individuals will remain confidential; identities will be recorded by sample code given by the project coordinator. This information will be kept strictly private and will under no circumstances be divulged without permission of the volunteer. No names of individuals will be associated with the analytical results. Volunteers are entitled to be informed of the BDE209 concentration in their own blood by requesting the information from the project coordinator, but the information will not be divulged to anyone else.

Volunteers will have always the right to stop the procedure without giving any reason. Blood samples will be collected by highly trained and qualified medical staffs who are accredited for blood collection.

Sample vial description
Samples will be taken in the BD Vacuvials® with brown caps supplied by IRAS. The volume of these evacuated sterile blood collection tube for whole blood haematology determination, is 10 ml. The tubes are made of medical grade PET and feature a BD Hemogard™ safety closure. The dimensions are 16 x 100 mm; K₂EDTA spray is applied to the inside of the tube.

Vacuvials for sampling
Prior to sampling, each partner laboratory will receive from IRAS by post 6 x 40 vacuvials of this description (plus extras). IRAS will provide also with labels with a sample code; the participating laboratory will weigh the vacuvials before and after sampling.

Sampling procedure and safety considerations
Blood sampling should be performed under safety conditions in order to not get in direct contact with the blood. It should be treated as (potentially) hazardous material. The blood sampling will take an estimated 10-15 min per donor.

In order to decrease possible variability on triglyceride content in blood, samples should be taken in the morning or in the afternoon at least 4h after the last meal (before lunch or dinner). Information record about sampling will include what time/date the person last ate.
Number of samples per donor

Per donor, a minimum of 6 vacuvials should be filled and labelled. Donors for whom less than 6 full vials are obtained, cannot be counted towards the 40 samples necessary for the study.

Immediately after sampling / Serum fraction isolation

Immediately upon drawing the blood into a vacuvial, invert (do not shake) the vacuvial 10 times in order to mix the EDTA coated on the inside walls of the vacuvial with the sample. Do NOT expose the blood samples to sunlight, as this will degrade the analyte.

For serum fraction analysis, the cells must be separated from the serum. The blood samples will be taken in Vacuvials with brown caps. Blood clots on its own, allowing serum to be separated from cells.

Location of all blood sample work = biological laboratory, fume hood nearest the centrifuge and door.

Centrifugation. To obtain serum, place collection tube upright in rack and allow blood to clot at room temperature (30 minutes). If the centrifugation cannot be performed directly after 30 minutes, the samples should be put in the refrigerator until centrifugation (e.g. until the following day).

When clot has formed, centrifuge the tube (swing bucket centrifuge) for 10 minutes at 1800 g (Karin Vroonhof, VUMC, pers.comm.) with caps on in a centrifuge that is well-balanced (using extra tubes filled with water if necessary). BD vacuvials should not break at g-forces up to 10000 g (pers. comm. with supplier).

Note: Do not use a centrifuge in which sediment or sewage sludge samples have just been centrifuged as this may be contaminated with DBDE.

NB: Prolonged centrifugation may cause hemolysis and must be avoided. Allow the centrifuge to come to a complete stop and remove tubes carefully without disturbing the red cells at the bottom. If cells become disturbed, repeated centrifugation can be attempted but usually cell lysis occurs. A signal that cell lysis has occurred is a pink colour in the serum, which normally is straw colour. Do not use serum contaminated with cells or intracellular contents.

Do not freeze samples before centrifuging as cellular contents will leak into the serum phase.

Removing serum from cell fraction.

· The specimen should only come into contact with carefully cleaned and solvent-rinsed glassware. Toluene should be used for rinsing since DBDE dissolves the best in this solvent.
· Do not allow dust to fall in the sample as dust contains a high amount of DBDE.
· Work in the fume hood in designated area of biological laboratory.
· Keep samples out of the light (DBDE degrades in the light).
· Keep workspace clean and sterile.

Holding the tube upright, carefully remove the stopper. The supernatant is the serum fraction. Transfer serum from this top layer to a properly labeled sample container (glass tube) with a disposable glass pipette (first rinse tubes x 3 with toluene, dry on air and be sure that all the toluene is gone before use). Do not disturb the cell layer or allow any cells into the pipette. Do not pour the serum or invert the tube. Do not allow the serum to sit in the centrifuge for more
than 30 minutes before transferring the serum to new sample vials. If a thin "buffy coat" layer
between cells and serum forms, allow this to stay with the cells, to avoid disturbing the cell
layer. NB. The weight of the serum fraction and the cellular fraction per person must be
known in order to do mass balances. Should a few cells be accidentally pipeted out with the
serum, weigh this for calculating the total g of serum, but do not add any cells to the serum
sample for analysis.

Information to record during sampling

The following sample information should be collected and stored in an excel file (see appendix)
which is to be sent by email to IVM. Each sample code is thereby linked to relevant information
for analysis of the samples.

- Sample code
- Weight of empty vacuvial (g)
- Weight of full vacuvial (g)
- Weight of blood sample (g)
- Order of the draw (i.e. tube sampled 1st, 2nd, 3rd, 4th, 5th or 6th etc in the series)
- Donor identification code
- Sampling date
- Sampling time point in 24 hour clock / time the person last ate
- Sample location
- Weight of empty serum glass vacuvial (g)
- Weight of full serum glass vacuvial (g)
- Identification code of the qualified person(s) who drew and handled the blood during
  sampling
- Storage conditions (temperature, dark, upright, etc.)
- Any other comments

Timing of Sampling

All blood samples for this study should be taken in 2007. In connection with laboratory
analysis planning for such a large set of samples, it is imperative to inform the Sampling
Coordinator of the date of sample availability as soon as this is known.

Expected dates:
- Spain    July - September 2007
- UK       August - September 2007
- Norway   August - September 2007
- NL       July – August 2007

Serum Sample Storage and Sending Instructions

Serum samples should be kept in the dark at all times and stored at –20 °C in an upright
position. Do not store samples in the same freezer (or other area) as samples that contain high
BDE209 concentrations (such as river or marine sediments or sewage sludge samples) to
prevent cross contamination.
Sending Instructions

Samples of blood should be sent frozen by express courier (e.g. DHL, see www.dhl.com) on dry ice to ensure the samples to not thaw during transport.

IATA (International Air Transport Association) regulates transport of blood by carrier.

http://www.iata.org/nr/rddonlyres/88834d9f-8ea2-42a0-8da6-bed8cd2e744/0/sampleissg7thed.pdf

Include in the package:
- one set of unused sampling devices identical to the ones used for blood sampling,
- at least 2 unused BD vacuials for the analysis of background BDE209 contamination
- at least 2 unused serum glass vacuials/tubes for the analysis of background BDE209 contamination.

Sending on dry ice requires special packaging instructions. Consult your local courier for advice. We include the following sending instructions as an extra guide. Use the appropriate stickers (available from DHL).

- A proforma invoice should be included describing the contents as “biological specimens for diagnostic analysis”
- Package the samples in leak-proof inner packages. This package should be placed in a second larger package, which is then put in the outer box with address label for sending. Use cushioning plastic bubble paper where necessary.
- Follow the “IATA Packing Instructions 650”
- Place a sticker on the box reading: “DRY ICE”
- Place a sticker on the box reading: “Diagnostic Specimens” and “for scientific use only”
- Describe the contents on the courier form
- Mark the package with the code "UN3373" and "contents packed in compliance with IATA Packing Instructions 650"
- Include a letter inside the box.
- Seal the box(es) well with tape.
- Note the length, width and height of the outer package (in cm) plus the weight (in kg).

Please courier samples and materials to this address:

Attention: Mr. B. van der Horst, Head of Laboratory
Institute for Environmental Studies (IVM)
Vrije Universiteit
De Boelelaan 1087
1081 HV Amsterdam
The Netherlands
Tel. +31-20-598 95 97
Tel. Secretariat +31-20-598 95 39
Fax. ++31-20-598 95 53
E-mail: bert.vanderhorst@ivm.falw.vu.nl

NB Please notify IVM of the date of arrival of your samples and send your sample list electronically by email:

To: Heather.leslie@ivm.vu.nl
Cc: bert.van.der.horst@ivm.vu.nl and r.fernandezcanton@iras.uu.nl
Contact Information of Project Sampling and Analysis Coordinators

Coordinator of Sampling and Epidemiological Study:

Drs. Rocío Fernández Cantón
Cellular and Molecular Toxicology
Institute for Risk Assessment Sciences (IRAS)
Cellular and Molecular Toxicology
P.O. Box 80.176
3508 TD Utrecht
The Netherlands
Tel. +31-30- 253 36 31
Tel. Secretariat: +31-30- 253 54 00
Fax. +31-30- 253 5077
Email: r.fernandezcanton@iras.uu.nl

Coordinator of Laboratory Analyses:

Dr. Heather A. Leslie
Institute for Environmental Studies (IVM)
Vrije Universiteit
De Boelelaan 1087
1081 HV Amsterdam
The Netherlands
Tel. +31-20-598 95 97
Tel. Secretariat: +31-20-598 95 55
Fax. ++31-20-598 95 53
E-mail: heather.leslie@ivm.falw.vu.nl
# International Survey of Decabromodiphenylether (DecaBDE) in Human Blood

## Questionnaire for participants

**CONFIDENTIAL**

<table>
<thead>
<tr>
<th>Section 1: personal information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
</tr>
<tr>
<td><strong>Address</strong></td>
</tr>
<tr>
<td><strong>Phone number:</strong></td>
</tr>
<tr>
<td><strong>e-mail:</strong></td>
</tr>
<tr>
<td><strong>Interviewer ID</strong></td>
</tr>
<tr>
<td><strong>Place of interview</strong></td>
</tr>
<tr>
<td><strong>Participant ID</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Has informed consent been signed?</strong></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Is the participant currently a non-smoker?</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
Section 2: screening

1a. Please indicate the country of your birth

1b. How many years have you lived in your current home? ...... Years

2. When did you move into your current home (mm/yyyy) ........../...........

3a. Do you have children, if yes, how many? Yes ☐ No ☐

(Heavy) (go to Section 3, Q4)

3b. For each of your child can you please indicate the date of birth and whether (and for how long) you breastfed each child?

<table>
<thead>
<tr>
<th>Date of Birth (dd/mm/yyyy)</th>
<th>Breastfed? (including expression of breastmilk)</th>
<th>Duration of breastfeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Section 3: health aspects

4. Please specify your height (cm) and weight (kg)

Height ............cm Weight ............ kg

............Feet/inches ............Stone/pounds

5. Please specify in the list below what would best describe your usual diet

☐ normal diet

☐ vegetarian but with fish

☐ strictly vegetarian

☐ vegan (go to 8)

☐ other, specify .................................................................

6a. Please indicate how often, on average, you consumed the following food products in the past month. Please, put one ‘X’ in every line.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food Group</td>
<td>never</td>
<td>less than once a week</td>
<td>on 1 or 2 days per week</td>
<td>on 3 to 5 days per week</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------</td>
<td>-----------------------</td>
<td>-------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Milk and milk products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seafood other than fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat and poultry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes:........................................................................................................................................

6b. Name the 3 fish or seafood products you consume most often, starting at 1) with the most often consumed kind. (e.g. tuna, cod, haddock, salmon, shrimp, mussels, sole, lobster)

1) ........................................................
2) ........................................................
3) ........................................................
### Section 4: Household characteristics

7. How many people live in your household?  

8. Please indicate in the table below the presence, number and average duration of use for the following electrical goods in your household.  
   ‘use’ means that the equipment is turned on and used, either by you or a household member.

For example, if there are 2 television sets in your house, and one is used daily for 2 hours per day on average, and the other for half an hour per day, and your DVD player is used once per week for 3 hours, the table should look like this:

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>number present in household</th>
<th>hours of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Television</td>
<td>X</td>
<td></td>
<td>2</td>
<td>2.5/day/week/month</td>
</tr>
<tr>
<td>video/CD/DVD player/recorder</td>
<td>X</td>
<td></td>
<td>1</td>
<td>3/day/week/month</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>yes</th>
<th>no</th>
<th>number present in household</th>
<th>Hours of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Television</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>video/CD/DVD player/recorder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>computer/labtop/notebook</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>radio/tuner/amplifier</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>micro wave</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kettle/coffee maker</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>food processor/blender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric oven</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dish washer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washing machine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tumble dryer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fridge/freezer?? Or do we assume that they have these??</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equipment</td>
<td>Usage Time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>--------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vacuum cleaner</td>
<td>...../day/week/month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric shower??</td>
<td>...../day/week/month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair driers/hair straighteners /curlers/ razors ??</td>
<td>...../day/week/month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric heaters??</td>
<td>...../day/week/month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>...../day/week/month</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In case more kinds of equipment fall into one category, usage time should be added up*
9a. Please indicate the presence of the following items in your living room, and specify the type of material, e.g. wool, synthetic, cotton, vinyl, etc. Please, also give the age in years.

<table>
<thead>
<tr>
<th>Item</th>
<th>Present</th>
<th>Material</th>
<th>Age (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Textile wall to wall floor covering</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rug(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upholstered easy chair(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upholstered sofa(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9b. State the presence of the following materials in your bedroom, and specify the type of material, e.g. wool, down, synthetic, etc.

<table>
<thead>
<tr>
<th>Item</th>
<th>Present</th>
<th>Material</th>
<th>Age (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Textile wall to wall floor covering</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rug(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duvet, blanket(s) / comforter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mattress(es)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pillow(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9c. How often, on average, is your living room and bedroom floor vacuumed?

<table>
<thead>
<tr>
<th>Surface</th>
<th>Frequency</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Living room floor</td>
<td></td>
<td>(times)</td>
</tr>
<tr>
<td>Bedroom floor</td>
<td></td>
<td>(times)</td>
</tr>
</tbody>
</table>
10. Please, specify the type of clothing material you choose to wear for clothes worn in direct contact with your skin.

<table>
<thead>
<tr>
<th>Mostly Natural</th>
<th>Mostly Synthetic</th>
<th>Mostly mixed materials</th>
<th>No preference</th>
<th>Don’t know</th>
</tr>
</thead>
</table>

underwear
trousers, skirts
blouses, dresses
socks, stockings

11. On average, how many times a day do you wash your hands? .................... times
### Section 5: Occupational environment

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>12a. Do you currently work?</td>
<td>Yes □ No □</td>
</tr>
<tr>
<td>12b. Do you mainly work from home?</td>
<td>Yes □ No □</td>
</tr>
<tr>
<td>12c. How many hours do you work per day?</td>
<td>................................. hours</td>
</tr>
<tr>
<td>12d. How many days do you work per week?</td>
<td>................................. days</td>
</tr>
<tr>
<td>12e. Which percentage of your worktime do you work with electrical equipment?</td>
<td>................................. percent</td>
</tr>
<tr>
<td>12f. Please, give a short description of your job, and the materials you work with:</td>
<td>.................................</td>
</tr>
<tr>
<td>13. Do use a car regularly (either as a driver or passenger)?</td>
<td>Yes □ No □</td>
</tr>
<tr>
<td>If yes:</td>
<td>.................................</td>
</tr>
<tr>
<td>On average per day, how much time do you spend in your car?</td>
<td>................................. hours</td>
</tr>
<tr>
<td>14. Please indicate how often you travel by plane?</td>
<td></td>
</tr>
<tr>
<td>□ Never</td>
<td></td>
</tr>
<tr>
<td>□ Ones per year</td>
<td></td>
</tr>
<tr>
<td>□ 1 – 4 times per year</td>
<td></td>
</tr>
<tr>
<td>□ 5 – 19 times per year</td>
<td></td>
</tr>
<tr>
<td>□ 20 times per year or more</td>
<td></td>
</tr>
</tbody>
</table>
• BDE-209 was measured in female serum from Norway, United Kingdom, The Netherlands and Spain.
• Median serum concentrations were highest in the Netherlands and United Kingdom.
• No consistent relationships between diets, household, clothes, electronics and occupation were observed.
• Compared with toxicity data the margin of exposure for these women ranges between one and three orders of magnitude for BDE-209.
• The small differences between serum levels from different countries do not influence the risk assessment
To determine possible effects of lifestyle, diet, housing and professional activities on differences in individual levels of decabromodiphenyl ether (BDE-209) in serum of women, 20 to 40 years of age, in the Netherlands, the United Kingdom, Norway and Spain.

BDE-209 was measured in serum of 145 female volunteers with no known occupational exposure from Norway, United Kingdom, The Netherlands and Spain. Blood levels of BDE-209 in a subgroup of 40 Dutch women were determined twice at a six months' interval. An extensive questionnaire was used to obtain detailed information about lifestyle factors that might contribute to BDE-209 exposure. Serum levels were used to determine margin of systemic exposure compared with a 28d rat toxicity study.

Median BDE-209 serum concentrations were highest in the Netherlands and United Kingdom, respectively 8.8 and 9.3 pg/g w.w. or 2.6 and 2.8 ng/g lipid. Median levels in Spain and Norway were lower, respectively 7.4 and 5.2 pg/g w.w. or 3.3 and 0.8 ng/g lipid. Maximum levels in individual women were higher by one order of magnitude than the mean or median. The country of residence was the only variable significantly associated with BDE-209 levels; we found that the differences between countries could not be explained by any of the investigated exposure variables, and that these did not explain differences between individuals either. No consistent relationships were determined between diets, household, clothes, number and duration of use of electronics and occupational activities for the whole study group.

We could not identify which of the multiple sources of exposure accounted for individual differences in blood levels. Although small differences in mean BDE-209 serum levels were recognized between countries, these differences are unlikely to cause a differential result with respect to risk assessment.
Serum levels of decabromodiphenyl ether (BDE-209) in women from different European countries and possible relationships with lifestyle and diet.

Martin van den Berg1#, Remko Houba1, Heather A. Leslie2, Rocio F. Canton1, Cathrine Thomsen3, Georg Becher3, Mar Alvarez-Pedrerol4, Jordi Sunyer Deu4, Markus Steiner5, Martie van Tongeren6, Bert Brunekreef4, Jacob de Boer2

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Disclosure

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Abstract
To determine possible effects of lifestyle, diet, housing and professional activities on differences in individual levels of decabromodiphenyl ether (BDE-209) in serum of women, 20 to 40 years of age, in the Netherlands, the United Kingdom, Norway and Spain. BDE-209 was measured in serum of 145 female volunteers with no known occupational exposure from Norway, United Kingdom, The Netherlands and Spain. Blood levels of BDE-209 in a subgroup of 40 Dutch women were determined twice at a six months’ interval. An extensive questionnaire was used to obtain detailed information about lifestyle factors that might contribute to BDE-209 exposure. Serum levels were used to determine margin of systemic exposure compared with a 28d rat toxicity study. Median BDE-209 serum concentrations were highest in the Netherlands and United Kingdom, respectively 8.8 and 9.3 pg/g w.w. or 2.6 and 2.8 ng/g lipid. Median levels in Spain and Norway were lower, respectively 7.4 and 5.2 pg/g w.w. or 3.3 and 0.8 ng/g lipid. Maximum levels in individual women were higher by one order of magnitude than the mean or median. The country of residence was the only variable significantly associated with BDE-209 levels; we found that the differences between countries could not be explained by any of the investigated exposure variables, and that these did not explain differences between individuals either. No consistent relationships were determined between diets, household, clothes, number and duration of use of electronics and occupational activities for the whole study group. We could not identify which of the multiple sources of exposure accounted for individual differences in blood levels. Although small differences in mean BDE-209 serum levels were recognized between countries, these differences are unlikely to cause a differential result with respect to risk assessment.

KEY WORDS: DecaBDE, BDE-209, PBDEs, serum, lifestyle, diet, household, risk assessment
Introduction

In modern life, flame retardants have become part of efforts to protect society against injuries, death and economic damage due to fires. A wide range of chemicals have been developed as flame retardants, from which the polybrominated diphenyl ethers (PBDEs) have been commonly used for many decades. Various PBDE products have been in production and use for several decades, commercial PentaBDE, OctaBDE and DecaBDE mixtures comprising diphenyl ethers of varying bromination degree. Some PBDEs have physico-chemical properties that promote environmental persistence and accumulation in food chains and humans (Darnerud et al. 2001; de Wit 2002; Frederiksen et al. 2009; Tanabe et al. 2008; Zhu et al. 2009). Certain lower brominated congeners have been reported to have long half-lives in humans, wild life and experimental animals, indicating a distinct role of bromine atoms in reducing metabolic rates of these compounds (Geyer et al. 2004; Gill et al. 2004; Toms et al. 2009b). As a result, levels of tetra- to heptabrominated BDEs in environmental biota and humans can equal those for PCBs in many industrialized countries (Haraguchi et al. 2009; Hites 2004; Schecter et al. 2005).

In the European Union, the commercial Penta- and OctaBDE mixtures were taken off the market in 2005 because of adverse effects observed in experimental animals (Directive 2003/11/EC). In North America these commercial formulations were voluntarily withdrawn from the market by industry in 2004 (BSEF 2009). Further, since May 2009, tetra- to heptabrominated diphenyl ethers have been listed in the UN Stockholm Convention on Persistent Organic Pollutants (http://www.pops.int). In contrast, commercial DecaBDE is still use as flame retardant for plastics and textiles when our study was done (BSEF 2009; Harrad et al. 2008; Public Health England 2009; 2013). The commercial mixture consists primarily of the fully brominated diphenyl ether (BDE-209) and smaller amounts of nonabrominated BDE (0.3-21.8%) and octabrominated BDE (0-0.04%). Although its use in electrical and electronic equipment had been banned in the EU in 2008 (BSEF 2009) and the production and sales of commercial DecaBDE (c-DecaBDE) has been phased out in North America (BSEF 2016), there is ongoing human exposure from dust in indoor environments (Harrad et al. 2006, Law et al., 2014) and from diet, particularly seafood (Shaw et al. 2009). Presently c-DecaBDE is still under consideration for restriction and elimination under EU’s REACH regulation and UN’s Stockholm Convention, respectively (http://chm.pops.int/Default.aspx?tabid=5171). In order to evaluate the result of these regulations in reducing human exposure, it is of great importance to establish good biomonitoring data for DecaBDE in particular.
Because of the adverse properties and effects of the lower brominated BDEs, commercial Penta- and OctaBDE have been phased out in European countries in the early 2000s and were globally banned by the UN Stockholm Convention in 2009. As a result, the increasing temporal trends of levels of tetra- to heptabrominated BDEs in human blood and milk have leveled off in the late 1990s in Europe and have declined since, this (Fängström et al. 2008; Thomsen et al. 2007), but is less distinct for North America (Law et al. 2014). Furthermore, an upward trend for decaBDE has been observed in the same time period (Law et al. 2014). It is well established that levels of lower brominated PBDEs in humans may vary strongly among geographical regions, e.g., mean total PBDE levels in North America are about one order of magnitude higher than in Europe (Frederiksen et al. 2009; Fromme et al. 2016; Hites 2004). In non-occupational situations the relative contribution of decabromodiphenyl ether (BDE-209) in humans constitute a variable part of the total amount of PBDE body burden (Antignac et al. 2009; Frederiksen et al. 2009; Gomara et al. 2007; Thuresson et al. 2005). The geographical differences might partly be explained by different regional fire safety regulations and use of decaBDE containing flame-retardants in consumer products (Harrad et al. 2008). Also, within countries individual differences in PBDE levels can be quite substantial and may easily exceed more than one order of magnitude in human blood and milk (Frederiksen et al. 2009; Hites 2004).

At present, the cause for this strong variability in human levels is unclear, but lifestyle factors have been suggested as a contributing factor. Although, food is an important pathway for human exposure to PBDEs (Fromme et al. 2009; Meng et al. 2007; Schecter et al. 2006; Schecter et al. 2008; Voorspoels et al. 2007; Wu et al. 2007), the ingestion of house dust is also considered to be an important exposure pathway, especially for BDE-209 (Harrad et al. 2006; Harrad et al. 2008; Jones-Otazo et al. 2005; Sjodin et al. 2008; Toms et al. 2009a).

Many in vivo toxicokinetic and toxicological studies with PBDEs with different degrees of bromination were done over the last decade to support risk assessment for humans and wildlife (Birnbaum and Cohen Hubal 2006; Darnerud 2003; Staskal et al. 2008). As a result, multiple toxic and biological effects have been identified (Darnerud 2003; He et al. 2009; Kuriyama et al. 2005), which show similarities between the lower brominated PBDEs and decaBDE (Dingemans et al. 2016). These include interactions with the pregnane X (PXR) and sex steroid receptors (Dang et al. 2007; Fery et al. 2009; Mercado-Feliciano and Bigsby 2008; Pacyniak et al. 2007), steroidogenesis (Canton et al. 2006; Canton et al. 2008) and thyroid hormone homeostasis (Lema et al. 2008; Talsness et al. 2008). In addition, effects on neurodevelopment and behavior in mammalian test systems have been observed for these compounds, including
BDE-209 (Viberg et al. 2003; Viberg et al. 2006; Viberg et al. 2008; Viberg 2009a, b); these
effects bear similarity with non-dioxin-like PCBs (Eriksson et al. 2006; He et al. 2009). With
respect to mechanism of action involvement of metabolites has also been determined for various
endpoints such as sex steroid hormone receptors, steroidogenesis (Canton et al. 2006; Canton
et al. 2008; He et al. 2008) and regulation and interference with calcium homeostasis in
neuronal cells (Alm et al. 2006; Bocio et al. 2003; Dingemans et al. 2008). There is also
emerging evidence that exposure to PBDEs in early human life stage can influence endocrine
and neurobehavioral development (Sagiv et al. 2015; Harley et al. 2017; Zota et al. 2011).
Recently, it has been argued that risk assessment for PBDEs and non-dioxin PCBs should be
combined (Dingemans et al. 2016) Earlier studies suggested that PBDEs can have a dioxin-like
mechanism of action, but this is now attributed to contamination of commercial PBDE mixtures
with brominated dibenzo-p-dioxins and dibenzofurans (Luthe et al. 2008; Peters et al. 2004;
Peters et al. 2006; Van den Berg et al. 2006).

Many lower brominated PBDEs bioaccumulate in the aquatic and human food chain and in
the past, bioaccumulation of BDE-209 was assumed to be low due to the large molecular size,
extreme hydrophobicity and low bioavailability (Darnerud et al. 2001; Debruyn et al. 2009;
Drouillard et al. 2007; Hardy et al. 2009; Huwe et al. 2008b; Kelly et al. 2008; Shaw et al.
2008). However, recent results from both aquatic and terrestrial food web studies demonstrate
that BDE-209 bioaccumulates, i.e., bioaccumulation factors and trophic magnification factors
above 1 (Chen et al. 2007; Chen et al. 2008; Law et al. 2006; UNEP 2015). Further,
environmental levels of BDE-209 can be up to lower ppm levels in abiotic compartments like
sediment and house dust (Harrad et al. 2008; Song et al. 2005a; Song et al. 2005b; Xiang et al.
2007; Zegers et al. 2003). Thus, risk assessment of PBDEs is complicated by significant
differences among congeners with respect to toxicokinetics, toxicology as well as differences
between species, including humans (Birnbaum and Cohen Hubal 2006). Our present study
was conducted to determine systemic exposure via blood of BDE-209 in women in four
different European countries (the Netherlands, United Kingdom, Norway and Spain) and to
study factors influencing those levels, for example differences in life style and fire safety
regulations. So far, there are few systematic studies that have focused on systemic exposure of
DecaBDE in residents and their households. Blood samples were collected from a group of
volunteers, women 20 to 40 years of age. In view of the uncertainties in human exposure to c-
DecaBDE, a questionnaire was designed to obtain broad and specific information regarding
possible sources of exposure, including lifestyle, use of electrical and electronic devices, diet
and country of residence. This questionnaire was compiled based on the information by the
1 BSEF or EU on the (possible) use of DecaBDE in household products (cf EFSA 2011). The
2 combined information might explain any individual differences in levels of BDE-209 in serum
3 and elicit the possible manner in which human exposure to c-DecaBDE occurs in non-
4 occupational situations. The present report describes the results of a first study of an originally
5 planned 10-year human monitoring program in Europe that would provide the authorities with
6 insight into the long term serum levels of BDE-209 in humans and possible causal relationships
7 with specific exposure scenarios.
Materials and Methods

Blood sampling and data collection

In view of different European dietary and lifestyle factors Norway, Spain and The Netherlands were selected from the Nordic, Mediterranean and West European Regions. In addition, the United Kingdom was included because of more stringent fire safety regulations compared with the rest of the European Union. A total of 145 women, age 20 to 40 years were recruited to participate in the study. This particular population was chosen in order to determine the range in systemic exposure of women around the (theoretical) age of first pregnancy and the initial first sampling round focused on blood of these women. A requirement for the first round was that no breastfeeding had occurred in the six months prior to sampling, so as to avoid a possible depletion of the body burden due to lactation. Later, it turned out that 15 women did not fulfill this criterion and statistical analyses were done with and without this subgroup.

All volunteers completed a questionnaire that contained questions related to lifestyle, work and diet that might have been related to the exposure to c-DecaBDE. Topics were selected based on the knowledge available at the time of the first sampling with respect to use, exposure and occurrence of c-DecaBDE in the environment and products. The questionnaire is included in the supplementary information.

In all four participating countries, the Netherlands (NL), Norway (NO), United Kingdom (UK) and Spain (ES) the approval of a medical ethical committee was obtained as well as informed consents from the women, before sampling of the blood. By December 2007, the sampling in NO and the NL (first and second round) was completed, while those in ES and the UK were finalized in May and June 2008, respectively. The Institute for Risk Assessment Sciences (IRAS) of Utrecht University, the Netherlands, coordinated this study and was also responsible for the collection of two rounds of serum samples from the same volunteers (n=40) in the Netherlands with a six-month interval. The interval analyses were done to collect information on variation in time for non-occupational exposed individuals. Collection of the blood samples in the UK (n=40), Norway (n=40) and Spain (n=25) was done respectively by the Institute of Occupational Medicine, (Edinburgh, UK), Division of Environmental Medicine, Norwegian Institute of Public Health (Oslo, Norway) and Municipal Institute for Medical Research (Barcelona, Spain). The Institute for Environmental Studies (VU University, Amsterdam, the Netherlands) analyzed the serum samples for BDE-209 as described below. A detailed protocol of the collection, storage and handling of the blood samples is included in the supplementary information.
Sample preparation and chemical analysis

BDE-209 was extracted from 5 g of serum using an automated solid phase extraction (SPE) technique, followed by an acid silica column clean up step. Extracts were analyzed by gas chromatography with electron capture negative ionization mass spectrometry (GC/ECNI-MS) using a short DB-5 column (15 m, internal diameter 0.2 mm, film thickness 0.01 mm).

Before starting the analysis of samples, multiple blank analyses were performed to ensure a minimal background contamination with BDE-209. For each series of 10 serum samples, several blank analyses (at least three) were performed using calf serum that contained no detectable BDE-209. In each series of 10 samples, one sample was performed in duplicate, demonstrating low variability. Duplicate analyses included samples at very low levels such as 4.1 (s.d. 0.7) and 3.9 (s.d. 0.43) pg/g. 13C-labelled BDE-209 was used as internal standard and serum samples were analyzed at least in duplicate. BDE-209 was quantified using 13C-BDE-209 as internal standard. The recoveries of the 13C decaBDE internal standard were on average 79% (median 76%), with an relative standard deviation of 32%.

Concentrations in serum samples reported have all been corrected for the mean blank value in the control chart at the time the series was analyzed. The limit of detection (LOD) was calculated as 3 times the standard deviation of the average of blank values. In all sample series, the LOD varied between 3.8 and 4.2 pg/g ww serum. The limit of quantification (LOQ) was defined as 10 times the standard deviation of the blanks. The LOQ in all series varied between 12.6 and 13.8 pg/g ww serum.

To determine the lipids in the serum samples, cholesterol and triglycerides were measured enzymatically by the Clinical Chemistry Laboratory at the VU Medical Centre. Total lipids (TL) were calculated based on the formula TL (g/l) = 1.12 × CHOL + 1.33 × TG + 1.48 used by Covaci et al. (2006).

Data analysis:

Questionnaire data from all four participating countries were checked intensively, converted to a uniform format where necessary, and merged with serum BDE-209 concentrations into one single dataset. Following the method proposed by Baccarelli and co-workers all samples below the LOD were assigned 1/√2 of the value of the LOD (approx. 2/3 of LOD) (Baccarelli et al. 2005). BDE-209 levels were strongly skewed and therefore log-transformed for statistical analyses to better satisfy the assumptions of normality. Statistical analyses were performed using concentrations on wet weight basis as in vitro and in vivo studies
have shown that BDE-209 binds to serum albumin and accumulates primarily in plasma and liver and not in fat tissue (Huwe et al. 2008a; Wang et al. 2014).

Statistical analyses were performed using SAS software (SAS System for Windows version 9.1, SAS Institute, Cary, NC). First, BDE-209 levels were tabulated in different categories of the potential explanatory variables. Second, BDE-209 levels were log-transformed before further analysis in view of the skewness of their distribution. Finally, multivariate regression analyses (PROC REG) were done with all potential explanatory variables summarized in groups and in tertiles. The log transformed data in the five data sets were not normally distributed. A Kruskal-Wallis test indicated significant differences. Making a parameter-free comparison using Anova of ranked data and significant difference between Norway and UK (<0.000), and Norway and NL 2nd round (p<0.01) data sets was detected. Graphpad Prism 6 was used to calculate the Spearman correlation coefficients between the DecaBDE serum levels in the first and the second round from The Netherlands and to construct the figure 1.
Results

Serum concentrations of BDE-209.

In Table 1 the mean and median serum concentrations based on either on wet weight (ww) or lipid weight (lw) are presented. Only minor differences were observed in median concentrations on a wet weight basis (all within a factor of 2), with UK having the highest (9.8 pg/g ww) and Norway having the lowest (5.2 pg/g ww). The UK and Dutch second round data sets were both statistically significantly higher than the Norway data set (p<0.000 and p<0.01 resp.). No differences among any other data sets were statistically significant. Based on the difference observed between the mean concentrations and the medians, it can be concluded that for all countries positively skewed distributions occur. A noticeable aspect of the individual serum samples was the extreme variation expressed by very high maximum levels, which could easily be one order of magnitude above the mean concentrations.

Although mean levels of BDE-209 for UK and the Netherlands were almost similar, respectively 12.6 vs. 12.4 pg/g ww, the highest individual levels of BDE-209 were observed from the Netherlands. This was consistent for both rounds of sampling within a six months’ interval in this country. With the limit of detection being around 4 pg/g ww, the highest number of non-detects (38%) was found in samples from Norway (See table 1 for details). Of all the serum samples analyzed, 75% had BDE-209 concentrations above 5 pg/g ww. In deviation from the original study design, 15 women had ceased breastfeeding less than six months prior sampling. When these women were excluded from the study population the overall pattern between countries did not change significantly, with the UK still having the highest mean levels of BDE-209, closely followed by the Netherlands and with Spain and Norway having the lowest levels (data not shown).

Variables explaining individual variability of BDE-209 levels

There were no meaningful correlations between BDE-209 levels and any of the dietary variables (Table 2), use of electronic equipment (Table 3) or use of synthetic clothing (Table 4). The type of diet was examined for its influence on the BDE-209 serum levels (Table 2). Most women (n=128) reported having a normal diet including meat, with only few individuals being vegetarian. The type of diet (normal, vegetarian, vegan) did not show any statistically significant association with BDE-209 levels. However, mean serum concentrations of vegetarians with or without fish consumption (n=7) were somewhat higher than those with a
normal diet (See table 2 for details), but this was not statistically significant. The possible influence of specific dietary components was also taken into account, but no statistically significant contribution of eggs, dairy products, fish or meat could be established (Table 2).

We also investigated if the presence or use of electronic equipment in the household was related to the individual serum concentrations (See table 3 for more details). No correlations were found with any type of equipment, except for coffeemakers and electric water kettles. With respect to the duration electronic equipment was used some positive trends were observed, e.g. hours of use of TV’s, radio’s and CD/DVD/video player, but none of these were statistically significant (Table 3).

The type of materials used in the living and bedroom and in clothes (natural vs. synthetic) was also included as covariate to explain individual differences in serum levels. While the abundance of synthetic material on floors or in furniture did not explain any variability in BDE-209 levels, it was observed that the use of synthetic fabric in clothes appeared to be associated with approximately 25% lower serum levels of BDE-209 compared to natural clothes. For these comparisons the mean serum levels in Norwegian women, being the lowest in the study, were used as a base line (See table 4 for details)

The multivariate regression analyses results are shown in table 5 which contains the variables that have been studied in relation to clothing, electronic appliances, diet and parity. Compared to low exposed Norwegian women, BDE-209 levels were significantly higher in the UK when expressed in pg/g wet weight, and significantly higher in the UK and Spain when expressed in ng/g lipid weight. Use of synthetic clothes was associated with lower BDE-209 levels when expressed in pg/g wet weight but not when expressed in ng/g lipid weight.

For the group of Dutch participants, serum concentrations from two rounds of blood sampling were included in the study to examine individual differences within a six months’ time interval (Table 1). The serum concentrations (wet and lipid weight) were plotted against each other on a logarithmic scale in figure 1. These relationships were only (weakly) statistically significant (p < 0.02) for the wet weight analyses, which indicates that serum levels of DecaBDE within an individual can vary significantly over a six months time period.
Discussion

Results in global context.

Of the three commercial PBDE mixtures only one, c-DecaBDE, is still applied as a flame retardant. This study describes BDE-209 serum levels in women from four different European countries, aged 20-40 years, who were non-occupationally exposed. Samples were collected between mid 2007 and mid 2008. Median serum concentrations were in the same range in all four countries, between 5 and 10 pg/g ww. Nevertheless, subtle differences were observed and the country of residence contributed most to the variation in BDE-209 serum concentrations of all factors examined. The highest concentrations were found in the UK and the Netherlands with mean serum levels around 12.5 pg/g ww or 2.2 to 3.5 ng/g lw. The apparent lack of a real difference in serum levels between both countries is notable, as the UK has stricter national fire safety regulations, comparable with that in some US states (Harrad et al. 2008). UK and Dutch BDE-209 serum levels in the present study are slightly higher than mean or median concentrations in other studies from Europe, e.g. France, Spain, Norway and Sweden (Antignac et al. 2009; Frederiksen et al. 2009; Gomara et al. 2007; Thuresson et al. 2005). Median BDE-209 levels from Spain determined in this study are three fold higher than those reported earlier from this country (Gomara et al. 2007), but still in the very low ng/g lw range. Median serum levels of BDE-209 from Norway were around 1.5 ng/g lw (Thomsen et al. 2008), which is almost one order of magnitude lower than those determined in pooled serum samples from the period 1998 to 2003 (Thomsen et al. 2007), but rather similar as those reported from Sweden (Hites 2004). In a French study, median concentrations of BDE-209 in women were in the same range as in our study, respectively 5.8 versus 1 to 4 ng/g lw. (Antignac et al. 2009). BDE-209 blood levels reported in one study for the U.S. are in the same range as those observed in our study (Schecter et al. 2005). This observation is in contrast with mean blood levels of the lower brominated PBDEs, which can be one order of magnitude higher as those in Europe (Hites 2004; Schecter et al. 2005). This noticeable difference between both continents has been explained by the significantly higher use of commercial PentaBDE and OctaBDE mixtures in North America in the past, while the use of c-DecaBDE is in the same range for both continents (Harrad et al. 2004; Hites 2004). Several studies from Japan and China have reported BDE-209 levels in blood from non-occupational exposed individuals. In Japan the mean or median blood levels were similar to our study, around 1 to 7 ng/g lw. (Inoue et al. 2006; Kawashiro et al. 2008). However, two studies from China reported serum levels that were on average one to two orders of magnitude higher than in our study (Jin et al. 2009; Zhu et al. 2009). One of these studies reported the levels in residents living close to a BDE-209 production
area (Jin et al. 2008) and further investigations are needed to clarify whether these results are indeed representative for general background exposure in China. Mean serum levels of BDE-209 in Australia are in the same range as those observed in our study (Toms et al. 2009b). For a more detailed recent analysis of global temporal and spatial time trends see Law et al. (2014).

Possible DecaBDE sources.

In our study, consumption frequencies of major food groups were included as a possible contributing factor to differences in serum levels of BDE-209, but no association with any type of food was found. A vegetarian diet with or without fish was associated with some higher, but not statistically significant, serum levels in our study (Table 2). However, as our study included only 7 participants on a vegetarian diet and therefore it may well be that this observation is underpowered to detect a statistical association. Possibly, the consumption of eggs could have contributed slightly to the observed levels in our study, but a consistent relationship was not observed (Table 3). Studies from Belgium and the US indicate that BDE-209 can be a common contaminant in eggs (Covaci et al. 2009; Schecter et al. 2006), but our study did not unequivocally confirm this as a major contribution to exposure of our volunteers.

BDE-209 may easily enter the home as an additive frequently used in household electronic equipment, carpets and furniture upholstery. As house dust has been established as a major source for human exposure to PBDEs, including DecaBDE, it may very likely originate from these household articles (Harrad et al. 2008; Jones-Otazo et al. 2005; Sjodin et al. 2008; Stapleton et al. 2005). We did not detect any statistically significant correlation between serum BDE-209 levels and the number of electronic devices nor duration of their use in the households of the participants (Table 3). Serum levels of BDE-209 in our multi-country study are much lower than those of workers in electronic waste dismantling facilities, who have serum levels that are one to two orders of magnitude higher than observed in individuals with only background exposure (Bi et al. 2007; Jakobsson et al. 2002; Thuresson et al. 2005). The contact with electronic equipment for our study population should also be considered as common.

The abundance of textiles in households, e.g. carpets and upholstery, was also used in the analysis of variance for the whole study population, but again no relationship with serum levels could be found in our study. The use of synthetic clothing was included as an exploratory parameter in our study, because the use of flame retardants in their production process is unknown (pers. comm. BSEF). However, no relationship was found between the use of synthetic clothing and DecaBDE blood levels (See table 4). A study from California reported high levels of lower brominated PBDEs in house dust suggesting this as an explanation for the
observed high serum levels in Californian populations. However, it should be noted that a limitation of this study is the lack of direct comparison of individual serum levels and dust from the same household at the time of sampling (Zota et al. 2008).

For the Dutch participants, BDE-209 concentrations were measured in two separate serum samples collected with a six months’ time interval. A low, but statistically significant correlation between both results was observed. A possible explanation for this could be the relative short half-life (~two weeks) found in humans for BDE-209 (Thuresson et al. 2005; Thuresson et al. 2006) where fluctuations in exposure may change body burdens within the six-months period considerably. With such relatively short half-life fluctuations in exposure to specific (unknown) point sources could easily and significantly change body burdens within the six months’ time period.

Implications for human health.

The question can be raised to which extent our observed serum levels of BDE-209 in these female volunteers can be compared with results from experimental toxicity studies. In a review of the toxicology and human health risks of BDE-209, a significant number of toxicity studies were evaluated (Hardy et al. 2009). Three studies with endpoints on liver, spleen, maternal and developmental toxicity were used to determine a reference dose (RfD) of 4 mg/kg-day (Hardy 2002; NTP 1986; Silberberg et al. 2009). This RfD value is similar to the one determined by the National Academy of Sciences (NAS) using the NTP study (NTP 1986). In contrast, the US EPA derived a much lower RfD of 0.007 mg/kg-day (EPA 2008), but its derivation has been the subject of scientific debate (Goodman 2009; Hardy et al. 2009). If these RfD values are used in combination with a recent estimate for background dietary intake of 1 – 5 ng/kg-day in Europe (EFSA 2011; Frederiksen et al. 2009; Fromme et al. 2009; Knutsen et al. 2008; Lorber 2008), a margin of exposure (MOE) between 1.4 and $800 \times 10^3$ would be calculated. However, this range of MOE does not take into account other routes of exposure e.g. ingestion of house dust, which might be especially relevant for children due to their increased hand-to-mouth activity (Stapleton et al. 2012). Recently, adult exposure to PBDEs, including BDE-209, in the US was summarized and the daily uptake was estimated to be about 2 ng/kg/day for an adult of 70 kg. Daily intake for US children aged 1-5, 6-11 and 12-19 years was estimated significantly higher, respectively 49, 14 and 9 ng/kg/day (Lorber 2008). Even with the higher infant exposure levels the MOE with the RfD still remains above a factor 100. However, if the maximum serum levels reported in our study are considered, this MOE could be approximately a factor of 10 lower.
Our study did not focus on uptake but blood levels of BDE-209, which would be a better proxy for systemic concentrations observed in experimental studies. The only animal study so far which has calculated systemic concentrations of BDE-209 in plasma on lipid weight basis in relation to observed effects, is a subchronic 28-days study with rats (Van der Ven et al. 2008). The hepatic benchmark dose levels (BMDLs) for the most sensitive effect was converted to a plasma level of 1.5 – 3.2 μg/g lw. Assuming comparable levels in serum and plasma on a lipid basis for BDE-209 (Frederiksen et al. 2009), this can be used for a comparison with our study. Using a mean and median serum concentration of 1 – 5 ng/g lw it provides a MOE for these women of approximately 300 to 3000 compared with the benchmark dose derived from the rat study. However, our study also indicates that in this non-occupationally exposed group of women maximum serum concentrations could be up to one order of magnitude above the mean or median serum concentrations in which case the MOE with the BMDL from the rat study would be lower than 100. Such a situation can also occur for occupational exposed individuals who may have BDE-209 blood levels one to two orders of magnitude higher than those found in background exposed populations. Theoretically, a comparison between human blood levels and experimental rat data should provide fewer uncertainties for risk assessment, but it has to be recognized that the bench mark dose derived from this 28-day rat study for BDE-209 has been criticized from a methodological point of view with respect to the computational model used (Hardy et al. 2008; Van der Ven et al. 2008)

With respect to risk assessment and an MOE derived solely for BDE-209 it should also be recognized that human exposure is not confined solely to BDE-209, but also to other PBDE congeners. It has been estimated that BDE-209 exposure in adults may contribute only 20 to 30% of total PBDE exposure (Fromme et al. 2009; Lorber 2008). It can therefore be discussed whether or not risk assessment should be based on single congeners only, such as BDE-209, or on the whole mixture of congeners. A number of experimental studies indicate similar mechanisms of action for different PBDE congeners (Canton et al. 2006; Fery et al. 2009; Pacyniak et al. 2007; Sanders et al. 2005; Viberg et al. 2006), which could provide an argument for using the complete PBDE mixture in the risk assessment proposing additivity as default method. Clearly such a mixture approach would have influence on the risk assessment compared with that based on individual congeners.

Finally, the question can be asked to which extent the observed serum concentrations in the female participants of our study would be related to the levels in their breast milk, as all these women were in childbearing age. Several studies have shown that there is a discrepancy between lipid adjusted BDE-209 concentrations in serum and milk, with breast milk levels
being about one order of magnitude lower (Darnerud et al. 2015; Frederiksen et al. 2009). To perform a risk assessment of BDE-209 exposure for breastfed infants, concentrations in breast milk as well as maternal blood should be investigated in future studies. In view of the usually higher sensitivity of the fetus, neonate and infants to organohalogen compounds, breastfeeding women should be given priority in future studies with BDE-209 to determine the MOE in relation to experimental studies.

**Conclusions**

The present study determined concentrations of BDE-209 in serum from women aged 20-40 years in the Netherlands, UK, Norway and Spain. Samples were collected in 2007-2008 prior to major restrictions on used of commercial DecaBDE. Mean and median serum levels were within the same range for all four groups, around 5 – 15 pg/g ww or 1 – 10 ng/g lw. Maximum serum levels were one order of magnitude higher. Lifestyle, type of clothes, diet and professional activities could not explain the individual differences observed in the total study group, but country of residence was a significant predictor. In view of the virtual absence of the role of lifestyle factors, type of household, use of electronic devices, diet and occupational activities in individual serum levels, it is likely that multifactorial exposures from different sources may account for individual differences in blood levels. In view of the established role of house dust for PBDE exposure, future studies should focus on this matrix and blood levels of residents in the same household. If mean serum levels are compared with the most sensitive endpoints in an experimental study the MOE for average exposure is still two to three orders of magnitude. However, this MOE would be one order of magnitude lower for women with the highest serum level observed in our study.

**Acknowledgements:** The authors like to thank dr. Timo Hamers from the Department of Environment and Health, Faculty of Earth and Life Sciences (FALW), Vrije Universiteit in Amsterdam for assistance with the statistical analysis.
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DecaBDE in serum of European Women
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Table 1  BDE-209 concentrations in human serum samples (pg/g wet weight and ng/g lipid).

<table>
<thead>
<tr>
<th></th>
<th>The Netherlands</th>
<th>Norway</th>
<th>Spain</th>
<th>UK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Round 1</td>
<td>Round 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pg/g serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>11.6</td>
<td>12.4</td>
<td>7.3</td>
<td>8.5</td>
</tr>
<tr>
<td>s.d.</td>
<td>15.5</td>
<td>18.5</td>
<td>9.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Median</td>
<td>6.5</td>
<td>8.8</td>
<td>5.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Minimum</td>
<td>&lt; 3.9</td>
<td>&lt; 4.5</td>
<td>&lt; 3.8</td>
<td>&lt; 4.7</td>
</tr>
<tr>
<td>Maximum</td>
<td>79.0</td>
<td>120.0</td>
<td>62.0</td>
<td>19.0</td>
</tr>
<tr>
<td>n</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>n&lt;LOD</td>
<td>4</td>
<td>8</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>ng/g lipid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.2</td>
<td>4.6</td>
<td>1.3</td>
<td>3.5</td>
</tr>
<tr>
<td>s.d.</td>
<td>3.0</td>
<td>7.6</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Median</td>
<td>1.2</td>
<td>2.6</td>
<td>0.8</td>
<td>3.3</td>
</tr>
<tr>
<td>Minimum</td>
<td>&lt; 0.7</td>
<td>&lt; 1.5</td>
<td>&lt; 0.5</td>
<td>&lt; 0.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>16.0</td>
<td>49.1</td>
<td>11.8</td>
<td>7.7</td>
</tr>
<tr>
<td>n</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>n&lt;LOD</td>
<td>4</td>
<td>8</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

1 Non-detects have been included in the calculation of the mean and s.d. as LOD/√2
Table 2  Mean serum concentrations of BDE-209 in individuals in relation to different types of diets.

<table>
<thead>
<tr>
<th>Type of diet</th>
<th>Mean (sd) pg/g ww</th>
<th>Mean (sd) ng/g lw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet (N=128)</td>
<td>10.0 (10.8)</td>
<td>2.4 (2.5)</td>
</tr>
<tr>
<td>Vegetarian with fish (N=4)</td>
<td>20.2 (25.3)</td>
<td>8.1 (13.8)</td>
</tr>
<tr>
<td>Strictly vegetarian (N=3)</td>
<td>16.8 (7.9)</td>
<td>3.7 (1.0)</td>
</tr>
<tr>
<td>Veganist (N=1)</td>
<td>4.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Other (N=4)</td>
<td>6.8 (1.2)</td>
<td>1.8 (1.0)</td>
</tr>
</tbody>
</table>

Egg consumption

<table>
<thead>
<tr>
<th>Egg consumption</th>
<th>Mean (sd) pg/g ww</th>
<th>Mean (sd) ng/g lw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never (N=7)</td>
<td>17.9 (19.9)</td>
<td>7.0 (10.5)</td>
</tr>
<tr>
<td>&lt; 1 / week (N=51)</td>
<td>8.5 (5.6)</td>
<td>2.1 (1.8)</td>
</tr>
<tr>
<td>1-2 / week (N=75)</td>
<td>10.6 (11.7)</td>
<td>2.5 (2.4)</td>
</tr>
<tr>
<td>3-5 / week (N=7)</td>
<td>4.5 (2.3)</td>
<td>1.2 (1.1)</td>
</tr>
<tr>
<td>6-7 / week (N=4)</td>
<td>21.4 (27.3)</td>
<td>4.1 (5.1)</td>
</tr>
</tbody>
</table>

Food consumption (milk, fish, shellfish, meat, eggs) in tertiles

<table>
<thead>
<tr>
<th>Food consumption</th>
<th>Mean (sd) pg/g ww</th>
<th>Mean (sd) ng/g lw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (N=41)</td>
<td>11.4 (10.3)</td>
<td>3.1 (4.7)</td>
</tr>
<tr>
<td>Medium (N=55)</td>
<td>9.1 (9.3)</td>
<td>1.9 (1.8)</td>
</tr>
<tr>
<td>High (N=47)</td>
<td>10.5 (13.7)</td>
<td>2.9 (3.0)</td>
</tr>
</tbody>
</table>

N = Number of samples within each category
Table 3  Mean serum concentrations of BDE-209 in individuals in relation to the use of electronic equipment

<table>
<thead>
<tr>
<th></th>
<th>Mean (sd) pg/g ww</th>
<th>Mean (sd) ng/g lw</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of TV’s</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (N=6)</td>
<td>12.0 (11.9)</td>
<td>2.8 (2.1)</td>
</tr>
<tr>
<td>1 (N=90)</td>
<td>9.5 (9.7)</td>
<td>2.5 (3.6)</td>
</tr>
<tr>
<td>2 (N=27)</td>
<td>10.7 (14.4)</td>
<td>2.6 (3.1)</td>
</tr>
<tr>
<td>3 (N=13)</td>
<td>10.3 (4.9)</td>
<td>2.9 (2.2)</td>
</tr>
<tr>
<td>4 or more (N=8)</td>
<td>14.4 (19.9)</td>
<td>2.5 (2.5)</td>
</tr>
</tbody>
</table>

| **Number of coffee makers & water kettles** |                  |                  |
| 0 (N=20)                            | 15.5 (16.7)      | 5.4 (6.2)        |
| 1 (N=77)                            | 8.8 (5.5)        | 2.3 (2.0)        |
| 2 or more (N=47)                    | 10.2 (14.3)      | 1.8 (2.5)        |

| **Number of dishwashers**          |                  |                  |
| 0 (N=74)                            | 12.0 (13.3)      | 2.9 (4.2)        |
| 1 (N=70)                            | 8.3 (7.9)        | 2.2 (1.9)        |

| **Hours of use TV and computers in tertiles** |                  |                  |
| Low (less than 120 hours; N=42)           | 11.4 (13.1)      | 3.1 (4.9)        |
| Medium (120-210 hours; N=46)              | 10.6 (11.8)      | 2.5 (2.7)        |
| High (> 210 hours; N=47)                  | 9.3 (9.6)        | 2.1 (1.9)        |

| **Hours of use radio and CD/DVD/Video in tertiles** |                  |                  |
| Low (less than 25 hours; N=47)              | 8.3 (4.6)        | 2.1 (1.8)        |
| Medium (25-50 hours; N=45)                  | 9.9 (10.3)       | 2.4 (2.8)        |
| High (> 50 hours; N=47)                     | 12.2 (15.6)      | 3.1 (4.7)        |

| **Number of other electrical apparatuses (microwave, coffee maker, electrical water kettle, blender, oven, dishwasher, washer, dryer, vacuum cleaner)** |                  |                  |
| Low (1-5; N=39)                           | 12.2 (13.2)      | 3.3 (4.8)        |
| Medium (6-7; N=58)                        | 9.4 (6.2)        | 2.4 (2.2)        |
| High (8-11; N=48)                         | 9.6 (13.7)       | 2.1 (2.7)        |

N  = Number of samples within each category
### Table 4  Mean serum concentrations of BDE in individuals in relation to the use of synthetic and natural clothes

<table>
<thead>
<tr>
<th>Use of synthetic blouses</th>
<th>Mean (sd)</th>
<th>Mean (sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pg/g ww</td>
<td>ng/g lw</td>
<td></td>
</tr>
<tr>
<td>1: 100% natural materials (N=29)</td>
<td>15.1 (14.8)</td>
<td>4.1 (5.6)</td>
</tr>
<tr>
<td>2 (N=42)</td>
<td>7.5 (4.9)</td>
<td>2.0 (1.9)</td>
</tr>
<tr>
<td>3 (N=51)</td>
<td>10.5 (13.4)</td>
<td>2.5 (2.9)</td>
</tr>
<tr>
<td>4 (N=18)</td>
<td>8.6 (4.8)</td>
<td>1.9 (1.4)</td>
</tr>
<tr>
<td>5: 100% synthetics (N=4)</td>
<td>5.4 (2.2)</td>
<td>1.5 (1.0)</td>
</tr>
</tbody>
</table>

**Use of synthetic clothes (underwear, pants, blouses, socks) in tertiles**

<table>
<thead>
<tr>
<th>Low (sum score 4-7; N=38)</th>
<th>Medium (sum score 8-9; N=45)</th>
<th>High (sum score 10-17; N=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.7 (13.4)</td>
<td>8.0 (5.5)</td>
<td>9.6 (12.3)</td>
</tr>
<tr>
<td>3.4 (5.0)</td>
<td>2.1 (1.9)</td>
<td>2.3 (2.7)</td>
</tr>
</tbody>
</table>

N = Number of samples within each category
Table 5  Multivariate analysis of geometric means of BDE-209 concentrations in A) pg/g wet weight and B) ng/g lipid weight

<table>
<thead>
<tr>
<th>A</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline = geometric mean serum BDE-209 pg/g wet weight in Norwegian women</td>
<td>7.7</td>
</tr>
<tr>
<td>Netherlands</td>
<td>1.42</td>
</tr>
<tr>
<td>UK</td>
<td><strong>1.74</strong></td>
</tr>
<tr>
<td>Spain</td>
<td>1.45</td>
</tr>
<tr>
<td>Milk, fish, shellfish meat &amp; eggs, 2\textsuperscript{nd} tertile</td>
<td>0.97</td>
</tr>
<tr>
<td>Milk, fish, shellfish meat &amp; eggs, 3\textsuperscript{d} tertile</td>
<td>1.03</td>
</tr>
<tr>
<td>Television &amp; computer use, 2\textsuperscript{nd} tertile</td>
<td>1.07</td>
</tr>
<tr>
<td>Television &amp; computer use, 3\textsuperscript{d} tertile</td>
<td>0.91</td>
</tr>
<tr>
<td>Radio, CD, DVD &amp; video use, 2\textsuperscript{nd} tertile</td>
<td>0.93</td>
</tr>
<tr>
<td>Radio, CD, DVD &amp; video use, 3\textsuperscript{d} tertile</td>
<td>1.01</td>
</tr>
<tr>
<td>N of other electric appliances, 2\textsuperscript{nd} tertile</td>
<td>0.93</td>
</tr>
<tr>
<td>N of other electric appliances, 3\textsuperscript{d} tertile</td>
<td>0.93</td>
</tr>
<tr>
<td>Use of synthetic clothes, 2\textsuperscript{nd} tertile</td>
<td><strong>0.72</strong></td>
</tr>
<tr>
<td>Use of synthetic clothes, 3\textsuperscript{d} tertile</td>
<td><strong>0.73</strong></td>
</tr>
<tr>
<td>Having children, yes vs. no</td>
<td>0.96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline = geometric mean serum BDE-209 ng/g lipid weight in unexposed Norwegian women</td>
<td>1.39</td>
</tr>
<tr>
<td>Netherlands</td>
<td>1.41</td>
</tr>
<tr>
<td>UK</td>
<td><strong>2.47</strong></td>
</tr>
<tr>
<td>Spain</td>
<td><strong>3.68</strong></td>
</tr>
<tr>
<td>Milk, fish, shellfish meat &amp; eggs, 2\textsuperscript{nd} tertile</td>
<td>0.89</td>
</tr>
<tr>
<td>Milk, fish, shellfish meat &amp; eggs, 3\textsuperscript{d} tertile</td>
<td>1.14</td>
</tr>
<tr>
<td>Television &amp; computer use, 2\textsuperscript{nd} tertile</td>
<td>1.02</td>
</tr>
<tr>
<td>Television &amp; computer use, 3\textsuperscript{d} tertile</td>
<td>0.90</td>
</tr>
<tr>
<td>Radio, CD, DVD &amp; video use, 2\textsuperscript{nd} tertile</td>
<td>0.96</td>
</tr>
<tr>
<td>Radio, CD, DVD &amp; video use, 3\textsuperscript{d} tertile</td>
<td>1.03</td>
</tr>
<tr>
<td>N of other electric appliances, 2\textsuperscript{nd} tertile</td>
<td>0.85</td>
</tr>
<tr>
<td>N of other electric appliances, 3\textsuperscript{d} tertile</td>
<td>0.88</td>
</tr>
<tr>
<td>Use of synthetic clothes, 2\textsuperscript{nd} tertile</td>
<td>0.85</td>
</tr>
<tr>
<td>Use of synthetic clothes, 3^rd terile</td>
<td>0.76</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Having children, yes vs. no</td>
<td>0.81</td>
</tr>
</tbody>
</table>

**Bold:** statistically significant at p<0.05
Figure 1. Possible associations between serum concentrations of BDE-209 (upper panel: pg/g wet weight; lower panel: ng/g lipid weight) measured twice, 6 months apart, in Dutch participants.
Supplementary information:

Decabromodiphenylether Monitoring in Human Blood Project

Instructions for Whole Blood Sampling and Sample Transport

A sampling guide for partner laboratories in Spain, UK, Norway and the Netherlands

June 2007

by

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(Coordinator of Chemical Analysis)

Institute for Environmental Studies (IVM)
VU University Amsterdam

and

Drs. R. Fernández Cantón
(Coordinator of Sampling)

Institute for Risk Assessment Sciences (IRAS)
Utrecht University
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Introduction

Decabromodiphenylether (also called BDE209 or decaBDE) is a brominated flame retardant used to reduce the flammability of various products in the EU and in countries around the world. It is currently the subject of a risk assessment in the EU. As part of the input for the risk assessment, the Bromine Science Environment Form, BSEF is sponsoring a 10-year human biomonitoring project in which the levels of BDE209 will be followed in human subjects. As part of the project, blood samples will be collected in 2007 from donors in Spain, the UK, Norway and the Netherlands (40 blood samples per country). The samples will be analysed to determine the concentration of BDE209. The blood samples will be collected by health care professionals at partner laboratories in each of these countries.

The concentrations of BDE209 currently being found in humans are very low, and are often reported in the picogram\(^1\) per gram range. The analysis of this compound in human blood therefore requires a high level of laboratory expertise. However, sampling of the blood is equally as important to successful determination of BDE209 levels in humans as the analytical part of the determination process. Particularly because of the ubiquitous nature of BDE209, (which is present at high levels in dust particles for instance), special care must be taken during the sampling process in order not to introduce BDE209 from outside the body into the sample during the sampling and sample handling process. This means working very “cleanly” during all steps of the process, starting with clean, dust-free sampling equipment. One particle of dust in a blood sample can significantly affect the concentration of BDE209 that we measure in a blood sample.

On the other hand, care must also be taken to prevent the BDE209 in the sample from degrading before it can be analysed. BDE209 will degrade if exposed to sunlight. Therefore, samples must be kept in the dark. In addition the samples should be preserved by freezing. These and other important aspects are covered in the sampling protocol provided in this document.

In addition, this document will provide you with useful information concerning the transport of the blood samples to the laboratory of the Institute for Environmental Studies (IVM) at the VU University in Amsterdam, the Netherlands. The IVM will be performing the chemical analysis of all samples taken. The role of the Institute for Risk Assessment Sciences (IRAS) at Utrecht University in this project is to coordinate the blood sampling and compile the epidemiological data. IVM and IRAS cooperate closely on the project. Drs. Rocío Fernández Cantón will be coordinating the sampling; Dr. Heather Leslie (IVM) will be coordinating the chemical analysis.

Safety Considerations

This project involves working with human blood and risks of infection are always present. These must be minimized by strictly observing professional safety rules for working with human blood. The analytical methods used for the detection of BDE209 are not automated. In the interest of maximizing laboratory safety, the blood donors to be selected by partner laboratories should be generally healthy with low risk of infectious diseases (such as HIV or hepatitis B).

\(^1\) One picogram is 0.000000000001 g.
Blood Sampling Protocol

Selection of donors
The preferred target group is healthy women of (20-40 yr) without occupational exposure and with non-smoking habits. Subjects working in offices where some exposure may occur will not be excluded. Occupational exposures during production and further handling of BDE209 will not be accepted; however factors as lifestyle and living environment are likely to play a key role in the possible uptake of BDE209 by humans. These influencing parameters will be studied as accurately as possible by using detailed questionnaires about life style and living environment for the human volunteers.

Each partner laboratory must supply blood samples of the prescribed volume from 40 different individual donors. (Keep in mind the possibility that the required volume may not be possible to draw from some of the donors for whatever reason. Therefore, it is advisable to keep a few qualified donors in reserve in order to be sure that a total of 40 blood samples will be available).

Privacy of Donors
Before starting volunteer donors will be fully informed of the sampling procedure by the scientists at the partner laboratories involved in the study, who will answer any questions they might have.

The identity of the individuals will remain confidential; identities will be recorded by sample code given by the project coordinator. This information will be kept strictly private and will under no circumstances be divulged without permission of the volunteer. No names of individuals will be associated with the analytical results. Volunteers are entitled to be informed of the BDE209 concentration in their own blood by requesting the information from the project coordinator, but the information will not be divulged to anyone else.

Volunteers will have always the right to stop the procedure without giving any reason. Blood samples will be collected by highly trained and qualified medical staffs who are accredited for blood collection.

Sample vial description
Samples will be taken in the BD Vacuvials® with brown caps supplied by IRAS. The volume of these evacuated sterile blood collection tube for whole blood haematology determination, is 10 ml. The tubes are made of medical grade PET and feature a BD Hemogard™ safety closure. The dimensions are 16 x 100 mm; K$_2$EDTA spray is applied to the inside of the tube.

Vacuvials for sampling
Prior to sampling, each partner laboratory will receive from IRAS by post 6 x 40 vacuvials of this description (plus extras). IRAS will provide also with labels with a sample code; the participating laboratory will weigh the vacuvials before and after sampling.

Sampling procedure and safety considerations
Blood sampling should be performed under safety conditions in order to not get in direct contact with the blood. It should be treated as (potentially) hazardous material. The blood sampling will take an estimated 10-15 min per donor.

In order to decrease possible variability on triglyceride content in blood, samples should be taken in the morning or in the afternoon at least 4h after the last meal (before lunch or dinner). Information record about sampling will include what time/date the person last ate.
**Number of samples per donor**

Per donor, a minimum of 6 vacuials should be filled and labelled. Donors for whom less than 6 full vials are obtained, cannot be counted towards the 40 samples necessary for the study.

**Immediately after sampling / Serum fraction isolation**

Immediately upon drawing the blood into a vacuivial, invert (do not shake) the vacuivial 10 times in order to mix the EDTA coated on the inside walls of the vacuivial with the sample. Do NOT expose the blood samples to sunlight, as this will degrade the analyte.

For serum fraction analysis, the cells must be separated from the serum. The blood samples will be taken in Vacuials with brown caps. Blood clots on its own, allowing serum to be separated from cells.

Location of all blood sample work = biological laboratory, fume hood nearest the centrifuge and door.

**Centrifugation.** To obtain serum, place collection tube upright in rack and allow blood to clot at room temperature (30 minutes). If the centrifugation cannot be performed directly after 30 minutes, the samples should be put in the refrigerator until centrifugation (e.g. until the following day).

When clot has formed, centrifuge the tube (*swing bucket centrifuge*) for 10 minutes at 1800 g (Karin Vroonhof, VUMC, pers.comm.) with caps on in a centrifuge that is well-balanced (using extra tubes filled with water if necessary). BD vacuials should not break at g-forces up to 10000 g (pers. comm. with supplier).

Note: Do not use a centrifuge in which sediment or sewage sludge samples have just been centrifuged as this may be contaminated with DBDE.

NB: Prolonged centrifugation may cause hemolysis and must be avoided. Allow the centrifugate to come to a complete stop and remove tubes carefully without disturbing the red cells at the bottom. If cells become disturbed, repeated centrifugation can be attempted but usually cell lysis occurs. A signal that cell lysis has occurred is a pink colour in the serum, which normally is straw colour. Do not use serum contaminated with cells or intracellular contents.

Do not freeze samples before centrifuging as cellular contents will leak into the serum phase.

Removing serum from cell fraction.

- The specimen should only come into contact with carefully cleaned and solvent-rinsed glassware. Toluene should be used for rinsing since DBDE dissolves the best in this solvent.
- Do not allow dust to fall in the sample as dust contains a high amount of DBDE.
- Work in the fume hood in designated area of biological laboratory.
- Keep samples out of the light (DBDE degrades in the light).
- Keep workspace clean and sterile.

Holding the tube upright, carefully remove the stopper. The supernatant is the serum fraction.

Transfer serum from this top layer to a properly labeled sample container (glass tube) with a disposable glass pipette (*first rinse tubes x 3 with toluene, dry on air and be sure that all the toluene is gone before use*). Do not disturb the cell layer or allow any cells into the pipette. Do not pour the serum or invert the tube. Do not allow the serum to sit in the centrifuge for more
than 30 minutes before transferring the serum to new sample vials. If a thin "buffy coat" layer between cells and serum forms, allow this to stay with the cells, to avoid disturbing the cell layer. NB. The weight of the serum fraction and the cellular fraction per person must be known in order to do mass balances. Should a few cells be accidentally pipeted out with the serum, weigh this for calculating the total g of serum, but do not add any cells to the serum sample for analysis.

Information to record during sampling

The following sample information should be collected and stored in an excel file (see appendix) which is to be sent by email to IVM. Each sample code is thereby linked to relevant information for analysis of the samples.

- Sample code
- Weight of empty vacuvial (g)
- Weight of full vacuvial (g)
- Weight of blood sample (g)
- Order of the draw (i.e. tube sampled 1st, 2nd, 3rd, 4th, 5th or 6th etc in the series)
- Donor identification code
- Sampling date
- Sampling time point in 24 hour clock / time the person last ate
- Sample location
- Weight of empty serum glass vacuvial (g)
- Weight of full serum glass vacuvial (g)
- Identification code of the qualified person(s) who drew and handled the blood during sampling
- Storage conditions (temperature, dark, upright, etc.)
- Any other comments

Timing of Sampling

All blood samples for this study should be taken in 2007. In connection with laboratory analysis planning for such a large set of samples, it is imperative to inform the Sampling Coordinator of the date of sample availability as soon as this is known.

Expected dates:
- Spain July - September 2007
- UK August - September 2007
- Norway August - September 2007
- NL July – August 2007

Serum Sample Storage and Sending Instructions

Serum samples should be kept in the dark at all times and stored at –20 ºC in an upright position. Do not store samples in the same freezer (or other area) as samples that contain high BDE209 concentrations (such as river or marine sediments or sewage sludge samples) to prevent cross contamination.
Sending Instructions

Samples of blood should be sent frozen by express courier (e.g. DHL, see www.dhl.com) on dry ice to ensure the samples to not thaw during transport.

IATA (International Air Transport Association) regulates transport of blood by carrier. [Link to IATA guidelines]

Include in the package:
- one set of unused sampling devices identical to the ones used for blood sampling,
- at least 2 unused BD vacuviels for the analysis of background BDE209 contamination
- at least 2 unused serum glass vacuviels/tubes for the analysis of background BDE209 contamination.

Sending on dry ice requires special packaging instructions. Consult your local courier for advice. We include the following sending instructions as an extra guide. Use the appropriate stickers (available from DHL).

- A proforma invoice should be included describing the contents as “biological specimens for diagnostic analysis”
- Package the samples in leak-proof inner packages. This package should be placed in a second larger package, which is then put in the outer box with address label for sending. Use cushioning plastic bubble paper where necessary.
- Follow the “IATA Packing Instructions 650”
- Place a sticker on the box reading: “DRY ICE”
- Place a sticker on the box reading: “Diagnostic Specimens” and “for scientific use only”
- Describe the contents on the courier form
- Mark the package with the code "UN3373" and "contents packed in compliance with IATA Packing Instructions 650"
- Include a letter inside the box.
- Seal the box(es) well with tape.
- Note the length, width and height of the outer package (in cm) plus the weight (in kg).

Please courier samples and materials to this address:

Attention: Mr. B. van der Horst, Head of Laboratory
Institute for Environmental Studies (IVM)
Vrije Universiteit
De Boelelaan 1087
1081 HV Amsterdam
The Netherlands
Tel. +31-20-598 95 97
Tel. Secretariat +31-20-598 95 39
Fax. ++31-20-598 95 53
E-mail: bert.vanderhorst@ivm.falw.vu.nl

NB Please notify IVM of the date of arrival of your samples and send your sample list electronically by email:

To: Heather.leslie@ivm.vu.nl
Cc: bert.van.der.horst@ivm.vu.nl and r.fernandezcanton@iras.uu.nl
Contact Information of Project Sampling and Analysis Coordinators

Coordinator of Sampling and Epidemiological Study:

Drs. Rocío Fernández Cantón
Cellular and Molecular Toxicology
Institute for Risk Assessment Sciences (IRAS)
Cellular and Molecular Toxicology
P.O. Box 80.176
3508 TD Utrecht
The Netherlands
Tel. +31-30-253 36 31
Tel. Secretariat: +31-30-253 54 00
Fax. +31-30-253 5077
Email: r.fernandezcanton@iras.uu.nl

Coordinator of Laboratory Analyses:

Dr. Heather A. Leslie
Institute for Environmental Studies (IVM)
Vrije Universiteit
De Boelelaan 1087
1081 HV Amsterdam
The Netherlands
Tel. +31-20-598 95 97
Tel. Secretariat: +31-20-598 95 55
Fax. ++31-20-598 95 53
E-mail: heather.leslie@ivm.falw.vu.nl
# International Survey of Decabromodiphenylether (DecaBDE) in Human Blood

## Questionnaire for participants

**CONFIDENTIAL**

### Section 1: personal information

<table>
<thead>
<tr>
<th>Name</th>
<th>Date of birth (dd/mm/yyyy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phone number:</th>
<th>e-mail:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interviewer ID</th>
<th>Date of interview (dd/mm/yyyy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Place of interview</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Participant ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Has informed consent been signed?</th>
<th>Yes ☐</th>
<th>No ☐</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is the participant currently a non-smoker?</td>
<td>Yes ☐</td>
<td>No ☐</td>
</tr>
</tbody>
</table>
Section 2: screening

1a. Please indicate the country of your birth .................................................................

1b. How many years have you lived in your current home? ...... Years

2. When did you move into your current home (mm/yyyy) .........../............

3a. Do you have children, if yes, how many? Yes ☐ No ☐
(If yes, go to Section 3, Q4)

3b. For each of your child can you please indicate the date of birth and whether (and for how long) you breastfed each child?

<table>
<thead>
<tr>
<th>Date of Birth (dd/mm/yyyy)</th>
<th>Breastfed? (including expression of breastmilk)</th>
<th>Duration of breastfeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Section 3: health aspects

4. Please specify your height (cm) and weight (kg)

   Height .......... cm               Weight .......... kg
               .......... Feet/inches            .......... Stone/pounds

5. Please specify in the list below what would best describe your usual diet

☐ normal diet

☐ vegetarian but with fish

☐ strictly vegetarian

☐ vegan (go to 8)

☐ other, specify .................................................................

6a. Please indicate how often, on average, you consumed the following food products in the past month. Please, put one ‘X’ in every line.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
</table>

44
### 6b. Name the 3 fish or seafood products you consume most often, starting at 1) with the most often consumed kind. (e.g. tuna, cod, haddock, salmon, shrimp, mussels, sole, lobster)

1) ..............................................
2) ..............................................
3) ..............................................
**Section 4: Household characteristics**

7. How many people live in your household? ....................................... persons

8. Please indicate in the table below the presence, number and average duration of use for the following electrical goods in your household.

   ‘use’ means that the equipment is turned on and used, either by you or a household member.

   For example, if there are 2 television sets in your house, and one is used daily for 2 hours per day on average, and the other for half an hour per day, and your DVD player is used once per week for 3 hours, the table should look like this:

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>number present in household</th>
<th>hours of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td></td>
<td>2</td>
<td>2.5/day/week/month</td>
</tr>
</tbody>
</table>

   **EXAMPLE:**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>number present in household</th>
<th>hours of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td></td>
<td>1</td>
<td>3/day/week/month</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Yes</th>
<th>no</th>
<th>number present in household</th>
<th>Hours of use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Television</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>video/CD/DVD player/recorder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>computer/labtop/notebook</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>radio/tuner/amplifier</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>micro wave</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kettle/coffee maker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>food processor/blender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric oven</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dish washer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washing machiner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tumble dryer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fridge/freezer?? Or do we assume that they have these??</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equipment Type</td>
<td>Frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vacuum cleaner</td>
<td>..... /day/week/month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric shower</td>
<td>..... /day/week/month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair dryers/hair straighteners</td>
<td>..... /day/week/month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>/curlers/ razors</td>
<td>..... /day/week/month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric heaters</td>
<td>..... /day/week/month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>..... /day/week/month</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*) In case more kinds of equipment fall into one category, usage time should be added up.
9a. Please indicate the presence of the following items in your living room, and specify the type of material, e.g. wool, synthetic, cotton, vinyl, etc. Please, also give the age in years.

<table>
<thead>
<tr>
<th>Item</th>
<th>Present</th>
<th>Material</th>
<th>Age (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Textile wall to wall floor covering</td>
<td>□ yes</td>
<td>□ no</td>
<td></td>
</tr>
<tr>
<td>Rug(s)</td>
<td>□ yes</td>
<td>□ no</td>
<td></td>
</tr>
<tr>
<td>Upholstered easy chair(s)</td>
<td>□ yes</td>
<td>□ no</td>
<td></td>
</tr>
<tr>
<td>Upholstered sofa(s)</td>
<td>□ yes</td>
<td>□ no</td>
<td></td>
</tr>
</tbody>
</table>

9b. State the presence of the following materials in your bedroom, and specify the type of material, e.g. wool, down, synthetic, etc.

<table>
<thead>
<tr>
<th>Item</th>
<th>Present</th>
<th>Material</th>
<th>Age (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Textile wall to wall floor covering</td>
<td>□ yes</td>
<td>□ no</td>
<td></td>
</tr>
<tr>
<td>Rug(s)</td>
<td>□ yes</td>
<td>□ no</td>
<td></td>
</tr>
<tr>
<td>Duvet, blanket(s) / comforter</td>
<td>□ yes</td>
<td>□ no</td>
<td></td>
</tr>
<tr>
<td>Mattress(es)</td>
<td>□ yes</td>
<td>□ no</td>
<td></td>
</tr>
<tr>
<td>Pillow(s)</td>
<td>□ yes</td>
<td>□ no</td>
<td></td>
</tr>
</tbody>
</table>

9c. How often, on average, is your living room and bedroom floor vacuumed?

<table>
<thead>
<tr>
<th>Floor</th>
<th>(Number of times) per (week, month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Living room</td>
<td></td>
</tr>
<tr>
<td>Bedroom floor</td>
<td></td>
</tr>
</tbody>
</table>
10. Please, specify the type of clothing material you choose to wear for clothes worn in direct contact with your skin.

<table>
<thead>
<tr>
<th>Mostly Natural</th>
<th>Mostly Synthetic</th>
<th>Mostly mixed materials</th>
<th>No preference</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td>underwear</td>
<td>trousers, skirts</td>
<td>blouses, dresses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>socks, stockings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11. On average, how many times a day do you wash your hands? ....................... times
### Section 5: Occupational environment

12a. Do you currently work?  
   - Yes [ ]  
   - No [ ]  
   (go to Q13)

12b. Do you mainly work from home?  
   - Yes [ ]  
   - No [ ]

12c. How many hours do you work per day?  
   [ ] ……………………………..hours

12d. How many days do you work per week?  
   [ ] ……………………………..days

12e. Which percentage of your worktime do you work with electrical equipment?  
   [ ] …………………………………percent

12f. Please, give a short description of your job, and the materials you work with:  
   ……………………………………………………………………………………………  
   ……………………………………………………………………………………………

13. Do you use a car regularly (either as a driver or passenger)?  
   - Yes [ ]  
   - No [ ]

   If yes:  
   On average per day, how much time do you spend in your car?  
   [ ] ……………hours

14. Please indicate how often you travel by plane?  
   - [ ] Never  
   - [ ] Ones per year  
   - [ ] 1 – 4 times per year  
   - [ ] 5 – 19 times per year  
   - [ ] 20 times per year or more