EXPOSURE ASSESSMENT OF FETUS AND NEWBORN TO BROMINATED FLAME RETARDANTS IN FRANCE: PRELIMINARY DATA

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Introduction

The impact of brominated flame retardants (BFR) on the environment and their potential risk for animal and human health is a present time concern for the scientific community. Their effects on vulnerable population groups are a particularly critical issue. Indeed , some of these compounds are considered as endocrine disruptors with potential adverse effects in case of exposure at critical stages of development (fetus, newborn). Though some exposure assessment studies have already been conducted in Northern Europe, fewer data is available from Southern Europe in general, and from France in particular. In this context, an analytical strategy was developed for the multi-residue analysis of hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA) and polybromodiphenylethers (PBDE including tri- to decaBDE) from various human biological matrices (serum, adipose tissue, breast milk). The proposed methodology was validated, and applied to more than 100 biological samples (maternal and newborn tissues) collected during caesarean deliveries.

Material and Methods

Samples

Samples were collected by the Centre Hospitalier Universitaire de Toulouse, during a research project financially supported by the Agence Française de Sécurité Sanitaire Environnementale et du Travail (AFSSET). All these samples (including maternal and umbilical serum, maternal adipose tissue and breast milk) were obtained from volunteer women during caesarean deliveries. This first part of the project concerned 26 mother/newborn dyads included in the study in 2005. The protocol was approved by an ethical committee, in accordance with French regulations.

Reagents and chemicals

All solvents were Picograde® quality and provided by LGC Promochem (Wesel, Germany), Sigma (Steinheim, Germany), Aldrich (Steinheim, Germany) or Solvents Documentation Synthesis (Peypin, France). β-glucuronidase from *Helix pomatia* (H5 type) was provided by Sigma. Enzymatic kit from Randox Laboratories Ltd. (Crumlin, UK) was used for total lipids determination in blood serums. Oasis HLB SPE cartridges (500 mg, 6 mL) were provided by Waters (Milford, MA, USA) and SiOH SPE cartridges (1 g, 6 mL) were purchased from United Chemical Technologies (Bristol, UK) or Interchim (Montluçon, France). Silica gel (G60) was provided by Fluka (Buchs, Switzerland). All reference ^{12}C-native and ^{13}C-labelled standard solutions were purchased from Cambridge Isotope Laboratories (Andover, USA) or Wellington Laboratories (Guelph, Canada). ^{13}C-labelled compounds used as internal standards for quantification included: γ-HBCD, TBBPA, BDE-28, 47, 99, 154, 153, 183 and 209. ^{13}C-BDE-139 was used as external standard.

Sample preparation

The developed sample preparation procedure, described elsewhere[1], was used pending minor modifications. Briefly, a first liquid extraction with ethyl acetate for serum and dichloromethane/acetone for milk was applied. For all samples, a liquid-liquid partitioning (acetonitrile/*n*-hexane) was then applied, allowing the separation of PBDE from the other BFR. The PBDE fraction was purified using two successive SPE (an Oasis HLB cartridge followed by a classical multilayer H_2SO_4 activated silica). The TBBPA+HBCD fraction was submitted to an enzymatic hydrolysis in order to deconjugate the potential TBBPA phase II metabolites and was also purified according to two successive SPE cartridges (an Oasis HLB followed by a SiOH stationary phase). The first Oasis HLB SPE also permitted to separate TBBPA from HBCD. Finally, the HBCD fraction was injected in LC-MS/MS, while the TBBPA and PBDE fractions were separately analysed by GC-HRMS.

LC-MS/MS and GC-HRMS measurements

Separation of the α-, β- and γ-HBCD stereoisomers was achieved using an Alliance® 2690 HPLC pump (Waters, Milford, MA, USA). A Symmetry C_{18} stationary phase (150 x 2.1 mm, 3.5 µm + guard column 10 x 2.1 mm) from Waters (Milford, USA) was used. Elution solvents were methanol (A), acetonitrile (B) and water containing 0.5% (v/v) acetic acid (C). Mobile phase composition (A:B:C, v/v/v) was 30:10:60 from 0 to 1 min and 50:50:0 at 4.5 min. Flow rate and injected volume were set at 0.25 $mL.min^{-1}$ and 20 µL. MS^2 was performed on a QuattroLC® triple quadrupole instrument (Micromass, Manchester, UK) operating in negative electrospray ionization and multiple reaction monitoring acquisition mode. Monitored product ions were the bromine atoms (m/z = 79, 81) produced after fragmentation of the $[M-H]^-$ precursor ion. Separation of TBBPA and PBDE was achieved using a Hewlett Packard 5890 (Palo Alto, CA, USA) gas chromatograph with capillary column (15 m x 0.25 mm x 0.10 µm) coated with low bleeding diphenyl (5%)-dimethylpolysiloxane (95%) copolymer (UB5-P, Interchim, Montluçon, France). The temperature gradient started from 120 °C (2 min), rose to 280 °C (10 $°C.min^{-1}$) and then to 320 °C (20 $°C.min^{-1}$, 8 min). Injected volume was 2 or 3 µL (splitless mode). Helium was used as carrier gas at 1 $mL.min^{-1}$. Detection was performed on a SX-102A (TBBPA) or a 700D (PBDE) (Jeol, Tokyo, Japan) double focusing electromagnetic instrument (R=10,000). Electron ionization energy was set at 70 eV (TBBPA) or 42 eV (PBDE). Monitored ions were the two most intense ones among the $[M-CH_3]^+$, $[M]^{+\bullet}$ or $[M-Br_2]^{+\bullet}$ ion clusters, respectively for TBBPA, tri- to pentaBDE and hexa- to decaBDE.

Results and Discussion

General comments

This preliminary study concerned 26 maternal serum samples, 26 umbilical serum samples, 26 maternal adipose tissue samples and 23 breast milk samples, which were collected from Mars to September 2005. For serum, some practical and ethical limitations led to very restricted collected volumes. Thus, serum weight was no higher than 4 g per sample, which may appear critical considering the very low fat content of this matrix. For adipose tissue and milk, samples weights were around 0.5 g and 1 g dry matter, respectively. Women included in the study were between 24 and 46 year old (mean = 34).

Hexabromocyclododecane

In adipose tissue, α-HBCD was identified in approximately 50% of the analysed samples, with concentration values globally ranging from 1 000 $pg.g^{-1}$ fat (limit of detection) to 3 000 $pg.g^{-1}$ fat. For three samples, higher values (6 000 to 12 000 $pg.g^{-1}$ fat) were measured. No correlation was found with the values measured for PBDE. In milk samples, only seven samples had α-HBCD concentration above the limit of detection, varying from 2 500 to 5 000 $pg.g^{-1}$ fat. For serum samples, the insufficient sample amount as well as the performance of the LC-MS/MS equipment did not allowed any measurement of HBCD. An alternative MS system is planned to be used in the future in order to authorize the quantification of HBCD with a higher efficiency.

Tetrabromobisphenol A

TBBPA was not detected in none of the adipose tissue samples. In milk samples, TBBPA was quantified at levels varying from 34 to 9 400 $pg.g^{-1}$ fat (median value = 172 $pg.g^{-1}$ fat). No correlation was noticed with the concentrations of α-HBCD or PBDE, respectively. In serum samples, the estimated median and average values were 7 and 54 $pg.g^{-1}$ fw in maternal serum, and 10 and 152 $pg.g^{-1}$ fw in umbilical serum, respectively. No correlation was observed with the other BFR measured in serum. At present, only a poor correlation was observed between the levels measured for maternal and umbilical serum samples. Now, additional data has to be collected to interpret the latter results.

Tri- to heptabromobiphenylethers

For serum samples, the limited sample amount as well as the very low fat content which is characteristic of this biological matrix led to analytical results below the detection limit for most samples. Considering this sensitivity limitation linked to practical issues, PBDE congeners 28, 47, 99 and 100 were identified in only 2 maternal serum samples, with median values equal to 67, 964, 1 105 and 169 $pg.g^{-1}$ fat, respectively. On the contrary, congeners 154 and 183 were sufficiently abundant to be measured in nearly all the analysed sera. PBDE-153 was present at concentration ranging from 211 to 1 906 $pg.g^{-1}$ fat (median value = 689 $pg.g^{-1}$ fat) in maternal serum, and from 137 to 1 083 $pg.g^{-1}$ fat (median value = 408 $pg.g^{-1}$ fat) in umbilical serum. The average concentrations measured for this congener were the higher for fat (1 161 $pg.g^{-1}$ fat), followed in decreasing order by milk (828 $pg.g^{-1}$ fat), maternal serum (728 $pg.g^{-1}$ fat) and umbilical serum (436 $pg.g^{-1}$ fat), with significant correlations between these different matrices (R^2 ranging from 0.57 to 0.88).

In maternal adipose tissue samples (Fig. 1), the 7 major PBDE congeners (28, 47, 99, 100, 153, 154 and 183) were unambiguously identified. The sum of the concentrations calculated for these 7 compounds was in the 1 228 – 14 908 pg.g^{-1} fat range (median value = 2 515 pg.g^{-1} fat), which appears relatively close to other published European data. The contributions of the different congeners were: 153 (47%), 47 (25%), 183 (8.8%), 99 (8.2%), 100 (6.2%), 28 (2.8%) and 154 (1.8%). The high proportion of BDE-153 detected in these samples compared to its proportion in the PentaMix formulation (5%) could point out a selective bioaccumulation of PBDE in fat, but the eventual contribution of the highly brominated compounds (through debromination reactions) remained to be investigated. Other minor congeners (37, 49, 75, 85, 118, 155 and 190) were also identified in these samples, the sum of their concentrations reaching around 4% of the concentration observed for the 7 major congeners.

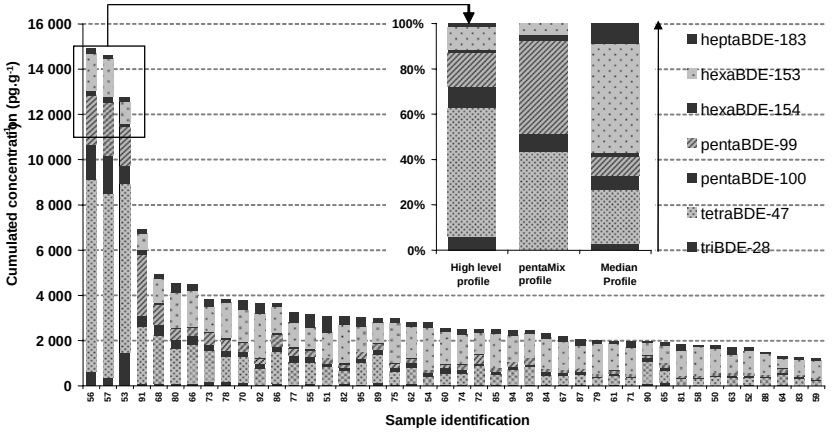

Fig. 1: Contamination profiles observed for tri- to heptaBDE in adipose tissue.

In breast milk samples (Fig. 2), the same 7 major PBDE congeners were also identified, with total cumulated concentrations ranging from 1 388 to 11 626 pg.g^{-1} fat (median value = 2 655 pg.g^{-1} fat). These values appear to be in the same range as the ones calculated for adipose tissue samples, and are comparable with other European published data. However, the contamination profiles observed in the two matrices are quite different. The correlations between the concentrations found in fat and milk samples are strong for BDE-28 and 100, but less obvious for the other congeners (Fig. 3). Other minor congeners (37, 49, 75, 85, 118, 155 and 190) were also identified, the sum of their concentrations reaching around 4.2% of the concentration measured for the 7 previous major congeners.

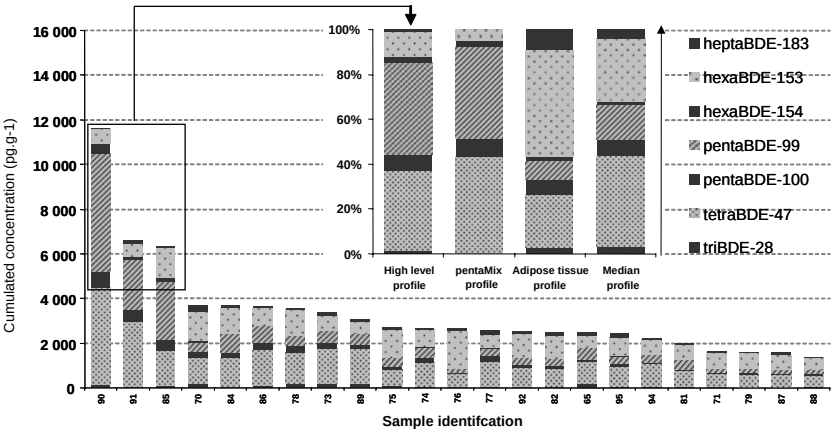

Fig. 2: Contamination profiles observed for tri- to heptaBDE in breast milk.

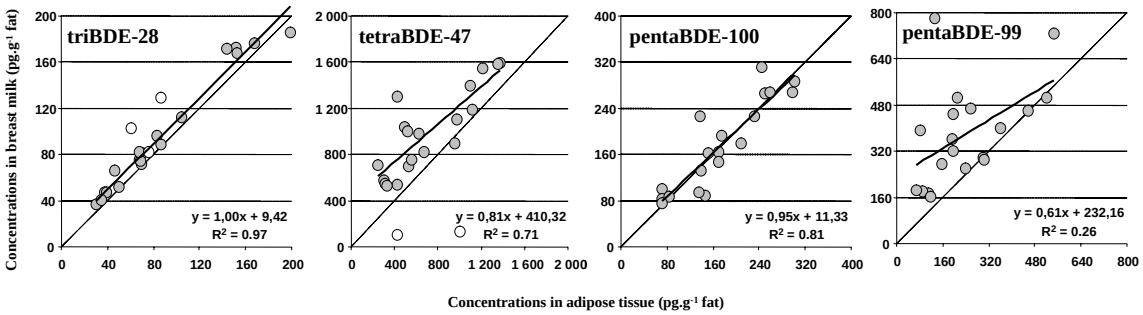

Fig. 3: Correlations between the concentrations measured for several tri- to hexaBDE in adipose tissue and milk.

Octa- to decabromodiphenylethers

In adipose tissue (Fig. 4), decaBDE-209 was quantified in all samples, with concentrations varying from 256 to 2 310 pg.g^{-1} fat (median value = 840 pg.g^{-1} fat), excepted one sample presenting an atypically high contamination level (16 856 pg.g^{-1} fat). Three nonaBDE and five octaBDE congeners were also quantified in these samples, with cumulated minimum / maximum / median values equal to 183 / 1 826 / 1 255 and 520 / 2 349 / 820 pg.g^{-1} fat for this two homologue groups, respectively. One nona- congener (BDE-207) and one octa- congener (#3) accounted for 80% and 70% of these total estimated concentrations, respectively.

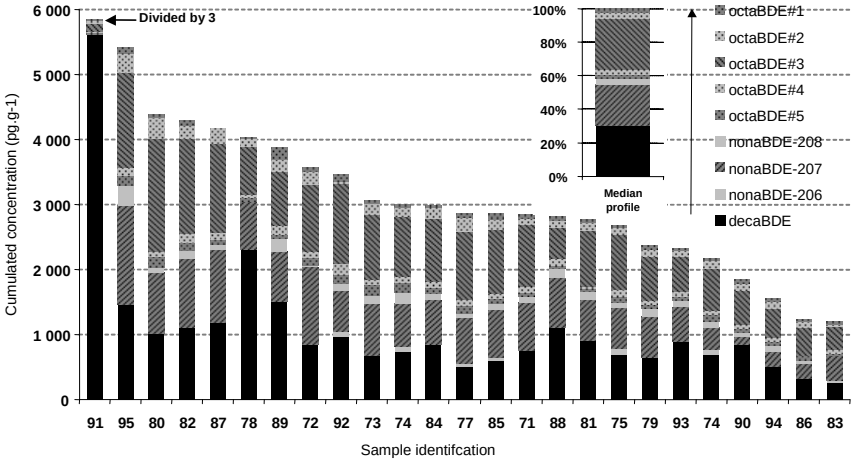

Fig. 4: Contamination profiles observed for octa- to decaBDE in maternal adipose tissue samples.

In milk samples, decaBDE-209 was quantified at concentrations ranging between 390 and 6 796 pg.g^{-1} fat (median value = 1 504 pg.g^{-1} fat). Nona- and octaBDE were also measured, with cumulated minimum / maximum / median values equal to 113 / 5 921 / 742 and 210 / 1 743 / 736 pg.g^{-1} fat for this two homologue groups, respectively. For serum samples, the median values estimated for decaBDE / nonaBDE / octaBDE were 7 404 / 2 898 / 1 560 pg.g^{-1} fat in maternal serum, and 28 769 / 6 263 / 808 pg.g^{-1} fat in umbilical serum, respectively. The high difficulty linked to measurements of PBDE and total lipid determination from very reduced sample size, as well as the poor correlations observed between maternal and umbilical serum regarding the presence of these highly brominated congeners indicate that the data set should be completed by additional samples before attempting to interpret the results.

Conclusion

A multi-residue analytical method was developed and validated for measuring brominated flame retardants (including tri- to decaBDE, TBBPA and HBCD) in various human biological matrices (serum, adipose tissue, breast milk). The application of the proposed methodology to more than 100 biological samples (maternal and newborn tissues) collected during caesarean deliveries provides the first exposure assessment data for a French population. Results demonstrate (1) a significant exposure of both the mothers and the foetus, especially to PBDE and TBBPA, and (2) a significant risk of overexposure of newborns through breast milk. Data regarding highly brominated PBDE congeners appear particularly informative and non commonly reported. Data collection as well as metabolism investigation is carried on, in order to provide complementary information.

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References

1. Cariou R, Antignac JP, Marchand P, Berrebi A, Zalko D, Le Bizec B, André F. *J Chromatogr A* 2005;1100:144.