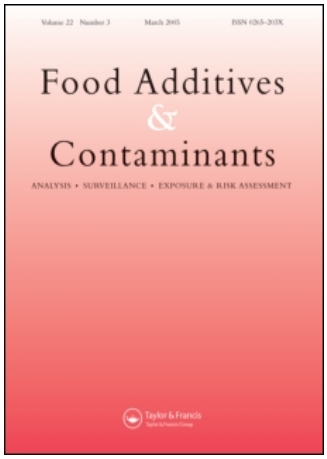

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Development and application of a simplified clean-up procedure for the determination of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in horse fat by gas chromatography-tandem mass spectrometry (GC-MS/MS)

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Abstract
A simplified clean-up procedure was developed in combination with gas chromatography-tandem mass spectrometry (GC-MS/MS) for the determination of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in adipose tissue. Clean-up was performed by the successive application of a Mega Bond Elut® silica column and a Bond Elut® PCB column. Validation of the method was conducted according to European Union Commission Decision 2002/657/EC. In order to evaluate the applicability of the method, 44 horse fat samples were analysed. The total PCB concentration (sum of PCBs 28, 52, 101, 118, 138, 153 and 180) ranged from 5.35 to 140 ng g^{-1} lipid weight. The total PBDE concentration (sum of BDEs 28, 47, 99, 100, 153, 154 and 183) ranged from below the decision limit to 6.34 ng g^{-1} lipid weight.

Keywords: *Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), solid-phase extraction, horse fat*

Introduction

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

Polychlorinated biphenyls (PCBs) were mainly produced between the 1930s and the 1970s as complex mixtures, containing many different congeners. Good thermal and chemical stability and electrical insulating properties favoured use of PCBs in a variety of industrial applications (WHO/IPCS 1992).

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

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According to Directive 2002/95/EC or the RoHS Directive (European Commission 2003a), European Union Member States should ensure that from 1 July 2006 new electrical and electronic equipment put on the market does not contain any PBDEs. Deca-BDE was exempted from the RoHS Directive on 15 October 2005 on the basis of the conclusions of a 10-year European Union environmental and human health risk assessment (European Commission 2005). Before this, the use of penta- and octa-BDE in all applications for the European market was banned from 15 August 2004 (European Commission 2003b). PCBs are one of the 12 persistent organic pollutants (POPs) that have been listed by the Stockholm Convention (2006). Belgian legislation has set maximum limits for the total sum of seven marker PCB congeners (28, 52, 101, 118, 138, 153 and 180) in different food matrices.

There is a need for a simple and sensitive analytical methodology for these two groups of components. Most methods, developed for their determination in biological samples and more specific in adipose tissue, have the disadvantage of being very laborious. They usually consist of a lipid-extraction step using a non-polar and/or polar solvent. Further clean-up and removal of co-extracted lipids is performed by gel permeation chromatography, adsorption chromatography and/or multilayer silica clean-up with sulphuric acidic treatment. The final determination is generally carried out by GC coupled with high- or low-resolution mass spectrometry (MS) or electron-capture detection (ECD) (De Boer et al. 2001; De Boer and Cofino 2002; Pirard et al. 2002; Ramos et al. 2004; Saito et al. 2004).

The aim of the work reported herein was to develop a practical, non-laborious, yet sensitive analytical method for the determination of PCBs and PBDEs in adipose tissue. In order to evaluate the newly developed method, horse fat samples were analysed. Within the European Union the Italians consume most horse meat per capita, followed by the Belgians (Hertrampf 2003). In 2004, Belgium was the main importer of horse meat and the second largest exporter of horse meat in terms of quantity (Food and Agricultural Organization of the United Nations 2004). Horses for human consumption have been mainly working animals or horses reared for recreational riding or racing (Martuzzi et al. 2001; Gill 2005). Horses may be exposed to environmental contaminants through the ingestion of contaminated feed, contaminated soil or contaminated water (Rhind 2002). Considering that horses for human consumption have had a long life-span and come from different backgrounds makes them interesting cases to study POPs.

Materials and methods

Chemicals

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

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

Apparatus

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

Sample collection and storage

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

Table I. Sample Collection details and concentrations (ng g^{-1} lipid weight) of PCBs and PBDEs in horse fat samples ($n=44$).

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 March 2006	–	26.6	0.98	23	4	02 May 2006	Foal	23.2	1.37
2	2	10 April 2006	–	31.4	1.74	24	2	06 June 2006	–	9.84	< CCα
3	3	04 April 2006	–	27.7	1.09	25	5	08 May 2006	Adult	7.43	< CCα
4	4	14 April 2006	–	20.2	1.02	26	6	02 May 2006	Foal	20.5	0.93
5	2	18 April 2006	–	16.7	0.92	27	2	25 April 2006	Foal	17.8	1.75
6	5	27 April 2006	–	75.4	1.32	28	2	08 May 2006	Foal	8.98	< CCα
7	2	21 March 2006	–	41.4	4.22	29	2	24 April 2006	–	67.6	< CCα
8	2	11 April 2006	–	40.7	3.77	30	2	24 June 2006	Foal	37.6	4.61
9	2	21 March 2006	–	24.2	1.70	31	2	04 May 2006	Adult	8.15	1.65
10	2	27 March 2006	–	16.5	1.14	32	2	06 June 2006	–	14.6	< CCα
11	2	18 April 2006	–	44.7	3.45	33	2	30 May 2006	–	65.9	1.87
12	2	11 April 2006	–	17.2	6.34	34	4	29 May 2006	Adult	140	4.70
13	4	30 March 2006	–	32.6	1.23	35	2	22 June 2006	Foal	5.35	< CCα
14	2	27 March 2006	–	40.0	1.56	36	4	23 June 2006	Foal	32.3	1.03
15	5	16 May 2006	Adult	73.8	2.98	37	2	29 June 2006	Foal	7.81	0.87
16	2	22 May 2006	Foal	22.6	1.92	38	2	30 May 2006	Foal	79.4	5.73
17	2	22 May 2006	Foal	32.3	3.63	39	5	12 June 2006	Adult	30.2	0.68
18	2	22 May 2006	Foal	15.4	1.92	40	5	24 April 2006	Adult	49.3	1.23
19	4	11 May 2006	Adult pony	19.6	2.11	41	2	13 June 2006	–	36.2	1.93
20	2	15 May 2006	Foal	36.3	3.74	42	2	13 June 2006	Foal	12.4	0.93
21	2	15 May 2006	Foal	37.0	1.06	43	2	13 June 2006	Foal	34.2	1.03
22	2	06 June 2006	–	31.7	2.12	44	5	07 June 2006	Adult	23.5	0.84

[a]Concentrations below the decision limit (CCα) were not taken into account for the calculations of the sum.

Extraction and clean-up

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

A Mega Bond Elut® silica column (2 g) was conditioned with 12 ml of *n*-hexane. Subsequently, the sample was passed through the column without applying vacuum. Another 9 ml of *n*-hexane were used to elute the analytes from the column. The eluate was evaporated under a gentle nitrogen stream at 40°C to approximately 1 ml. A Bond Elut® PCB column was conditioned with 4 ml of *n*-hexane. Consequently, the reduced eluate was applied to the column and eluted once more without applying vacuum. A total of 2 ml of *n*-hexane was used to elute the analytes under light vacuum. This step was repeated three additional times to an accumulated volume of 9 ml. After evaporation to approximately 4 ml and transfer to a graduated vial, injection standards PBB 155 (20 µl of 0.1 ng μl^{-1}) and mirex (50 µl of 0.1 ng μl^{-1}) and keeper solvent isooctane were added. This mixture was concentrated under a gentle nitrogen stream at 40°C to 100 µl and put into a GC-MS vial.

Gas chromatography/mass spectrometry

Aliquots of the final extract (2 µl) were injected in the splitless mode with a splitless period of 1 min following injection. The injection temperature was set at 300°C and the transfer line temperature was 275°C. The oven was programmed from 70°C for 1 min to 170°C at a rate of 30°C min^{-1}, then to 300°C (15 min) at a rate of 8°C min^{-1}. Optimization of GC-MS/MS parameters has been elaborately described elsewhere (Naert et al. 2004; 2006; 2007). Detection of all analytes was done in electron impact MS/MS mode. In order to achieve as much selectivity as possible, the most abundant ion of the molecular cluster was chosen as the precursor ion for collision-induced dissociation. Excitation voltage, excitation time and *q*-value are presented in Table II.

Validation

Validation of the method was carried out according to Commission Decision 2002/657/EC (European Commission 2002). Specificity, decision limit (CCα), detection capability (CCβ), recovery, precision, bias and trueness were determined as required for a quantitative confirmatory method. Because levels of PCBs 118, 138, 153 and 180 in the blank pool were occasionally higher than the decision limit, a blank subtraction was applied in order to correct validation and sample results accordingly.

Table II. Details of MS/MS parameters.

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

CCα and CCβ were established by a calibration curve procedure and this was repeated five times. Blank fat samples were fortified with a mixture of seven PBDE congeners at levels of 1, 2, 4, 8 and 15 ng g^{-1} per congener and a mixture of seven PCB congeners at 1, 5, 10, 20 and 50 ng g^{-1} per congener. Subsequently, the signal was plotted against the added concentration. The decision limit or CCα ($\alpha = 1\%$) corresponds to the concentration at the *y*-intercept plus 2.33 times the standard deviation of the within-laboratory reproducibility of the intercept. Detection capability or CCβ ($\beta = 5\%$) equals the concentration at the decision limit plus 1.64 times the standard deviation of the within-laboratory reproducibility of the mean measured at the decision limit.

The specificity for all congeners, except for PCBs 118, 138, 153 and 180, was investigated by analysing different blank samples for interferences. For the determination of recovery, bias, within-day and between-day precision, 18 blank fat samples were fortified at three levels (2, 4 and 8 ng g^{-1} per congener for PBDEs and 5, 10 and 20 ng g^{-1} per congener for PCBs). Recovery is defined as that fraction of mass of the analyte added to the sample which is present in the final extract. The responses of the fortified samples were compared with samples fortified after clean-up. Within-day and between-day precision were expressed as a coefficient of variation (CV%). Trueness was determined by means of analysis of purchased CRM material (ERM-BB446). Certified values for PCBs 28, 52, 101, 118, 138, 153 and 180 and an indicative value for BDE 47 were given. Measurement uncertainty was determined according to the EURACHEM/CITAC guide (Ellison et al. 2002). The global uncertainty was calculated as a combination of the uncertainty associated with precision and the uncertainty associated with accuracy. Although this is the combined uncertainty, the results are expressed as an expanded uncertainty, which corresponds to twice that value.

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

Results and discussion

Extraction and clean-up

An extensive clean-up procedure using glass columns filled with sodium sulphate, aluminium oxide and sulfuric acid was previously described elsewhere (Naert et al. 2004; 2006; 2007). Since this method was laborious and large volumes of solvent were consumed, it was the authors' intention to simplify this clean-up procedure by using disposable solid-phase extraction (SPE) columns. Fat removal properties of silica, florisil and alumina (activity III) were compared and the Mega Bond Elut® silica column (2 g) and yielded best results. Still further clean-up was required. A number of additional commercial SPE columns, e.g. Bond Elut® PCB (Varian), Mega Bond Elut® SCX (2 g) (Varian), Sep-Pak® florisil cartridge (Waters, Zellik, Belgium), Mega Bond Elut® silica (2 g) (Varian), PCB-A (Malinckrodt Baker, Boom, Diegem, Belgium), and an in-house made column (2 g) filled with silica, acidified silica or aluminium oxyde were assessed. The best results and cleanest chromatograms were obtained with a Mega Bond Elut® silica column (2 g) in combination with the Bond Elut® PCB column. This column is a dual-phase SPE column consisting of a strong cation exchange bed and a silica bed. It has been developed for the extraction of PCBs in transformer oil and organic matrices.

Validation

A summary of the validation results is presented in Table III. Decision limits for PCBs and PBDEs ranged from 0.53 to 1.73 ng g^{-1} lipid weight and from 0.30 to 1.07 ng g^{-1} lipid weight, respectively. Detection capabilities for PCBs and PBDEs varied between 1.23 and 3.65 ng g^{-1} lipid weight and between 0.78 and 1.90 ng g^{-1} lipid weight, respectively. Precision, expressed as the coefficient of variation (CV%), did not exceed 15.7%, indicating the good repeatability of the method. Recoveries were between 78.1 and 87.8% for PCBs and between 84.4 and 99.3% for PBDEs. No interfering

Table III. Summary of validation results.

Compound	CCα (ng g^{-1} lipid weight)	CCβ (ng g^{-1} lipid weight)	Precision (CV%) Within day[a] ($n=6$)	Precision (CV%) Between day[a] ($n=6$)	Bias[a] (%) ($n=6$)	Recovery[a] ($n=6$) (%)	Expanded measurement uncertainty (%)	Trueness ($n=4$) (%)	CV (%)
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 were obtained at the 4 ng g^{-1} level for PBDEs and at the 10 ng g^{-1} level for PCBs.

peaks for PBDEs and PCBs 28, 52 and 101 could be detected when analysing different blank fat samples. Specificity for PCBs 118, 138, 153 and 180 could not be established because of the difficulty of obtaining blank animal fat for these congeners. To ensure that separation of PCBs and PBDEs was adequate, separation of PCBs 28 and 31 was verified with every sample sequence.

In the concentration ranges 1–15 and 1–50 ng g^{-1} for PBDEs and PCBs, respectively, correlation coefficients (R^2) were all >0.990. The per cent bias was within the –30 to 10% and –20 to 10% ranges, as prescribed by Commission Decision 2002/657/EC (European Commission 2002), for concentrations ranging from >1 to 10 ng g^{-1} and ≥10 ng g^{-1}, respectively. Trueness, established by analysis of certified reference material (ERM-449), varied between 98.7 and 107.9% for PCBs. Trueness for BDE 47 was 109.4%. Expanded measurement uncertainty ranged from 19.1 to 37.2% for PCBs and from 18.1 to 34.9% for PBDEs.

The absolute matrix effect ranged from 62.9 to 105.9% for all compounds at three concentration levels. Furthermore, a calibration curve in matrix was prepared with each sample sequence to compensate for absolute matrix effects. Relative matrix effects were compensated by the use of an internal standard.

Identification criteria, as laid down by Commission Decision 2002/657/EC (European Commission 2002), were fulfilled. Signal-to-noise ratios for each diagnostic ion were ≥3. Relative retention times (RRTs) of the analyte did not differ by more than ±0.5% of the RRT of the calibration standard. The relative intensities of the detected ions, expressed as a percentage of the intensity of the most abundant ion, corresponded within the tolerances specified by Commission Decision 2002/657/EC.

Table IV. PCB and PBDE concentrations in horse fat samples ($n=44$).

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

Analysis of horse fat samples

In order to evaluate the method, 44 horse fat samples from a local slaughterhouse were analysed for seven indicator PCBs (28, 52, 101, 118, 138, 153 and 180) and seven PBDE congeners (28, 47, 99, 100, 153, 154 and 183). PCBs were identified in all samples and PBDEs were identified in all but six samples. A summary of the PBDE and PCB levels is listed in Tables I and IV. A PCB and a PBDE chromatogram of a horse adipose tissue

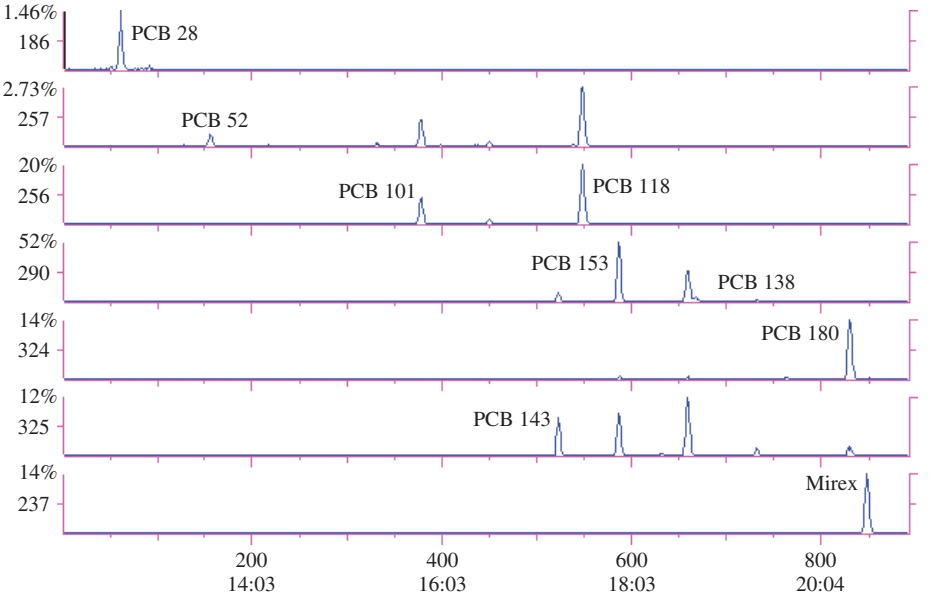

Figure 1. PCB chromatogram of a horse fat sample extract (sample number 38).

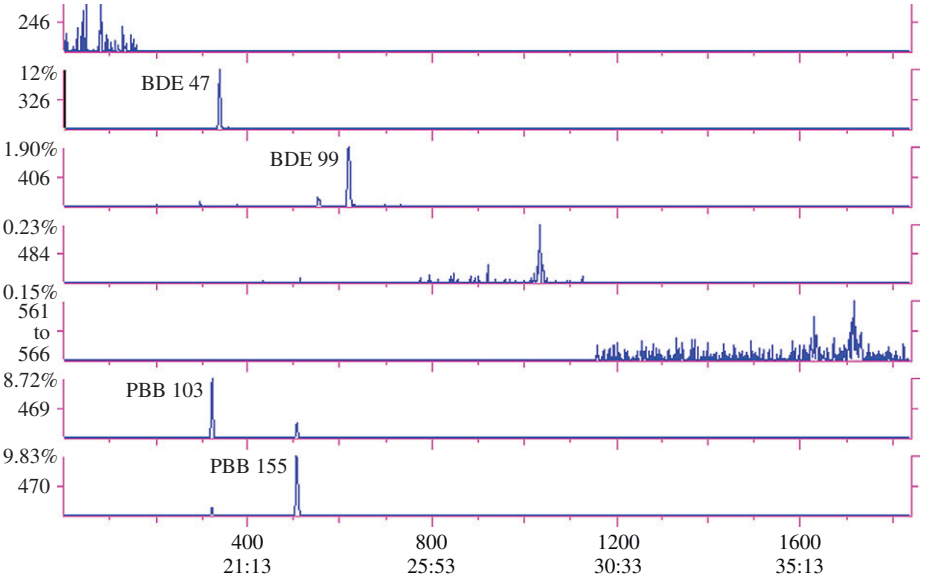

Figure 2. PBDE chromatogram of a horse fat sample extract (sample number 38).

extract (sample number 38) are presented in Figures 1 and 2.

The sum of the seven PCB congeners ranged between 5.35 and 140 ng g^{-1} lipid weight (median = 28.9 ng g^{-1} lipid weight). No sample exceeded the maximum permitted level of 200 ng g^{-1} fat as laid down by Belgian legislation. Levels were higher than those found in meat and meat products from Brazil (median = 10.30 ng g^{-1}) (Costabeber et al. 2006), in pork fat (range = 2.6–6.3 ng g^{-1}) and pork meat (range = 8.6–17.5 ng g^{-1}) from Romania (Covaci et al. 2004), and in beef (median = 0.284 ng g^{-1}), pork (median = 0.234 ng g^{-1}) and chicken (median = 0.241 ng g^{-1}) from South Korea (Kim et al. 2004). Levels corresponded extremely well with a study by Pirard et al. (2002), where poultry, horse, pork and beef samples were analysed and horse samples also showed higher background levels of marker PCBs (21.6 ng g^{-1} fat). It has been suggested that this might be due to the longer lifespan of horses compared with pigs, cattle or chickens (Focant et al. 2002). In the present study, no significant difference in PCB concentration was found between adult horses or ponies and foals (t-test = −1.070; p = 0.315). Perhaps diet can also be a major contributing factor because the composition

of the diet may vary according to age, breed and type of labour. This conclusion should, however, be interpreted with caution because only eight adult animals were included in this study.

PCB 153 was the predominant congener in every sample accounting for 43% of the total PCB concentration (range = 31–74%). This is in accordance with the notion that the proportion of the higher chlorinated congeners is larger in organisms situated at the top of the food chain (WHO/IPCS 1992).

The total PBDE concentration ranged from below the decision limit (CCα) to 6.34 ng g^{-1} lipid weight (median = 1.35 ng g^{-1} lipid weight). Comparisons with other studies are difficult because most studies are market basket-based studies. PBDE levels found in the present study are comparable with levels found by Huwe et al. (2002) in chicken and higher than levels found in meat products from Catalonia (Bocio et al. 2003). BDE 47 was the predominant congener in all but one sample (range = 64–100%). This is in contrast with a market basket-based study from the USA where BDE 99, followed by BDE 47, predominates in meat samples (Schecter et al. 2004).

No significant difference in PBDE concentration was found between adult horses or ponies and foals (t-test = 0.735; $p = 0.849$). Significant but low correlation could be established between the sum of PCBs and PBDEs (Spearman's rho = 0.531, $p < 0.0005$).

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