Polybromodiphenyl ethers in mothers and their newborns from a non-occupationally exposed population (Valencia, Spain)

Esther Vizcaino and Joan O. Grimalt*

Department of Environmental Chemistry. Institute of Environmental Assessment and Water Research (IDÆA-CSIC)) Jordi Girona, 18. 08034 Barcelona. Catalonia. Spain

Maria-José Lopez-Espinosa†, Sabrina Llop, Marisa Rebagliato and Ferran Ballester

Center for Public Health Research (CSISP), Conselleria de Sanitat, Generalitat Valenciana, Avda Catalunya 21, 46020 Valencia, Spain

Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain

*Corresponding author phone: +34934006118; fax: +34932045904
e-mail: joan.grimalt@idaea.csic.es
†Present address.* Department of Social and Environmental Health Research. London School of Hygiene and Tropical Medicine. Keppel Street, London WC1E 7HT. United Kingdom.

Abstract

Polybromodiphenyl ethers (PBDEs) were analyzed in blood serum of pregnant women and in cord blood serum of their newborns from a general population cohort (n = 174; Valencia, Spain). The most abundant PBDE congeners identified were BDE 47, BDE 99, BDE 153, BDE 154 and BDE 209. Their cord blood serum concentrations were about 45% of those in maternal serum but after lipid normalization median of total PBDEs was 9.6 ng/g lipid in cord serum (concentrations encompassing between not detected and 140 ng/g lipid) and 9.6 ng/ lipid in maternal serum(concentrations covering between not detected and 120 ng/g lipid). The distributions of these compounds were dominated by BDE 47 in both cases. In cord blood serum the decreasing order of abundance was BDE 47 > BDE 99 > BDE 209 > BDE 153 > BDE 154. The congener composition in maternal serum followed a similar trend: BDE 47 > BDE 153 > BDE 154 > BDE 209 > BDE 99. The congener concentrations exhibited a higher degree of correlation in cord blood than in maternal serum. Use of the maternal determinants for categorization of the observed maternal and fetal PBDE concentrations only showed significant associations for the levels in umbilical cord. Neonates from rural areas exhibited statistically significantly lower concentrations than those from urban, semi-urban or metropolitan sites. Maternal serum also showed this difference but the higher dispersion of the concentrations in maternal serum did not afford its recognition with statistical significance. The lower qualitative and quantitative variability in the PBDE concentrations of cord blood serum than maternal serum suggest that the latter is reflecting PBDE contributions from a wider diversity of sources than the former whereas cord blood sera seems to represent the long term standing stock of these compounds accumulated in the maternal tissues.

1. Introduction

Polybromodiphenyl ethers (PBDEs) are a group of brominated flame retardants (BFRs) commonly used to reduce flammability of polymers and plastics. They have been added to many types of commercial and household products. PBDEs have similar properties to organochlorine compounds. They are persistent, highly lipophilic, accumulate in organisms and biomagnify through the food chain. Accordingly, they have become ubiquitous in the environment and in human population (Hites 2004).

Three technical PBDE mixtures have been produced commercially. They are known by their average bromine composition. Penta-BDE, mainly pentabromodiphenyl congeners, was mainly used in flexible polyurethane foam for soft furnishing. Octa-BDE, mainly octabromodiphenyl congeners, was used in acrylonitrile butadiene styrene resins. Deca-BDE, mainly decabromodiphenyl ether, is used in a variety of polymeric materials (Alaee et al., 2003). Due to health concerns and their widespread occurrence in environmental and human samples, the pentaBDE and octaBDE technical mixtures were banned by the European Union (EU) in 2004 and their production ceased in North America. Recently, the Stockholm Convention on Persistent Organic Pollutants (SCPOPs) decided to include the following mixtures in their list: tetraBDE (defined as BDE 47), pentaBDE (defined as BDE 99), hexaBDE (defined as BDE 153 and BDE 154) and heptaBDE (defined as BDE 175 and BDE 183) (SCPOPS,2010). Production and use of decaBDE has continued although its use was restricted in Europe last year (EBFRIP, 2009).

Human exposure to these compounds is of concern due to their widespread occurrence and potential toxicity. PBDEs have been described to interfere on natural hormone activity causing neurotoxicological and negative reproductive effects (Eriksson et al., 2006; Legler, 2008). These toxic effects are potentially more harmful during brain development in the prenatal period than in the adult life. Endocrine disruption can lead to serious deficits in neuropsychological functions in infants (Weiss, 2000). Recent studies have shown neurodevelopment deficits related to background prenatal BDE levels (Herbstman et al., 2010; Roze et al., 2009). Moreover, other negative effects such as cryptorchidism in newborn boys (Main et al., 2007) and lower birth weight and length (Chao et al., 2008) have been associated to exposure to low PBDE doses.

The studies on PBDE concentrations in human tissues have been reviewed recently (Frederiksen et al., 2009a). Some of them concern both mothers and newborns (Antignac et al., 2009; Bi et al., 2006; Gomara et al., 2007; Kawashiro et al., 2008; Mazdai et al., 2003; Meironyte Guvenius et al., 2003). However, in some cases the number of subjects studied was small and many of them were focused on the lower brominated BDE congeners. Data of BDE 209 in prenatal exposure is scarce, probably due to analytical difficulties (Stapleton, 2006). In addition, these previous studies have shown dissimilar conclusions. In some cases, the same PBDE concentrations were found in mother and infant sera (Mazdai et al., 2003) and in others these concentrations were significantly different (Bi et al., 2006; Gomara et al., 2007; Meironyte Guvenius et al., 2003). Exposure to PBDEs is attributed to a combination of diet and indoor environment (Wilford et al., 2005). However, more information is needed on the influence of maternal determinants on PBDE accumulation.

Accordingly, the present study is addressed to investigate the occurrence of PBDEs, including low and high brominated congeners, in maternal and umbilical cord serum. The transfer of these compounds between mother and fetus is evaluated. The influence of demographic determinants such as maternal age, previous lactation, pre-pregnant body mass index (BMI), residence site, education level and others on PBDE concentrations have also been considered.

2. Material and Methods

2.1. Population and study design

The study sample was drawn from a cohort established in Valencia as part of the INMA (Environment and Childhood) study, a Spanish multicenter mother-child cohort study that analyzes the influence of prenatal environmental exposures on growth, development and health from early fetal life until childhood. The study design is reported elsewhere (Ribas-Fito et al., 2006). The inclusion criteria for mothers in the cohort were: at least 16 years old, singleton pregnancy, no assisted conception, no chronic hypertension, delivery foreseen at the reference hospital and no communication handicap. In addition, mothers receiving TH supplements or anti-TH drugs were excluded. The study protocol was approved by the Ethics Committee of the reference

hospital and informed consent was obtained for every participant. 855 women were enrolled in the study during 2003-2005. Maternal blood samples (n=806) were collected during the first trimester of gestation (median= 12 weeks; range= 10-13 wks). Women who withdrew, were lost to follow-up, had induced or spontaneous abortions and fetal deaths were excluded. A total sample of 787 women was followed until delivery between May 2004 and February 2006. 527 cord blood samples were successfully collected. For logistical reasons and due to budget limitations, we present data on the first consecutive 174 maternal and cord serum samples. No differences in maternal BDE concentrations are expected during pregnancy (Meijer et al., 2008).

2.2. PBDEs analysis

The laboratory analytical methods and quality control procedures have been described elsewhere (Vizcaino et al., 2009). A total of 14 PBDE congeners were analyzed: BDE 17 and BDE 28 (tribromo), BDE 47, BDE 66 and BDE 71 (tetrabromo), BDE 85, BDE 99 and BDE100 (pentabromo), BDE 153, BDE 154 and BDE 138 (hexabromo), BDE 183 and BDE 190 (heptabromo) and BDE 209 (decabromo).

Briefly, 1 ml of serum was spiked with the surrogate standard of decachlorobiphenyl (PCB 209) and vortex stirred for 30 s at 2,000 rpm. n-Hexane (3 ml) was added, followed by concentrated sulfuric acid (2 ml). After reaction, the mixture was stirred for 30 s and the supernatant n-hexane phase was separated by centrifugation. The remaining sulfuric acid solution was re-extracted two times with 2 ml of n-hexane (each by 30 s stirring and centrifugation). The combined n-hexane extracts (7 ml) were additionally cleaned with sulfuric acid (2 ml, stirring 30 s). Then, the n-hexane phase was separated by centrifugation and reduced to a small volume under a gentle nitrogen stream. The extract was transferred to gas chromatography (GC) vials using four 25 µl rinses of isooctane. BDE 118 (20 µl) and [^{13}C]-BDE 209 (10 µl) were added as internal standards before injection.

PBDEs were analyzed using an Agilent 6890N GC coupled to a 5975 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) operating in negative chemical ionization mode (NICI). The instrument was equipped with a low bleed SGE-BPX5 MS fused silica capillary column (15 m long, 0.25 mm internal diameter and 0.10 µm film thickness). Sample batches encompassed 9, 15 or 19 samples, depending on

157 laboratory material availability. One procedural blank was included in each sample
158 batch. PBDE levels in blanks were negligible with exception of BDE 47, BDE 99 and
159 BDE 209. In this case, blank levels were extracted from the corresponding sample
160 batch. On average, they accounted for 2.6%, 10% and 11% of BDE 47, BDE 99 and
161 BDE 209 of median concentrations. BDE compounds in the samples were considered as
162 "non detected" if the corresponding blank in the batch was equal or higher than 30% of
163 the compound measurements. Method detection limits (MDLs) were calculated as three
164 times the standard deviation of the procedural blank levels. Instrumental detection limits
165 were used for the congeners not detected in the procedural blanks. They were calculated
166 by injection of standard solutions as three times the value of the signal-to-noise ratio
167 (S/N). Detection limits ranged between 0.0006 and 0.006 ng/mL depending on the
168 PBDE congener. PBDEs identification was based on retention time and mass spectral
169 information. Quantification was performed by reference to linear calibration lines and
170 correction by the surrogate and injection standards (Vizcaino et al., 2009). Percent
171 recoveries of PCB 209 ranged between 41% and 101% (mean ± standard deviation = 64
172 ± 9.5). Final validation was made by analysis of reference material obtained from the
173 Arctic Monitoring and Assessment Program (AMAP). We participate regularly in the
174 AMAP Ring Test Proficiency Program for POPs in human serum (Centre de
175 Toxicologie Institut National de Santé Publique du Québec, Québec, Canada) and the
176 laboratory results usually were within 20% of the consensus values, including BDE 209
177 concentrations.
178
179 *2.3. Lipid determination*
180
181 Total cholesterol and triglycerides were determined using colorimetric
182 enzymatic methods in the General Biochemistry Laboratory of Hospital La Fe. Total
183 serum lipid concentrations were calculated as described by Phillips et al. (1989).
184
185 *2.4. Demographic variables*
186
187 Information on residence (urban, metropolitan, semiurban, rural), country of birth
188 (Spain, other countries), education (up to primary school, high school, university),
189 maternal age (<25, 25-29, 30-34, ≥35 years), pre-pregnancy BMI (underweight, normal,

overweight, obese) and previous lactation (<6, >6 months) were obtained from a questionnaire administered at 10-13 weeks of gestation. Maternal blood samples were collected at this time.

2.5. Data analysis

Descriptive statistics were calculated for concentrations of PBDE compounds in maternal and umbilical cord serum. PBDE levels were expressed in pg/mL or were lipid-adjusted (ng/g), dividing serum residue levels by total serum lipid concentrations for comparision with the literature but statistical calculations were performed on fresh weight PBDE concentrations (pg/mL). Values of half detection limits were introduced in the database when measurable quantities of the analytes were not detected. Only congeners found above quantification level in more than 30% of the samples analyzed were considered. PBDE concentrations were not normally distributed, they were right-skewed even after log-transformation. They did not show normal distribution so eventually we did not use any transformation. Normal distribution was examined according to the Kolmogorov-Smirnov method. Spearman correlations were used to examine associations between maternal and cord serum levels. Kruskal-Wallis and Mann-Whitney U tests were used to evaluate associations between PBDE levels and sociodemographic variables. Statistical significance was fixed at $p<0.05$ (two sided). Statistical analysis was done with the Statistical Package for Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Characteristics of the population included in the study

Six determinants of the mothers participating in the study are shown in Table 1. More than 90% of the total population was originally born in Spain. The proportion of those living in rural areas was small (2%). About 32% had a primary school degree, 39.1% had secondary degree and 25.9% an university degree. The mean age at delivery was 30.1(4.8). 48% were multiparous and 20% had previously breastfed for more than 6

months. Standardized BMI categories showed that 24.1% of the mothers were overweight and 10.9% were obese.

3.2. PBDEs concentrations in mothers and newborns

BDE 17, BDE 66, BDE 71, BDE 85 BDE 138 and BDE 190 were not detected in any of the samples analyzed. BDE 28 (LOD = 0.0006 ng/mL), BDE 100 (LOD = 0.0004 ng/mL) and BDE 183 (LOD = 0.0012 ng/mL) were found above limit of detection in less than 10% of the samples. These congeners were therefore not included in the following sections. Mean, median and concentration ranges of the most abundant PBDEs, BDE 47, BDE 99, BDE 153, BDE 154 and BDE 209, are shown in Table 2. Total PBDEs encompassed between not detected and 140 ng/g lipid in cord serum and between not detected and 120 ng/g lipid in maternal serum. BDE 47 was the dominant congener in both, cord and maternal serum accounting for 28% and 45% of total PBDEs respectively. Congener concentrations in the cord serum samples tended to decrease at higher number of bromine substituents except in the case of BDE 209 (Fig. 1) which was the third most abundant congener.

BDE 47 was also the predominant congener in maternal serum but BDE 153 and BDE 154 were present in higher concentrations than BDE 99. Serum PBDE distributions with BDE 47 predominance and higher relative proportion of BDE 153 than BDE 99 (but lower concentration of BDE 154 than BDE 99) have been found in Stockholm (Meironyte Guvenius et al., 2003), Kashiva (Japan; Kawashiro et al., 2008) and Guangzhou (Bi et al., 2006). In contrast, distributions with predominance of BDE 99 over BDE 153 and BDE 154 have been found in Indiana (Mazdai et al., 2003), Salinas Valley (Bradman et al., 2007) and Madrid (Gomara et al., 2007). In some cases, BDE 153 has been found to dominate in the PBDE distribution (Groningen; Meijer et al., 2008). These differences in composition can be interpreted to reflect diverse contributions of the above described PBDE commercial mixtures. In addition, the dominance of BDE 47 may reflect contributions from environmentally transformed mixtures in which this congener becomes predominant (Keum and Li, 2005; Eriksson et al., 2004; Robrock et al., 2008).

The concentrations found in the present cohort are similar to those reported in other Spanish cohorts, e.g. Madrid (mothers, n = 61, median 12 ng/g lipid; newborns, n = 44, median 17 ng/g lipid; Gomara et al., 2007), Menorca (newborns, n = 92; 6.2 ng/g

lipid; Carrizo et al., 2007), as well as in other European sites, e.g. Toulouse (n = 93; 8.8 ng/g lipid in mothers and 12 ng/g lipid in newborns; Antignac et al., 2009), or in Asian locations such as Guiyu (n = 102; 14 ng/g lipid in newborns; Wu et al., 2010). They are higher than in Stockholm (n = 15; 1.9 ng/g lipid in mothers and 1.3 ng/g lipid in newborns; Meironyte Guvenius et al., 2003), Groningen (n = 90; 3.3 ng/g lipid in mothers and 1.9 ng/g lipid in newborns; Meijer et al., 2008), Guangzhou (n = 27; 3.9 ng/g lipid; Bi et al., 2006), Chaonan (n = 51; 5.2 ng/g lipid in newborns; Wu et al., 2010), Kashiwa (n = 16; 4.6 ng/g lipid; Kawashiro et al., 2008) and Japan (n = 89; 2.9 ng/g lipid; Inoue et al., 2006). On the contrary, the concentrations from the Valencian cohort were much lower than concentrations reported in populations from north America, e.g. Indianapolis (n = 12; 37 ng/g lipid in mothers and 39 ng/g lipid in newborns; Mazdai et al., 2003), Salinas Valley (n = 24; 21 ng/g lipid in mothers; Bradman et al., 2007), Baltimore (n = 297; 27 ng/g lipid in newborns; Herbstman et al., 2007) and Manhattan (n = 210; 19 ng/g lipid in newborns; Herbstman et al., 2010).

In any case, there is a large variation of PBDE concentrations in the samples analyzed. A group of highly exposed mothers could be identified, 16 participants were in the highest decile of ΣPBDEs and their concentrations were five times higher than the average values of the other cohort participants (Table 3). This high variability has been observed in previous studies (Kawashiro et al., 2008). The group of the highest decile exhibits concentration values that are higher than those in the upper concentration range of previously described cases. This group of highly exposed individuals reflect specific PBDE sources since it exhibits a distribution also dominated by BDE 47 but higher concentrations of BDE 99 than BDE 153 and BDE 154. This distribution is different from the previously described average PBDE composition in the mothers (Table 2).

3.3 PBDE composition in umbilical cord vs maternal serum

All congener concentrations are lower in umbilical cord than in maternal serum when reported on fresh weight basis (pg/ml; Table 2). On average PBDEs in cord blood are about 45% of those in maternal serum. However, normalization of these concentrations on lipid basis provided quite similar levels in both matrices for all congeners (Table 2). It is important to highlight that the fresh weight concentrations of the congeners are not correlated with total lipid serum content (data not shown).

289 Accordingly, fresh weight concentrations were used for the calculations of correlations
290 and other tests.
291 In qualitative terms, similar distributions are found both in maternal and cord
292 sera. Thus, even BDE 209, which is characterized by a high molecular size due to its ten
293 bromine substituents (Sölder et al., 2009), is found in pre-natal children in average
294 concentrations that are about half those found in maternal serum. However, these
295 concentrations are even higher in cord blood serum when normalized to lipid content
296 (Table 2). High concentrations of this compound have also been found in placenta
297 (Frederikssen et al., 2009b). BDE 209 has also been found in cord serum in previous
298 studies, e.g. Guiyu (median 4.2 ng/g lipid; Bi et al., 2006), Chaonan (2.5 ng/g lipid; Bi
299 et al., 2006) and Toulouse (27 ng/g lipid; Antignac et al., 2009). Anyhow, taking into
300 account the short life of BDE 209 in humans (Thuresson et al., 2006) it seems that
301 mothers must be exposed to it continuously to keep the observed serum concentrations
302 and transfer to the fetus.
303 Significant correlation coefficients between the concentrations of the different
304 congeners in both mothers and newborns can be established (Table 4). In newborns all
305 congeners except BDE 209 are correlated with a high degree of significance ($p < 0.01$;
306 Table 4) although in some cases the coefficients are not very high. BDE 209 is
307 correlated with BDE 47 and BDE 99 but not with BDE 153 and BDE 154. In maternal
308 serum, significant correlations ($p < 0.01$) are found again between all PBDE congeners
309 except BDE 209. In this group of samples BDE 209 exhibit a more dissimilar trend than
310 in newborns which likely reflect a larger diversity of sources contributing to the PBDE
311 composition in the former than in the latter.
312 We only found statistically significant correlations between BDEs concentration
313 in maternal and cord serum for BDE 209 ($r^2 = 0.152$, $p<0.05$). Some authors found
314 strong correlations between paired cord and maternal concentrations (n = 12; Mazdai et
315 al., 2003), others only found weak correlations and not for all congeners (n = 21, Bi et
316 al., 2006; n = 16, Kawashiro et al., 2008) while others did not find any (n = 72-90,
317 Antignac et al., 2009; n = 44, Gómara et al., 2007). According to these antecedents,
318 significant correlations between paired mother-fetus BDE concentrations were only
319 found when the number of cases was small. The lack of correlation at larger sample
320 numbers could reflect different factors. Placenta could prevent the free pass of PBDEs
321 into the fetal compartment leading to different accumulation patterns of these
322 compounds (Frederikssen et al., 2009b). Differences between mother and fetus for

congener metabolization could also contribute to the lack of correlation since fetus do not have their detoxification mechanisms fully developed (Olsen, 2000). We think that this issue is still open.

3.4 Maternal determinants of prenatal PBDE exposure

Maternal socio-economic characteristics such as residence site, educational level, age, BMI and previous lactation were assessed in order to know their influence on PBDE concentrations. Significant associations were found for total PBDEs and residence site and BDE 153 and age in umbilical cord but not in maternal serum (Table 5). The lack of statistically significant correlation with BMI, lactation or educational level has already been observed in other studies (Antignac et al., 2009; Gomara et al., 2007; Mazdai et al., 2003; Meironyte Guvenius et al., 2003; Bradman et al., 2007). However, there is one previous study reporting one association between PBDE cord serum concentrations and BMI, previous lactation and education level (Herbstman et al., 2007).

Geographic differences have been found for PBDE concentrations in cord serum. Neonates from rural areas have lower concentrations than those born in urban, semi-urban or metropolitan sites (Table 5; Fig. 2). Although these data must be interpreted cautiously since the number of cases from rural areas was small (n = 4). These differences were also observed in breast milk and maternal serum from Japan (Inoue et al., 2005). In the cohort of Valencia the difference is observed in cord serum but not in maternal serum. PBDEs in maternal serum probably reflect contributions from a higher diversity of sources and therefore higher variability which hinders the observation of differences such as location. In contrast, cord serum likely records PBDE inputs from maternal sources that result from larger time period averages involving lower dispersion of concentrations and composition. This lower dispersion of PBDE concentrations in cord serum facilitates the observation of long term geographic pollution differences. Thus, PBDEs from newborns of mothers living in rural sites exhibit significant lower values than in urban, semi-urban or metropolitan sites whereas higher dispersion in the PBDE concentrations of the mothers does not afford a statistically significant identification of this difference (Fig. 2).

4. Conclusions

The most abundant PBDE congeners in both newborns and their mothers are BDE 47, BDE 99, BDE 153, BDE 154 and BDE 209. The summed concentrations of these congeners in cord blood serum are about 45% of those observed in maternal serum. However, when these measurements are normalized by lipid content about the same concentrations are observed.

In qualitative terms, the distributions of these major PBDEs are similar in both types of samples being dominated by BDE 47. In cord blood serum this distribution exhibits a progressive decay from this less brominated congener until BDE 154 involving relative concentration decreases at higher degree of bromination. This trend is not followed by BDE 209 that was the third most abundant congener. The congener composition in maternal serum follows a similar trend but BDE 153 is found in higher concentration than BDE 99. The concentrations between congeners exhibit a higher degree of correlation in cord blood than in maternal serum. These differences may indicate that maternal serum is reflecting PBDE contributions from a wider diversity of sources than cord blood serum which seems to represent the long term standing stock of these compounds accumulated in the maternal tissues.

Categorization of the observed PBDE concentrations by maternal determinants has only shown some significant associations for the levels in umbilical cord but not in maternal serum. Neonates from rural areas exhibit statistically significantly lower concentrations than those born in urban, semi-urban or metropolitan sites. Maternal serum also shows this difference but the higher dispersion of the concentrations in this type of samples does not afford its recognition with statistical significance.

References

Alaee M, Arias P, Sjödin A, Bergman A. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. Environ Int 2003;29:683-689.

Antignac JP, Cariou R, Zalko D, Berrebi A, Cravedi JP, Maume D, et al. Exposure assessment of French women and their newborn to brominated flame retardants: Determination of tri- to deca- polybromodiphenylethers (PBDE) in maternal

390 adipose tissue, serum, breast milk and cord serum. Environ Pollut 2009;157:164-
391 173.
392 Bi X, Qu W, Sheng G, Zhang W, Mai B, Chen D, et al. Polybrominated diphenyl ethers
393 in South China maternal and fetal blood and breast milk. Environ Pollut
394 2006;144:1024-1030.
395 Bradman A, Fenster L, Sjödin A, Jones RS, Petterson Jr DG, Eskenazi B.
396 Polybrominated diphenyl ether levels in the blood of pregnant women living in
397 an agricultural community in California. Environ Health Perspect 2007; 115:71-
398 74.
399 Bromine Science and Environmental Forum (BSEF). UN Stockholm Convention
400 concludes that only certain lower brominated diphenylethers are POPs.2009.
401 http://www.bsef.com/uploads/Documents/documents/BSEF%20Statement%20C
402 OP4.pdf.
403 Carrizo D, Grimalt JO, Ribas-Fito N, Sunyer J, Torrent M. Influence of breastfeeding in
404 the accumulation of polybromodiphenyl ethers during the first years of child
405 growth. Environ Sci Technol 2007;41:4907-4912.
406 Chao HR, Wang SL, Lee WJ, Wang YF, Papke O. Levels of polybrominated diphenyl
407 ethers (PBDEs) in breast milk from central Taiwan and their relation to infant
408 birth outcome and maternal menstruation effects. Environ Int 2007;33:239-245.
409 Eriksson J, Green N, Marsh G, Bergman A. Photochemical decomposition of 15
410 polybrominated diphenyl ether congeners in methanol/water. Environ Sci
411 Technol 2004;38:3119-3125.
412 Eriksson P, Fischer C, Fredriksson A. Polybrominated diphenyl ethers, a group of
413 brominated flame retardants, can interact with polychlorinated biphenyls in
414 enhancing developmental neurobehavioral defects. Toxicol Sci 2006;94:302-
415 309.
416 European Brominated Flame Retardant Industry Panel (EBFRIP). The RoHS Directive
417 and Deca-BDE. 2009. http://www.ebfrip.org/main-nav/european-regulatory-
418 centre/rohs-directive-restriction-of-the-use-of-certain-hazardous-substances-in-
419 electrical-and-electronic-equipment/the-rohs-directive-and-deca-bde/.
420 Frederiksen M, Thomsen M, Vorkamp K, Knudsen LE. Patterns and concentration
421 levels of polybrominated diphenyl ethers (PBDEs) in placental tissue of women
422 in Denmark. Chemosphere 2009a;76:1464-1469.

423 Frederiksen M, Vorkamp K, Thomsen M, Knudsen LE. Human internal and external
424 exposure to PBDEs - A review of levels and sources. Int J Hyg Environ Health
425 2009b;212:109-134.
426 Gómara B, Herrero L, Ramos JJ, Mateo JR, Fernández MA, Garcia JF, et al.
427 Distribution of polybrominated diphenyl ethers in human umbilical cord serum,
428 paternal serum, maternal serum, placentas, and breast milk from madrid
429 population, Spain. Environ Sci Technol 2007;41:6961-6968.
430 Herbstman JB, Sjödin A, Apelberg BJ, Witter FR, Patterson DG, Halden RU, et al.
431 Determinants of prenatal exposure to polychlorinated biphenyls (PCBs) and
432 polybrominated diphenyl ethers (PBDEs) in an urban population. Environ
433 Health Perspect 2007;115:1794-1800.
434 Herbstman JB, Sjödin A, Kurzon M, Lederman SA, Jones RS, Rauh V, et al. Prenatal
435 exposure to PBDEs and neurodevelopment. Environ Health Perspect
436 2010;118:712-719.
437 Hites RA. Polybrominated Diphenyl Ethers in the Environment and in People: A Meta-
438 Analysis of Concentrations. Environ Sci Technol. 2004;38:945-956.
439 Inoue K, Harada K, Takenaka K, Uehara S, Kono M, Shimizu T, et al. Levels and
440 concentration ratios of polychlorinated biphenyls and polybrominated diphenyl
441 ethers in serum and breast milk in Japanese mothers. Environ Health Perspect
442 2006;114:1179-1185.
443 Kawashiro Y, Fukata H, Omori-Inoue M, Kubonoya K, Jotaki T, Takigami H, et al.
444 Perinatal exposure to brominated flame retardants and polychlorinated biphenyls
445 in Japan. Endocr J 2008;55:1071-1084.
446 Keum Y-S, Li QX. Reductive debromination of polybrominated diphenyl ethers by
447 zerovalent iron. Environ Sci Technol. 2005;39,2280-2286.
448 Legler J. New insights into the endocrine disrupting effects of brominated flame
449 retardants. Chemosphere 2008;73:216-222.
450 Lopez-Espinosa MJ, Vizcaino E, Murcia M, Llop S, Espada M, Seco V, et al.
451 Association between thyroid hormone levels and 4,4'-DDE concentrations in
452 pregnant women (Valencia, Spain). Environ Res 2009;109:479-485.
453 Main KM, Kiviranta H, Virtanen HE, Sundqvist E, Tuomisto JT, Tuomisto J, et al.
454 Flame retardants in placenta and breast milk and cryptorchildism in newborn
455 boys. Environ Health Perspect 2007;115:1519-1526.

Mazdai A, Dodder NG, Abernathy MP, Hites RA, Bigsby RM. Polybrominated diphenyl ethers in maternal and fetal blood samples. Environ Health Perspect 2003;111:1249-1252.

Meijer L, Weiss J, Van Velzen M, Beouwer A, Bergman ÃKE, Sauer PJJ. Serum concentrations of neutral and phenolic organohalogens in pregnant women and some of their infants in the Netherlands. Environ Sci Technol 2008;42:3428-3433.

Meironyté Guvenius D, Aronsson A, Ekman-Ordeberg G, Bergman A, Norën K. Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols, and pentachlorophenol. Environ Health Perspect 2003;111:1235-1241.

Olsen, J. Prenatal exposures and long term health effects. Epidemiol. Rev. 2000;22:76-81.

Phillips DL, Pirkle JL, Burse VW, Bernert Jr JT, Henderson LO, Needham LL. Chlorinated hydrocarbon levels in human serum: Effects of fasting and feeding. Arch Environ Contam Toxicol 1989;18:495-500.

Ribas-Fitó N, Ramón R, Ballester F, Grimalt JO, Marco A, Olea N, et al. Child health and the environment: The INMA Spanish study. Paediatr Perinat Epidemiol 2006;20:403-410.

Robrock KR, Korytar P, Alvarez-Cohen L. Pathways for the anaerobic microbial debromination of polybrominated diphenyl ethers. Environ Sci Technol. 2008;42,2845-2852.

Roze E, Meijer L, Bakker A, Van Braeckel KNJA, Sauer PJJ, Bos AF. Prenatal exposure to organohalogen, including brominated flame reatrdants, influences motor, cognitive and behavioral performance at school age. Environ Health Perspect 2009;117:1953-1958

Sölder E, Rohr I, Kremser C, Hutzler P, Debbage PL. Imaging of placental transport mechanisms: a review. Eur J Obstet Gynecol Reprod Biol 2009;144 Suppl 1:S114-120.

Stapleton HM. Instrumental methods and challenges in quantifying polybrominated diphenyl ethers in environmental extracts: A review. Anal Bioanal Chem 2006;386:807-817.

488 Stapleton HM, Kelly SM, Pei R, Letcher RJ, Gunsch C. Metabolism of polybrominated
489 diphenyl ethers (PBDEs) by human hepatocytes in vitro. Environ Health
490 Perspect 2009;117:197-202.
491
492 Stockholm convention on Persistent Organic Pollutants (POPs). Programmes. New
493 POPs.http://chm.pops.int/Programmes/NewPOPs/The9newPOPs/tabid/672/lang
494 uage/en-US/Default.aspx
495 Thuresson K, Höglund P, Hagmar L, Sjödin A, Bergman A, Jakobsson K. Apparent
496 half-lives of hepta- to decabrominated diphenyl ethers in human serum as
497 determined in occupationally exposed workers. Environ Health Perspect
498 2006;114:176-181.
499 Vizcaino E, Arellano L, Fernandez P, Grimalt JO. Analysis of whole congener mixtures
500 of polybromodiphenyl ethers by gas chromatography-mass spectrometry in both
501 environmental and biological samples at femtogram levels. J Chromatogr A.
502 2009;1216:5045-5051.
503 Weiss B. Vulnerability of children and the developing brain to neurotoxic hazards.
504 Environ Health Perspect 2000;108:375-381.
505 Wilford BH, Shoeib M, Harner T, Zhu J, Jones KC. Polybrominated diphenyl ethers in
506 indoor dust in Ottawa, Canada: Implications for sources and exposure. Environ
507 Sci Technol 2005;39:7027-7035.

508 FIGURE CAPTION

509 Figure 1: Median concentrations (pg/mL) of the major BDE congeners found in
510 maternal and umbilical cord serum.
511
512 Figure 2: Relationship between PBDE levels in cord (a) and maternal (b) serum and
513 residence site in the INMAValencia cohort 2004-2006. Points refer to geometric means
514 and error bars represent 95% CI (n = 174; urban = 22, metropolitan = 82, semiurban =
515 66; rural = 4).