

## Feasibility of GC-NCI-MS for the trace determination of tri- to deca- brominated diphenyl ethers in human samples

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### Introduction.

During recent years, a marked increase in the levels of brominated flame retardants (BFRs), especially polybrominated diphenyl ethers (PBDEs), in human tissues all over the world have been observed (Scheter et al., 2000; Tomsen et al., 2002). It is mainly due to the following facts: their production and use have undergone a dramatic increase starting in the 1980s (Sjödin et al., 2004) and their persistence and lipophilic character which tend to concentrate in the food chain, and thus accumulate in the human body (de Wit, 2002). The consumption in European countries of the different PBDE commercial mixtures available was estimated to be 150 metric tons of Penta-, 400 metric tons of Octa- and 7000 metric tons of Deca-BDE technical products (BSEF, 2000), which made the higher brominated PBDEs, mainly the PBDE 209, a matter of special concern in European countries. However, the majority of the data available in the literature are focused on tetra- to hexa-BDE congeners while little information is available on PBDEs 183 and 209 (the major components of the commercial flame retardant mixture Octa-BDE and Deca-BDE, respectively). In the same way, the concentrations concerning impurities of technical formulations and/or degradation products of PBDE 209, such as PBDEs 184, 191, 196, and 197 (Dandenourd et al., 2001; Stapleton et al., 2003), are very scarce in the literature. This fact could be related to the determination difficulties for high brominated PBDEs mainly when gas chromatography coupled with low resolution mass spectrometry (GC-MS) is used. GC-MS is a more selective technique than GC coupled to electron capture detector (ECD) but electron impact-MS (EI-MS) ionisation mode is less sensitive than GC-ECD. In this direction, negative chemical ionisation-MS (NCI-MS) operating mode has emerged as a valuable and more sensitive technique than EI-MS for the determination of PBDEs, mainly those with high bromination degree. On the other hand, human serum, umbilical cord serum, breast milk, and placenta samples are non-destructive matrices adequate for monitoring human exposure to PBDEs indicating both parents and neonates body burden. Although there is a wide knowledge on organochlorine contaminants in human tissues like serum and breast milk (Costopoulou et al., 2006; Chen et al., 2006), information regarding PBDE concentrations, mainly those of high-brominated congeners, in this kind of samples, is still very scarce in the literature. We present here, for the first time, PBDE levels, including tri- to deca- substituted congeners, found in human samples from the Spanish population. The preliminary results of an extended study of PBDE concentrations in paternal, maternal and neonate serum, breast milk, and placenta samples from individuals living in the Community of Madrid (Spain) are shown.

### Material and Methods.

A total of 10 maternal serum samples, 10 paternal serum samples, 10 umbilical cord serum samples, 10 placenta samples, and 10 breast milk samples were collected in 2005-2006 from volunteers living in the Community of Madrid (Spain). Once at the laboratory, serum samples were frozen at  $-20^{\circ}\text{C}$  and breast milk and placenta samples were freeze-dried and stored at room temperature until analysis. Serum samples were extracted and purified using a semi-automated solid phase extraction and clean-up method previously described (Gómara et al., 2002). The method used for breast milk and placenta

samples briefly consisted on a matrix solid phase dispersion (MSPD) of 0.2 g of freeze-dried breast milk and 1.0 g placenta. Fatty extract, containing PBDEs, were eluted using a mixture of acetone:*n*-hexane (1:1, v:v). Further clean-up and lipid removal was achieved by using acid and basic impregnated silica gel multilayer columns and *n*-hexane was used as elution solvent.

All PBDE standard were purchased from Wellington Laboratories (Ontario, Canada) and the fifteen congeners selected (Nos. 17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 184, 191, 196, 197, and 209) were determined using a 6890N gas chromatograph coupled with a 5975 quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA) in the negative chemical ionisation mode (GC-NCI-MS). Standards and samples were injected in a hot splitless mode (300 °C, 1 µL; splitless time 2.0 min) applying a pressure pulse of 30 psi during 2.10 min. For separation, a low bleed GC capillary column DB-5MS (15 m, 0.2 mm i.d., 0.2 µm film thickness) purchased from J&W Scientific (USA) was used. The column temperature was programmed as follows: 110 °C (1.5 min) to 200 °C at a rate of 30 °C/min, then to 275 °C at 5 °C/min, then to 300 °C (10 min) at 40 °C/min, and then to 310 °C (2 min) at 10 °C/min. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. The temperature of the transfer line, ion source and quadrupole were set at 310 °C, 150 °C, and 150 °C, respectively and methane was selected as reagent gas. The identification of target compounds was based on the detection, at the corresponding retention time, of the selected *m/z* values experimentally determined and characteristic of each congener.

## Results and Discussion.

First of all, ions to be monitored were experimentally determined in order to achieve the highest signal to noise ratios (S/N) for each congener. The two ions of bromine (*m/z* 79 and 81) were, in all cases, the most abundant ones. In addition, two more ions characteristic of each congener were selected both as qualifier ions and for the quantification of the samples. Using those four specific ions, the analytical characteristics of the instrumental GC-NCI-MS method were studied in terms of repeatability, intermediate precision, instrumental limits of detection (LODs), and linear ranges. The repeatability was calculated as the relative standard deviation (RSD, %) of the areas corresponding to three consecutive injections and the intermediate precision was expressed as the RSD (%) of the areas of four injections carried out in different days along two weeks. The results obtained for repeatability and intermediate precision were below 6% and 11%, respectively, for all congeners investigated except PBDE 209, whose RSD were 9% and 19% for repeatability and intermediate precision, respectively. The instrumental LODs, calculated using standard solutions, were in the range of 6 to 507 fg. The ions monitored as well as the analytical characteristic of the method corresponding to each PBDE are shown in Table 1. The concentration range investigated (1-400 pg for all congeners except PBDE 209, 2-800 pg) was linear in all cases with regression coefficients higher than 0.991.

The developed instrumental method was then applied to the quantification of target PBDEs in maternal serum, paternal serum, umbilical cord serum, placenta, and breast milk samples. Table 2 summarised the preliminary results of an extended study concerning the concentrations of tri- to deca-BDE in serum, placenta and breast milk samples. The concentrations are expressed in pg/mL of serum and in pg/g wet weight (w.w.) for breast milk and placenta. Breast milk and umbilical cord serum samples exhibit the highest median total concentrations (1291 pg/g w.w. and 1291 pg/mL, respectively) among all sample types investigated, following by paternal serum (903 pg/mL), maternal serum (680 pg/mL) and placenta samples (287 pg/g w.w.).

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Table 1. Analytical characteristic of the GC-NCI-MS method used for tri- to deca-BDE determinations.

PBDE No.	Ions monitored (m/z)	Repeatability (% RSD, n = 3)	Intermediate precision (% RSD, n = 4)	LODs (fg)
17	79/81 [Br] <sup>-</sup> + 159/161 [HBr <sub>2</sub> ] <sup>-</sup>	1	5	20
28	79/81 [Br] <sup>-</sup> + 159/161 [HBr <sub>2</sub> ] <sup>-</sup>	2	4	27
47	79/81 [Br] <sup>-</sup> + 159/161 [HBr <sub>2</sub> ] <sup>-</sup>	1	5	12
66	79/81 [Br] <sup>-</sup> + 159/161 [HBr <sub>2</sub> ] <sup>-</sup>	1	4	44
100	79/81 [Br] <sup>-</sup> + 403/405 [M-HBr <sub>2</sub> ] <sup>-</sup>	1	2	28
99	79/81 [Br] <sup>-</sup> + 403/405 [M-HBr <sub>2</sub> ] <sup>-</sup>	2	3	18
85	79/81 [Br] <sup>-</sup> + 403/405 [M-HBr <sub>2</sub> ] <sup>-</sup>	2	3	49
154	79/81 [Br] <sup>-</sup> + 483/485 [M-HBr <sub>2</sub> ] <sup>-</sup>	2	4	47
153	79/81 [Br] <sup>-</sup> + 483/485 [M-HBr <sub>2</sub> ] <sup>-</sup>	3	4	80
184	79/81 [Br] <sup>-</sup> + 407/409 [M-HBr <sub>4</sub> ] <sup>-</sup>	3	8	6
183	79/81 [Br] <sup>-</sup> + 562/564 [M-HBr <sub>2</sub> ] <sup>-</sup>	3	6	27
191	79/81 [Br] <sup>-</sup> + 562/564 [M-HBr <sub>2</sub> ] <sup>-</sup>	4	6	15
197	79/81 [Br] <sup>-</sup> + 407/409 [M-HBr <sub>5</sub> ] <sup>-</sup>	4	11	45
196	79/81 [Br] <sup>-</sup> + 407/409 [M-HBr <sub>5</sub> ] <sup>-</sup>	6	8	9
209	79/81 [Br] <sup>-</sup> + 487/489 [M-HBr <sub>6</sub> ] <sup>-</sup>	9	19	507

Table 2. PBDE median concentrations and range (indicated in brackets) in serum, breast milk and placenta samples from volunteers living in Community of Madrid (Spain). The concentrations are expressed in pg/mL of serum and in pg/g wet weight (w.w.) for breast milk and placenta.

PBDE No.	Maternal serum (n=10)	Paternal serum (n=10)	Umbilical cord serum (n=10)	Placenta (n=10)	Breast milk (n=10)
17	ND	ND	ND	ND	ND
28	2.6 (ND–2.7)	10 (ND–45)	161 (ND–287)	ND	ND
47	102 (88–137)	103 (93–107)	166 (116–397)	24 (ND – 28)	65 (50–79)
66	ND	ND	ND	ND	ND
100	47 (43–50)	46 (44–49)	67 (54–133)	7.7 (5.4 – 9.2)	41 (28–47)
99	114 (107–138)	113 (108–202)	168 (144–310)	19 (14 – 22)	81 (61–88)
85	123 (ND–152)	113 (ND–118)	212 (ND–317)	16 (ND – 19)	80 (64–91)
154	83 (ND–93)	83 (ND–84)	118 (ND–240)	14 (ND – 15)	56 (48–69)
153	115 (ND–125)	110 (ND–110)	186 (ND–217)	19 (ND – 20)	75 (ND–142)
184	ND	ND	102 (ND–121)	ND	56 (ND–67)
183	120 (ND–144)	125 (ND–131)	172 (ND–339)	22 (16 – 28)	84 (68–383)
191	ND	ND	ND	ND	ND
197	123 (ND–126)	119 (ND–120)	218 (ND–233)	21 (15 – 24)	87 (73–590)
196	ND	169 (ND–169)	235 (ND–245)	20 (ND – 27)	115 (ND–170)
209	1128 (ND–1184)	1022 (ND–1328)	1566 (ND–1893)	167 (123 – 202)	788 (ND–2967)
<b>Median</b>					
<b>Total PBDEs</b>	<b>680 (446–1953)</b>	<b>903(339–1994)</b>	<b>1291 (606–3988)</b>	<b>287 (209 – 360)</b>	<b>1291 (432–4680)</b>