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Development and application of a simplified clean-up procedure for the determination of PCBs and PBDEs in horse fat by gas chromatography-tandem mass spectrometry

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3 1 **Development and application of a simplified clean-up procedure for the**
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6 2 **determination of PCBs and PBDEs in horse fat by gas chromatography-**
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9 3 **tandem mass spectrometry**
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12 4

13
14 5 **Abstract**
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17
18 7 A simplified clean-up procedure was developed in combination with GC-MS/MS for the
19
20 8 determination of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers
21
22 9 (PBDEs) in adipose tissue. Clean-up was performed by the successive application of a Mega
23
24 10 Bond Elut[®] silica column and a Bond Elut[®] PCB column. Validation of the method was
25
26 11 conducted according to EU Commission Decision 2002/657/EC. In order to evaluate the
27
28 12 applicability of the method, 44 horse fat samples were analysed. The total PCB concentration
29
30 13 (sum of PCBs 28, 52, 101, 118, 138, 153, 180) ranged from 5.35 to 140 ng g⁻¹ lipid weight.
31
32 14 The total PBDE concentration (sum of BDEs 28, 47, 99, 100, 153, 154, 183) ranged from
33
34 15 below the decision limit to 6.34 ng g⁻¹ lipid weight.
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41 17 **Keywords:** *PBDEs, PCBs, solid phase extraction, horse fat.*
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22 Introduction

23
24
25 Polybrominated diphenyl ethers (PBDEs) have been used to protect the public from accidental
26 fires by reducing the flammability of combustible materials, e.g. plastics and synthetic
27 polymers (WHO – EHC 162, 1994). Nowadays, they are a matter of concern because they
28 have been identified worldwide, in both aquatic and terrestrial compartments, as newly
29 identified environmental contaminants (Gama et al., 2006; Law et al., 2006; Hites, 2004).

30 Polychlorinated biphenyls (PCBs) have been mainly produced from the 1930s to the
31 1970s as complex mixtures, containing many different congeners. Good thermal and chemical
32 stability and electrical insulating properties favoured use of PCBs in a variety of industrial
33 applications (WHO – EHC 140, 1992).

34 Both PCBs and PBDEs bioaccumulate through the food chain (Domingo, 2004; Sjödin
35 et al., 2003) and as a consequence humans are significantly exposed to both types of
36 contaminants through their diet. The toxicological profile of PBDEs and especially of PCBs is
37 mainly dependent on the type of congener. Generally, PBDEs have been shown to act as
38 thyroid hormone disruptors (Meerts et al., 2000) and neuro-developmental toxicants (Branchi
39 et al., 2003). The IARC has classified PCBs as probable carcinogenic to humans (Group 2A)
40 (IARC, 1987). Moreover, they have also been shown to cause a number of non-cancerous
41 health effects, e.g. immunotoxicity (Levin et al. 2005), reproductive toxicity (Pocar et al.,
42 2006), neurotoxicity (Pabello et al., 2006) and endocrine disruption (Tabuchi et al., 2006).

43 According to Directive 2002/95/EC or the RoHS Directive (OJ L 37, 2003), EU
44 Member States should ensure that from the 1st of July 2006 onwards, new electrical and
45 electronic equipment put on the market, does not contain any PBDEs. Deca-BDE was
46 exempted from the RoHS Directive on the 15th of October 2005, on the basis of the
47 conclusions of a 10-year European Union environmental and human health risk assessment
48 (OJ L 271, 2005). Prior to this, the use of penta-BDE and octa-BDE in all applications for the

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2
3 49 European market has been banned since the 15th of August 2004 (OJ L 42, 2003). PCBs are
4
5 50 one of the 12 persistent organic pollutants (POPs) that have been listed by the Stockholm
6
7 51 Convention. Belgian legislation has set maximum limits for the total sum of seven marker
8
9 52 PCB congeners (PCBs 28, 52, 101, 118, 138, 153, 180) in different food matrices.

12 53 There is a need for simple and sensitive analytical methodology for these two groups
13
14 54 of components. Most methods, developed for their determination in biological samples and
15
16 55 more specific in adipose tissue, have the disadvantage of being very laborious. They usually
17
18 56 consist of a lipid extraction step using a non-polar and/or polar solvent. Further clean-up and
19
20 57 removal of co-extracted lipids is performed by gel permeation chromatography, adsorption
21
22 58 chromatography and/or multilayer silica clean-up with sulphuric acidic treatment. The final
23
24 59 determination is generally carried out by GC coupled with high or low resolution mass
25
26 60 spectrometry (MS) or electron capture detection (ECD) (de Boer et al., 2001; de Boer et al.,
27
28 61 2002; Pirard et al., 2002; Ramos et al., 2004; Saito et al., 2004).

30 62 The aim of the work reported here was to develop a practical, non-laborious, yet
31
32 63 sensitive analytical method for the determination of PCBs and PBDEs in adipose tissue. In
33
34 64 order to evaluate the newly developed method, horse fat samples were analysed. Within the
35
36 65 European Union the Italians are consuming most horse meat *per capita*, followed by the
37
38 66 Belgians (Hertrampf, 2003). In 2004, Belgium was the main importer of horse meat and the
39
40 67 second largest exporter of horse meat, in terms of quantity (Food and Agricultural
41
42 68 Organization of the United Nations, 2004). Horses for human consumption have been mainly
43
44 69 working animals or horses that were reared for recreational riding or racing (Martuzzi et al.,
45
46 70 2001; Gill, 2005). Horses may be exposed to environmental contaminants through the
47
48 71 ingestion of contaminated feed, contaminated soil or contaminated water (Rhind, 2002).
49
50 72 Considering that horses for human consumption have had a long life-span and come from
51
52 73 different backgrounds makes them interesting cases to study POPs.

74

75 Experimental*76 Chemicals*

77 All reagents and solvents were of analytical grade. Isooctane, n-hexane Suprasolv[®] and
78 anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany). Nonane was
79 purchased from Sigma Aldrich nv/sa (Bornem, Belgium). Bond Elut[®] SPE cartridges were
80 purchased from Varian Inc. (Sint-Katelijne-Waver, Belgium).

81 Individual PBDE standards (IUPAC Nos. 28, 47, 99, 100, 153, 154, and 183) were
82 purchased from Wellington Laboratories (Guelph, Ontario, Canada). Individual PCB
83 standards (IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180) together with PCB-Mix 3 (10 ng
84 μl^{-1} of each congener), internal standards (polybrominated biphenyl (PBB)103 and PCB 143)
85 and injection standards (mirex and PBB 155) were purchased from Dr. Ehrenstorfer
86 (Augsburg, Germany). Certified reference material, ERM-BB446, pork fat (sum of 7 PCBs:
87 $207 \pm 11 \text{ ng g}^{-1}$) was obtained from the Institute for Reference Materials and Measurements
88 (IRMM, Geel, Belgium). Stock solutions were stored between 2 and 8°C, protected from
89 light. Suitable PBDE and PCB working solutions were prepared daily by dilution in nonane
90 and isooctane, respectively

91

92 Apparatus

93 Analysis of cleaned-up extracts was carried out using a Finnigan GCQ gas chromatograph
94 coupled to a Finnigan GCQ mass spectrometer (Austin, Texas, USA). The autosampler was a
95 CTC 200 series injector (Zwingen, Switzerland). PCB and PBDE congeners were separated
96 on a HT-8 capillary column (25 m x 0.22 mm x 0.25 μm , SGE, Achrom, Zulte, Belgium).
97 Carrier and collision gas was Alphagaz 2 helium (Air Liquide, Liege, Belgium). Instrument
98 set points and data acquisition were under the control of GCQ software.

99

100 *Sample collection and storage*

101 Horse fat samples were collected from March until June 2006 from a local slaughterhouse and
102 stored at -20°C. Details of sample collection e.g. age and slaughter dates are presented in
103 Table 1. A pool of extracted chicken fat, from samples taken within the framework of the
104 Belgian monitoring programme for PCBs, was used as blank for validation and quality control
105 purposes. A sample of this pool was analysed for PBDEs and a new pool was prepared if
106 PBDE levels were above the decision limit ($CC\alpha$). Because levels of PCB 118, PCB 138,
107 PCB 153 and PCB 180 in the blank pool were occasionally higher than the decision limit,
108 validation and sample results were corrected accordingly.

109 [Insert Table 1 about here]

110

111 *Extraction and clean-up*

112 To prevent contamination, glassware was washed extensively and rinsed twice with hexane
113 (Acros, Geel, Belgium). Approximately 10 g of adipose tissue was placed on a folded Ederol
114 filter paper (VWR, Leuven, Belgium) in a glass funnel together with anhydrous sodium
115 sulphate (10 g). The fat was melted in a microwave oven (600 W, 2 min) and collected in a
116 glass vessel. Internal standards PCB 143 (50 μl 0.1 $\text{ng } \mu\text{l}^{-1}$) and PBB 103 (20 μl 0.1 $\text{ng } \mu\text{l}^{-1}$),
117 together with 1 ml n-hexane were added to 0.5 g of melted fat.

118 A Mega Bond Elut[®] silica column (2 g) was conditioned with 12 mL of n-hexane.
119 Subsequently, the sample was passed through the column without applying vacuum. Another
120 9 mL of n-hexane were used to elute the analytes from the column. The eluate was evaporated
121 under a gentle nitrogen stream at 40°C to approximately 1 mL. A Bond Elut[®] PCB column
122 was conditioned with 4 mL of n-hexane. Consequently the reduced eluate was applied to the
123 column and eluted once more without applying vacuum. Two mL of n-hexane were used to

1
2
3 124 elute the analytes under light vacuum. This step was repeated three additional times to an
4
5 125 accumulated volume of 9 mL. After evaporation to approximately 4 mL and transfer to a
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8 126 graduated vial, injection standards PBB 155 (20 μL 0.1 ng μL^{-1}) and mirex (50 μL 0.1 ng
9
10 127 μL^{-1}) and keeper solvent isooctane were added. This mixture was concentrated under a gentle
11
12 128 nitrogen stream at 40 °C to 100 μL and put into a GC-MS vial.
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15 129

17 130 *Gas chromatography/mass spectrometry*

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19 131 Aliquots of the final extract (2 μl) were injected in the splitless mode with a splitless period of
20
21 132 1 min following injection. Injection temperature was set at 300°C and the transfer line
22
23 133 temperature was 275°C. The oven was programmed from 70 °C for 1 min to 170 °C at a rate
24
25 134 of 30 °C min^{-1} , then to 300°C (15 min) at a rate of 8 °C min^{-1} . Optimisation of GC-MS/MS
26
27 135 parameters has been elaborately described elsewhere (Naert et al., 2004; Naert et al., 2006;
28
29 136 Naert et al.; accepted for publication in Chemosphere). Detection of all analytes was done in
30
31 137 electron impact MS/MS mode. In order to achieve as much selectivity as possible, the most
32
33 138 abundant ion of the molecular cluster was chosen as precursor ion for collision induced
34
35 139 dissociation. Excitation voltage, excitation time and q-value are presented in Table 2.
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41 140 [Insert Table 2 about here]
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46 142 *Validation*

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48 143 Validation of the method was carried out according to Commission Decision 2002/657/EC
49
50 144 (OJ L 221, 2002). Specificity, decision limit ($CC\alpha$), detection capability ($CC\beta$), recovery,
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52 145 precision, bias and trueness were determined as required for a quantitative confirmatory
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54 146 method. Because levels of PCB 118, PCB 138, PCB 153 and PCB 180 in the blank pool were
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56 147 occasionally higher than the decision limit, a blank subtraction was applied in order to correct
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58 148 validation and sample results accordingly.
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3 149 $CC\alpha$ and $CC\beta$ were established by calibration curve procedure and this was repeated 5
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5 150 times. Blank fat samples were fortified with a mixture of 7 PBDE congeners at a level of 1, 2,
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7
8 151 4, 8, 15 ng g^{-1} per congener and a mixture of 7 PCB congeners at 1, 5, 10, 20, 50 ng g^{-1} per
9
10 152 congener. Subsequently, the signal was plotted against the added concentration. The decision
11
12 153 limit or $CC\alpha$ ($\alpha = 1\%$) corresponds to the concentration at the y-intercept plus 2.33 times the
13
14 154 standard deviation of the within-laboratory reproducibility of the intercept. Detection
15
16 155 capability or $CC\beta$ ($\beta = 5\%$) equals the concentration at the decision limit plus 1.64 times the
17
18 156 standard deviation of the within-laboratory reproducibility of the mean measured at the
19
20 157 decision limit.

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24 158 Specificity for all congeners, except for PCB 118, PCB 138, PCB 153 and PCB 180,
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26 159 was investigated by analysing different blank samples for interferences. For the determination
27
28 160 of recovery, bias, within day and between day precision, 18 blank fat samples were fortified at
29
30 161 3 levels (2, 4 and 8 ng g^{-1} per congener for PBDEs and 5, 10 and 20 ng g^{-1} per congener for
31
32 162 PCBs). Recovery is defined as that fraction of mass of the analyte added to the sample which
33
34 163 is present in the final extract. The responses of the fortified samples were compared with
35
36 164 samples fortified after clean-up. Within-day and between-day precision were expressed as a
37
38 165 coefficient of variation (CV %). Trueness was determined by means of analysis of purchased
39
40 166 CRM material (ERM-446). Certified values for PCB 28, 52, 101, 118, PCB 138, PCB 153
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42 167 and PCB 180 and an indicative value for BDE 47 were given. Measurement uncertainty was
43
44 168 determined according to the EURACHEM/CITAC guide (Ellison et al., 2002). The global
45
46 169 uncertainty was calculated as a combination of the uncertainty associated with precision and
47
48 170 the uncertainty associated with accuracy. Although this is the combined uncertainty, the
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50 171 results are expressed as an expanded uncertainty, which corresponds to twice that value.

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53 172 The absolute matrix effect was assessed by ratio of the peak area of the analyte spiked
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55 173 to a blank extract after sample clean-up by the peak area obtained for standards.

174

175 Results and discussion

176

177 Extraction and clean-up

178 An extensive clean-up procedure using glass columns filled with sodium sulphate, aluminium
179 oxide and sulfuric acid was previously described elsewhere (Naert et al., 2004; Naert et al.,
180 2006; Naert et al., accepted for publication in Chemosphere). Since this method was laborious
181 and large volumes of solvent were consumed, it was the authors' intention to simplify this
182 clean-up procedure by using disposable solid phase extraction (SPE) columns. Fat removal
183 properties of silica, florisil and alumina (activity III) were compared and the Mega Bond
184 Elut[®] silica column (2 g) yielded best results. Still, further clean-up was required. A number
185 of additional commercial SPE columns, e.g. Bond Elut[®] PCB (Varian, Inc.), Mega Bond
186 Elut[®] SCX (2 g) (Varian, Inc.), Sep-Pak[®] florisil cartridge (Waters, Zellik, Belgium), Mega
187 Bond Elut[®] silica (2 g) (Varian, Inc.), PCB-A (Malinckrodt Baker, Boom, Diegem, Belgium),
188 and an in-house made column (2 g) filled with silica, acidified silica or aluminium oxyde
189 were assessed. Best results and cleanest chromatograms were obtained with a Mega Bond
190 Elut[®] silica column (2 g) in combination with the Bond Elut[®] PCB column. This column is a
191 dual phase SPE column consisting of a strong cation exchange bed and a silica bed. It has
192 been developed for the extraction of PCBs in transformer oil and organic matrices.

193

194 Validation

195 A summary of the validation results is presented in Table 3. Decision limits for PCBs and
196 PBDEs ranged from 0.53 - 1.73 ng g⁻¹ lipid weight and from 0.30 - 1.07 ng g⁻¹ lipid weight,
197 respectively. Detection capabilities for PCBs and PBDEs varied between 1.23 - 3.65 ng g⁻¹
198 lipid weight and between 0.78 - 1.90 ng g⁻¹ lipid weight, respectively. Precision, expressed as

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3 199 the coefficient of variation (CV %), did not exceed 15.7 %, indicating the good repeatability
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5 200 of the method. Recoveries were between 78.1 - 87.8 % for PCBs and between 84.4 - 99.3 %
6
7 201 for PBDEs. No interfering peaks for PBDEs and PCB 28, PCB 52 and PCB 101 could be
8
9 202 detected when analysing different blank fat samples. Specificity for PCB 118, PCB 138, PCB
10
11 203 153 and PCB 180 could not be established because of the difficulty to obtain blank animal fat
12
13 204 for these congeners. To ensure that separation of PCBs and PBDEs was adequate, separation
14
15 205 of PCB 28 and PCB 31 was verified with every sample sequence.

16
17 206 In the concentration ranges 1 - 15 ng g⁻¹ and 1 - 50 ng g⁻¹ for PBDEs and PCBs, respectively,
18
19 207 correlation coefficients (R²) were all higher than 0.990. The percent bias was within the - 30
20
21 208 % to +10 % range and - 20 % to +10 % range, as prescribed by Commission Decision
22
23 209 2002/657/EC, for concentrations ranging from >1-10 ng g⁻¹ and ≥ 10 ng g⁻¹, respectively.
24
25 210 Trueness, established by analysis of certified reference material (ERM-449) varied between
26
27 211 98.7 % and 107.9 % for PCBs. Trueness for BDE 47 was 109.4%. Expanded measurement
28
29 212 uncertainty ranged from 19.1 to 37.2 % for PCBs and from 18.1 to 34.9 % for PBDEs.

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36 213 [Insert Table 3 about here]

37
38 214 The absolute matrix effect ranged from 62.9 % to 105.9 % for all compounds at three
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40 215 concentration levels. Furthermore, a calibration curve in matrix is prepared with each sample
41
42 216 sequence to compensate for absolute matrix effects. Relative matrix effects are compensated
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44 217 by the use of an internal standard.

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47 218 Identification criteria, as laid down by Commission Decision 2002/657/EC (OJ L 221,
48
49 219 2002), were fulfilled. Signal-to-noise ratios for each diagnostic ion were larger than or equal
50
51 220 to three. Relative retention times (RRTs) of the analyte did not differ by more than +/- 0.5 %
52
53 221 of the RRT of the calibration standard. The relative intensities of the detected ions, expressed
54
55 222 as a percentage of the intensity of the most abundant ion, corresponded within the tolerances
56
57 223 specified by Commission Decision 2002/657/EC.

224

225 *Analysis of horse fat samples*

226 In order to evaluate the method, 44 horse fat samples from a local slaughterhouse were
227 analysed for 7 indicator PCBs (PCB 28, 52, 101, 118, 138, 153 and 180) and 7 PBDE
228 congeners (BDE 28, 47, 99, 100, 153, 154 and 183). PCBs were identified in all samples and
229 PBDEs were identified in all but 6 samples. A summary of the PBDE and PCB levels is listed
230 in Tables 1 and 4. [Insert Table 4 about here] A PCB and a PBDE chromatogram of a horse
231 adipose tissue extract (sample N° 38) are presented in Figures 1 and 2. [Insert Figures 1 and 2
232 about here]

233 The sum of the seven PCB congeners ranged between 5.35 and 140 ng g⁻¹ lipid weight
234 (median 28.9 ng g⁻¹ lipid weight). No sample exceeded the maximum permitted level of 200
235 ng g⁻¹ fat as laid down by the Belgian legislation. Levels were higher than levels found in
236 meat and meat products from Brazil (median 10.30 ng g⁻¹) (Costabeber et al., 2006), in pork
237 fat (range: 2.6 – 6.3 ng g⁻¹) and pork meat (range: 8.6 – 17.5 ng g⁻¹) from Romania (Covaci et
238 al., 2004) and in beef (median 0.284 ng g⁻¹), pork (median 0.234 ng g⁻¹) and chicken (median
239 0.241 ng g⁻¹) from South Korea (Kim et al., 2004). Levels corresponded extremely well with
240 a study by Pirard et al. (2002). In this study poultry, horse, pork and beef samples were
241 analysed and horse samples also showed higher background levels of marker PCBs (21.6 ng
242 g⁻¹ fat). It has been suggested that this might be due to the longer life-span of horses compared
243 to pigs, cattle or chickens (Focant et al., 2002). In the present study, no significant difference
244 in PCB concentration has been found between adult horses or ponies and foals (t-test = -
245 1.070; Sig. = 0.315). Perhaps diet can also be a major contributing factor because the
246 composition of the diet may vary according to age, breed and type of labour. This conclusion
247 should however be interpreted with caution because only eight adult animals were included in
248 this study.

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3 249 PCB 153 was the predominant congener in every sample accounting for 43 % of the
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5 250 total PCB concentration (range: 31 - 74 %). This is in accordance with the notion that the
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8 251 proportion of the higher chlorinated congeners is larger in organisms situated at the top of the
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10 252 food chain (WHO – EHC 140, 1992).

11
12 253 The total PBDE concentration ranged from below the decision limit (CC α) to 6.34 ng
13
14 254 g⁻¹ lipid weight (median 1.35 ng g⁻¹ lipid weight). Comparisons with other studies are difficult
15
16 255 because most studies are market basket based studies. PBDE levels found in this study are
17
18 256 comparable to levels found by Huwe et al. (Huwe et al., 2002) in chicken and higher than
19
20 257 levels found in meat products from Catalonia (Bocio et al., 2003). BDE 47 was the
21
22 258 predominant congener in all but one sample (range: 64 – 100 %). This is in contrast with a
23
24 259 market basket based study from the US where BDE 99, followed by BDE 47, predominates in
25
26 260 meat samples (Schechter et al., 2004).

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28
29 261 No significant difference in PBDE concentration was found between adult horses or
30
31 262 ponies and foals (t-test = 0.735; Sig. = 0.849). Significant but low correlation could be
32
33 263 established between the sum of PCBs and PBDEs (Spearman's rho = 0,531, Sig. < 0.0005).

34
35 264

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38
39 267

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Sample	Furnisher	Slaughter date	Age	Sum PCB ^a	Sum PBDE ^a	Sample	Furnisher	Slaughter date	Age	Sum PCB ^a	Sum PBDE ^a
1	1	27.03.06	-	26.6	0.98	23	4	02.05.06	foal	23.2	1.37
2	2	10.04.06	-	31.4	1.74	24	2	06.06.06	-	9.84	<CC α
3	3	04.04.06	-	27.7	1.09	25	5	08.05.06	adult	7.43	<CC α
4	4	14.04.06	-	20.2	1.02	26	6	02.05.06	foal	20.5	0.93
5	2	18.04.06	-	16.7	0.92	27	2	25.04.06	foal	17.8	1.75
6	5	27.04.06	-	75.4	1.32	28	2	08.05.06	foal	8.98	<CC α
7	2	21.03.06	-	41.4	4.22	29	2	24.04.06	-	67.6	<CC α
8	2	11.04.06	-	40.7	3.77	30	2	24.06.06	foal	37.6	4.61
9	2	21.03.06	-	24.2	1.70	31	2	04.05.06	adult	8.15	1.65
10	2	27.03.06	-	16.5	1.14	32	2	06.06.06	-	14.6	<CC α
11	2	18.04.06	-	44.7	3.45	33	2	30.05.06	-	65.9	1.87
12	2	11.04.06	-	17.2	6.34	34	4	29.05.06	adult	140	4.70
13	4	30.03.06	-	32.6	1.23	35	2	22.06.06	foal	5.35	<CC α
14	2	27.03.06	-	40.0	1.56	36	4	23.06.06	foal	32.3	1.03
15	5	16.05.06	adult	73.8	2.98	37	2	29.06.06	foal	7.81	0.87
16	2	22.05.06	foal	22.6	1.92	38	2	30.05.06	foal	79.4	5.73
17	2	22.05.06	foal	32.3	3.63	39	5	12.06.06	adult	30.2	0.68
18	2	22.05.06	foal	15.4	1.92	40	5	24.04.06	adult	49.3	1.23
19	4	11.05.06	adult pony	19.6	2.11	41	2	13.06.06	-	36.2	1.93
20	2	15.05.06	foal	36.3	3.74	42	2	13.06.06	foal	12.4	0.93
21	2	15.05.06	foal	37.0	1.06	43	2	13.06.06	foal	34.2	1.03
22	2	06.06.06	-	31.7	2.12	44	5	07.06.06	adult	23.5	0.84

^a Concentrations below the decision limit (CC α) were not taken into account for the calculations of the sum.

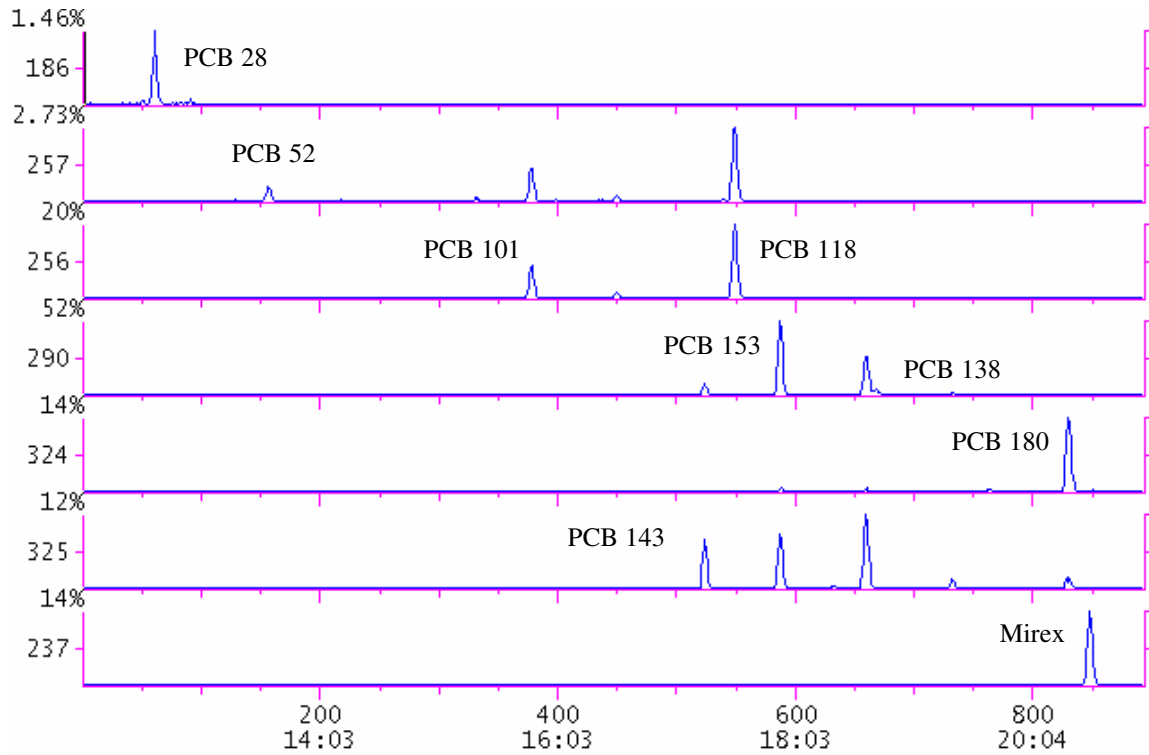
PCB congener	q-value	Excitation voltage (V)	Excitation time (ms)	PBDE congener	q-value	Excitation voltage (V)	Excitation time (ms)
PCB 28	0.450	1.8	15	BDE 28	0.300	1.5	15
PCB 52	0.450	1.8	15	BDE 47	0.300	2.0	15
PCB 101	0.450	2.0	15	BDE 99	0.300	2.0	15
PCB 118	0.450	2.0	15	BDE 100	0.300	2.0	15
PCB 138	0.450	1.8	15	BDE 153	0.300	2.5	15
PCB 153	0.450	1.8	15	BDE 154	0.300	2.5	15
PCB 180	0.450	1.8	15	BDE 183	0.300	3.0	15

Compound	CC α (ng g ⁻¹ lipid weight)	CC β (ng g ⁻¹ lipid weight)	Precision (CV %)		Bias ^a (%) (n = 6)	Recovery ^a (n = 6) (%)	Expanded measurement uncertainty (%)	Trueness (n = 4) (%)	CV (%)
			Within day ^a (n = 6)	Between day ^a (n = 6)					
PCB 28	0.53	1.25	4.77	11.4	-3.4	78.1	19.1	101	15.6
PCB 52	0.65	1.52	8.40	8.67	-2.3	82.5	27.4	98.7	15.1
PCB 101	0.59	1.23	7.44	9.94	-1.6	81.6	23.7	103	16.1
PCB 118	0.70	1.60	7.64	11.0	-0.1	83.2	36.3	104	15.4
PCB 138	0.60	1.46	8.02	7.58	-1.9	86.6	30.9	102	10.1
PCB 153	1.73	3.65	13.05	16.4	2.0	85.6	37.2	103	6.78
PCB 180	0.56	1.29	9.46	7.44	-5.1	87.8	24.7	108	10.1
BDE 28	0.41	0.78	5.74	7.02	0.5	94.3	18.1	109	3.98
BDE 47	0.60	1.14	4.98	13.9	4.5	96.3	27.0	-	-
BDE 99	0.30	0.85	3.61	8.80	-1.7	94.7	19.4	-	-
BDE 100	0.42	0.80	2.58	11.3	10	98.8	15.4	-	-
BDE 153	0.72	1.66	3.49	13.1	-5.2	96.0	22.4	-	-
BDE 154	0.50	1.10	10.9	12.1	-1.7	99.3	29.4	-	-
BDE 183	1.07	1.90	10.1	10.5	-2.4	84.4	34.9	-	-

^a Data obtained at the 4 ng g⁻¹ level for PBDEs and the 10 ng g⁻¹ level for PCBs.

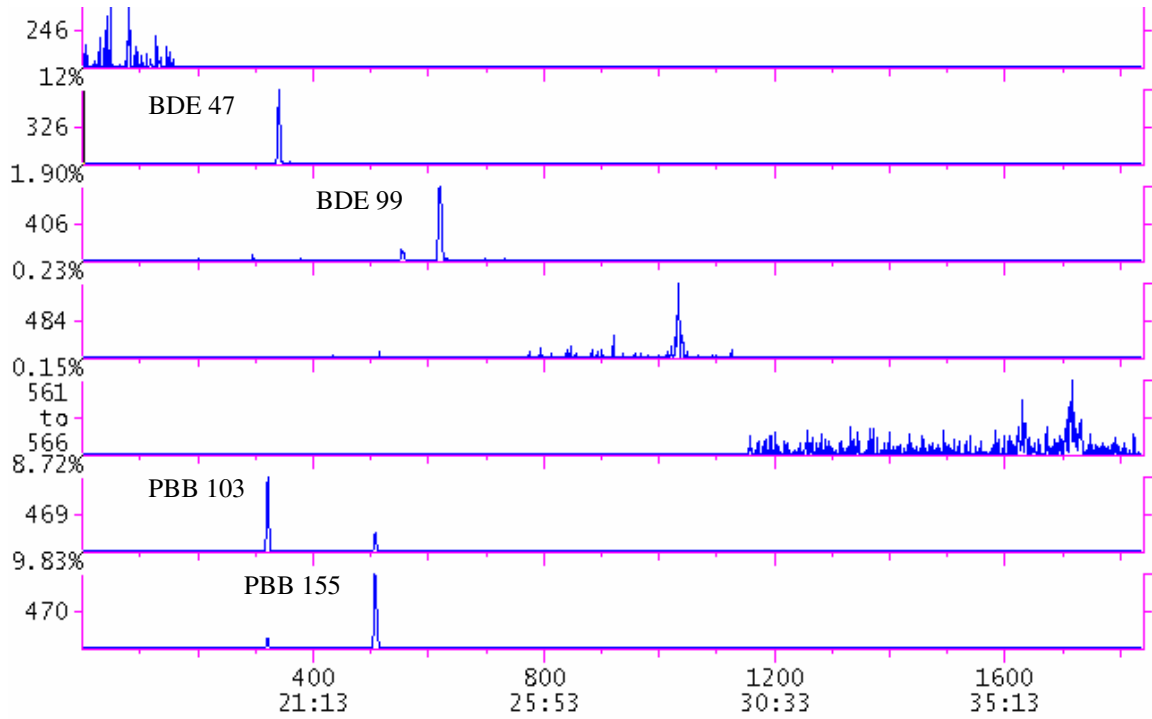
Compound	Mean	Median	Range
	(ng g ⁻¹ lipid weight)		
PCB 28	1.13	1.15	<CC α - 4.87
PCB 52	<CC α	<CC α	<CC α - 2.78
PCB 101	<CC α	<CC α	<CC α - 4.62
PCB 118	5.96	4.98	1.11 - 18.3
PCB 138	6.57	3.97	<CC α - 37.2
PCB 153	14.2	12.0	2.06 - 52.9
PCB 180	4.95	3.35	<CC α - 30.7
SUM	33.1	28.9	5.35 -140
BDE 28	<CC α	<CC α	<CC α
BDE 47	1.56	1.23	<CC α - 4.37
BDE 99	<CC α	<CC α	<CC α - 1.97
BDE 100	<CC α	<CC α	<CC α
BDE 153	<CC α	<CC α	<CC α - 0.74
BDE 154	<CC α	<CC α	<CC α
BDE 183	<CC α	<CC α	<CC α - 1.18
SUM	1.84	1.35	<CC α - 6.34

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