Inputs of polychlorinated biphenyl residues in animal feeds

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ABSTRACT

Animal nutrition constitutes an important issue for the animal production industry. Products intended for animal feed may contain undesirable substances which could endanger animal health or, because of their presence in livestock products, human health or the environment. In this sense, several incidents related with the presence of persistent organic pollutants, particularly with polychlorinated biphenyls (PCBs), have happen in food and feed additives. Animal feed and feed components are challenging matrices for the determination of residues and contaminants. The variability of these matrices is enormous. It ranges from relatively simple ones like those based on wheat to all kinds of by-products from agro and food industry, such as cereal oils.

Firstly, this article reviews and addresses the extraction efficiency of ultrasonic assisted solvent extraction (UASE) and focused ultrasonic solvent extraction (FUSE) for determining selected PCBs in animal feed and ingredients. Detection of these pollutants was carried out by gas chromatography (GC) coupled to electron capture detection (ECD); tandem mass spectrometry (MS/MS) was used as confirmatory technique. Recoveries ranged from 70% to 98% by UASE and from 75% to 106% by FUSE with estimated quantification limits between 0.11 and 0.3 μg/kg in feeds and ingredients and between 0.2 and 0.75 μg/kg in fats.

Once the method was optimised, it was applied to 18 feed samples as well as 16 ingredients. PCBs were detected in almost all the selected samples. As expected, the samples of animal origin as shell powder and fish oil showed the highest concentrations of 56 and 29 ng/g, which are equivalent to toxicological concentrations of 123 and 18 ng WHO-TEQ DL-PCBs/kg, respectively. Feeds and ingredients from vegetable origin ranged from non-detected to 7.1 μg/kg. PCB 77 and 169 were the discriminant congeners in the selected samples of feed and ingredients. Samples showed that the pattern of PCBs depends on the sources of contamination.

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1. Introduction

Public concern over the adverse health effects of exposure to polychlorinated biphenyls (PCBs) has grown because of incidents involving food and feed (Llerena, Abad, Caixach, & Rivera, 2003). PCBs have been recognised by the EPA as an environmental hazard, which are toxic and persistent. Once released into the environment, PCBs do not readily break down. Instead, they may accumulate in the environment and have the potential to migrate through the food chain. Depending on the number of chlorine atoms and their position, 209 PCB congeners are possible. Based on structural characteristics and toxicological effects, PCBs are divided into dioxin-like PCBs (DL-PCBs) showing toxicological properties similar to dioxins and non-dioxin-like PCBs (NDL-PCBs), which do not share dioxin's toxic mechanism. They are regulated under Toxic Substances Control Act in part because of their probable carcinogenicity and tendency to bioaccumulate in the food chain (Kelly, Ikonomou, Blair, Morin, & Gobas, 2007; Zuccato et al., 2008). Their production and use in commerce was banned by a series of legislative acts and, in this way, it has been assumed PCBs would eventually be eliminated (Hu, Martinez, & Hornbuckle, 2008). However, recently, a congener 3,3′-dichlorobiphenyl (PCB11), which is not produced as an Arochlor in the environment, was found unexpectedly.

The most important route by which persistent, bioaccumulative organic contaminants enter humans is, generally, via the ingestion of meat and dairy products (Darnerud et al., 2006; Duarte-Davidson & Jones, 1994; Kiviranta, Ovaskainen, & Vartiainen, 2004). Traceability of animals and animal products has become a priority for governments because intensive studies have shown animal feed and feed additives are major sources of contamination (Botaro et al., in press; Ábalos, Parera, Abad, & Rivera, 2008).

On 27 December 2010, the German authorities informed the European Commission’s Rapid Alert System for Food and Feed (RASFF) that a batch of fatty acids, which was meant to be used for technical purposes, was mixed with fat for the production of...
feed. The batch of fatty acids was produced by a biodiesel company and delivered to a feed fat producing company, and contained higher levels of dioxin and PCBs than allowed by EU law (EC, 2010). In response to the crisis, the EU intends to impose a strict separation between the production of industrial greases and fats, and those used in the manufacture of feed.


In the 1999 Belgian dioxin crisis, six indicator congeners and the mono-ortho substituted PCB 118 (2,3',4,4',5'-pentachlorobiphenyl PCB) were used to identify the composition of PCBs. These six congeners were PCB 28 (2,4,4'-trichlorobiphenyl), PCB 52 (2,2',5,5'-tetrachlorobiphenyl), PCB 101 (2,2',4,5,5'-pentachlorobiphenyl), PCB 138 (2,2',3,4,4',5'-hexachlorobiphenyl), PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl), PCB 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl) and PCB 209 (decachlorobiphenyl), which were present in most of the PCB-mixtures and environmental samples (Kim et al., 2004). Moreover, the Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) noted in its Scientific Opinion related to the presence of NDL-PCBs in feed and food that the sum of the six indicator PCBs represented about 50% of the total NDL-PCB in food (EFSA, 2010). The EU Commission is currently discussing to lay down maximum levels for the sum of the six indicator NDL-PCBs in food and feed. The detection of new PCBs could have important impacts on regulatory decisions in future, although further investigation is needed.

Ultrasonic radiation is a powerful tool to accelerate the analytical process in both solid and liquid samples. One reason for applying acoustic energy is that it enhances matrix washing by a mechanical mechanism, which also includes abrasion of suspended particles leading to surface removal of the contaminants. Ultrasonic assisted solvent extraction (UASE) and focused ultrasonic assisted extraction (FUSE) are an expeditious, inexpensive, and efficient alternative to conventional extraction techniques and, in some cases, even to supercritical fluid extraction (SFE) and microwave assisted extraction (MAE), as it was demonstrated for a wide range of environmental samples (Ashley, Andrews, Cavazos, & De-Mange, 2001; Priego-López & Luque de Castro, 2003).

Finally, the aim of this research was to evaluate the contamination caused by DL-PCBs (3,3',4,4'-tetrachlorobiphenyl PCB 77; 2,3',4,4',5'-pentachlorobiphenyl PCB 118; 3,3',4,4',5'-pentachlorobiphenyl PCB 126; 2,3',4,4',5'-hexachlorobiphenyl PCB 156 and 3,3',4,4',5,5'-hexachlorobiphenyl PCB 169) and NDL-PCBs (3,3'-dichlorobiphenyl PCB 11, PCB 28, 52, 101, 138, 153, 180, 209 and 2,2',3,3',4,4',5,5'-octachlorobiphenyl PCB 194) in different animal feeds and feed ingredients belonging to the different categories.

2. Experimental

2.1. Chemicals and materials

All reagents used for the analysis of PCBs were of trace analysis grade, n-hexane, dichloromethane, acetone, iso-octane were supplied from Panreac (Barcelona, Spain). A mixture containing several PCB congeners in iso-octane (PCB 28, 52, 101, PCB 138, PCB 153, PCB 180; and PCB 209, 10 μg/mL of each one) was obtained from Riedel de Haén (Seelze, Germany). As surrogate standard 2,4,6-trichlorobiphenyl PCB 30 was chosen. In addition, three individual non-ortho standards (PCB 77, PCB 126 and PCB 169), were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The mono-ortho substituted PCB 118 and PCB 156 were supplied also from Dr. Ehrenstorfer (Augsburg, Germany). Finally, PCB 11 and PCB 194 were selected as representatives of dichlorobiphenyls and octachlorobiphenyls, and were purchased from Sigma–Aldrich (Madrid, Spain) and from Dr. Ehrenstorfer (Augsburg, Germany), respectively.

Analytical grade C-45 nitrogen was supplied by Carburos Metálicos (Vigo, Spain). Additional equipment included a TurboVap evaporator (Caliper Life Sciences, Barcelona, Spain), an ultrasonic bath (P-Selecta, Barcelona, Spain), an oven (P-Selecta, Barcelona, Spain), an analytical precision scale (Sartorius, Madrid, Spain) and a vortex shaker (Heidelberg,olph, Barcelona, Spain). The ultrasonic processor UP50H (Hielsher, Germany) was used for FUSE experiments. Disposables used were nylon filters (0.45 μm), micro-petites (200–1000 μL), specific recipients for low sonication volume and injection vials (2 mL) furnished with screw caps and PTFE-lined butyl rubber septa and inserts (0.35 mL).

2.2. Feed samples

The sampling plan for the present study included 18 feeds as well as sixteen ingredients, currently employed as feed ingredients and considered as potential candidates for feedstuff. Selected samples were collected at local feed processing plants and reflected the representative feeding situation in Galicia (North West Spain). Moreover, such samples were classified, taking into account their origin and/or the industrial processes applied to recover them, as one of four different groups, namely feed containing animal fats (ANFA), vegetable oils (VEGO), fatty acids calcium soaps (FACS) and feed without added fat (WAFA). General characteristics of the selected feeds and ingredients are shown in Table 1.

2.3. Extraction procedures

2.3.1. Ultrasonic assisted solvent extraction (UASE)

Although different non-polar solvents, used previously for PCB extraction from different matrix (López-Avila, Benedico, Charan, Young, & Beckert, 1995; Ramil-Criado, Rodriguez-Pereiro, & Cela-Torrijos, 2003), were assayed, the selected solvents for PCB extraction in feeds and fats were hexane:acetone (1:1, v/v) and acetonitrile (ACN), respectively.

About 5 g of feed sample and feed ingredients (except fats) were extracted twice with 25 mL n-hexane:acetone (1:1, v/v) for 10 min. Fats (2 g) were extracted three times with 10 mL ACN for 10 min. The extracts obtained were centrifuged (2665 g) for 20 min and evaporated under nitrogen in a TurboVap to 0.5 mL. Once concentrated, the organic extracts were cleaned up.

2.3.2. Focused ultrasonic solvent extraction (FUSE)

Using n-hexane/acetone as solvent, the samples (5 g feeds and feed ingredients, and 2 g fats) were exposed to ultrasonic irradiation with the volume, time, and number of cycles fixed according to experimental design, and with the titanium tip of the probe immersed 1 cm from the upper surface of the feed samples.

The extract was concentrated to dryness under nitrogen. It was in 0.5 mL n-hexane to ensure the concentrated extract was enriched in a non-polar solvent before normal-phase solid-phase extraction (SPE) clean-up.

2.3.3. Clean-up

Glass columns containing anhydrous sodium sulphate (2 g), florisoril (3 g), acid silica gel (2 g) and basic alumina grade II (1 g), previously conditioned with 20 mL of n-hexane, were used to clean samples. NDL-PCBs were eluted with 20 mL n-hexane and DL-PCBs with 20 mL n-hexane:dichloromethane (7:3, v/v) then this extract concentrated under nitrogen to 0–0.25 mL and made up to 0.5 mL with n-hexane before injection into the chromatographic system.
2.4. Chromatographic and detection conditions

A Fisons (Rodano, Italy) GC 8000 series gas chromatograph equipped with an electron capture detector (ECD) was used. Chromatographic separations were performed using a DB-XLB capillary column (30 m × 0.25 mm i.d., 0.5 µm film thickness) from Agilent Technologies. The oven temperature was programmed as follows: (a) an initial temperature of 90 °C for 1 min; (b) programmed to 170 °C at 20 °C/min; (c) kept at 170 °C for 5 min; (d) programmed to 300 °C at 3 °C/min; and, (e) kept at 300 °C for 5 min. A split/splitless injector was used in the splitless mode (1.0 min) with a split ratio of 1/100. The carrier gas was helium at a constant column flow of 1.5 mL/min. Nitrogen (150 kPa) was used as the make-up gas. Injector and detector temperature were 280 °C and 250 °C, respectively.

Gas chromatographic (GC) analyses were carried out on a Trace GC Thermo Finnigan gas chromatograph (Rodano, Italy) equipped with a PolarisQ ITMS detection system, interfaced to a PC computer running the software Xcalibur 1.4, from Thermo Electron Corporation (Italy), using the same column. PTV was used for the 2 µL injection volume into a silcosteel liner without packing (120 mm × 2 mm i.d.). The temperature programming of the PTV was: 85 °C for 0.3 min; 600 °C/min to 270 °C and hold for 2 min; 840 °C/min to 300 °C; and hold for 5 min. The GC was set to constant pressure of 100 kPa. The transfer line temperature was 270 °C, and the ionisation source temperature was 250 °C. Mass detection was performed in full scan mode to determine clean-up efficiency. Selected reaction mode (SRM) was used in the recovery experiments and in the trade samples (Fernández-González, Martínez-Carballo, González-Barreiro, Rial-Otero, & Simal-Gándara, 2011).

2.5. Recovery experiments on matrix

In an attempt to elucidate the relationship of PCB recovery to the nature of the matrix, a series of recovery experiments were conducted following the spiking of a known amount of contaminant (i.e. mixed congener) on the matrix. Two PCB mixtures (5 µg/kg and 25 µg/kg) were prepared in hexane by mixing equal volumes of the selected PCBs and the resulted solutions were spiked on 5 g of feed or ingredient matrix and 2 g fats. These spiking levels were selected in accordance with the PCB levels typically found in this type of samples. Following the completion of spiking, 5 mL of hexane were added for complete wetting of the sample. The solution was up-and-down stirred for 1 h to obtain a homogeneous solution. After 48 h contact time, hexane was allowed to evaporate at 23 ± 2 °C and the PCB-spiked organic matrix was brought to dryness under nitrogen. Then, UASE and FUSE studies were conducted on spiked matrix systems. This set of samples was processed together with a reagent blank to test for contamination in the extraction process. So, as Fig. 1 shows, the selected method was quantitative enough to determine PCBs in feed samples.

2.6. Statistical treatment

2.6.1. Experimental design

Three factors were selected as potentially affecting the extraction efficiency of FUSE, namely: extraction time, volume and cycles of extraction. To screen the relative influence of these factors and their possible interactions in the experimental domain, a mixed level factorial design (3 × 2²) was chosen, which determines the effects of the selected factors in 12 experiments. The order of the experiments was randomised to protect against the effects of unknown variables. Two centerpoints were added to ensure enough degrees of freedom for error evaluation. The values corresponding to the upper (+) and low (−) levels taken by each variable in this design were: extraction time (0.5 and 4 min); volume (8 and 16 mL) and cycles (1 and 2). Data analysis was performed by means of the statistical package Statgraphics Plus for Windows V.5.1 (Academic Enterprise, StatPoint Inc, Herndon, Va, USA).

2.6.2. Principal component analysis (PCA) and discriminant regression

The principal component (PC) model was calculated on the auto-scaled data (namely, columns were mean-centred and scaled to unit variance) to focus the analysis on between sample varia-
tions, and establish the importance of each compound independent of its concentration. The model was further validated by cross-validation, visual inspection of loadings, and chemical interpretation to ascertain a meaningful interpretation for the PCs.

In the regression by stepwise discriminant function analysis, a model of discrimination is built up. At each step, all variables are reviewed and evaluated to determine which will contribute most to the discrimination between groups. These statistical analyses were carried out using also Statgraphics version 5.1 (Academic Enterprise, StatPoint Inc., Herndon, VA, USA).

3. Results and discussion

3.1. Ultrasonic energy

3.1.1. Ultrasonic assisted solvent extraction (UASE)

The pre-analytical treatment used in this work was based on a procedure for the determination of PCBs in ash samples obtained after the incineration process of mussel shells previously reported by the present authors (Fernández-González et al., 2011). In this way, UASE optimization was carried out taking into account parameters such as extraction solvent, number of consecutive extractions, time and extraction volume. The selected solvents were hexane, dichloromethane, toluene and mixtures between hexane/acetone. The decreasing order of recovery performance with feeds and feed ingredients was: toluene (92–98%) > hexane:acetone (1:1) (88–100%) > dichloromethane (75–90%) > hexane:dichloromethane (8:2) (70–85%) > hexane (60–80%) with two extractions with 25 mL each for 20 min. As it can be seen in Fig. 1, PCB recoveries were highest with hexane:acetone (1:1, v/v); toluene was rejected because its toxicity, and they proved to be nearly quantitative. In the case of fats, no quantitative recoveries were obtained because of the matrix effects. Therefore, and based on a procedure for the determination of polycyclic aromatic hydrocarbons in vegetable oil (Vázquez-Troche, García-Falcón, González-Amigo, Lage-Yusty & Simal-Lozano, 2000), ACN was used. In this case, three consecutive extractions of 10 mL each were enough to obtain quantitative recoveries for 2 g fat samples.

3.1.2. Focused ultrasonic solvent extraction (FUSE)

A progressive strategy was used because many factors could potentially be affecting the analytical process. Based on previous experiments, and the literature, the most important factors were identified (composition of the extraction solvent, ultrasonic (US) power, extraction temperature and extraction mass) and included in the selected mixed level factorial design \(3 \times 2^2\) type V resolution design using highest and lowest values from previous experimental work. With this design, the main effects and confounded two-factor interactions can be followed. Thus, with a reasonable number of experiments, the statistical significance of all the main factors could be clearly established.

Response was evaluated in terms of the selected PCB peak areas. The analysis of these results showed that not all the initially selected variables produced a significant effect and that no significant interactions between factors were apparent. Fig. 2 shows the main effect plots for FUSE of some of the target PCBs, for which some of the selected factors were significant. The main effect plot shows the estimated variable as a function of each experimental factor. In each plot, the factor of interest varies from its lowest level to its highest level, while all the other factors remain constant at their central values.

As it can be seen, extraction time and volume were the factors with greatest effect (in fact, the only statistically significant factors for some of the target PCBs). As it can be expected, some of the PCB peak areas increase when extraction volume increase. Instead, the highest PCB peak areas for almost all the target PCBs were obtained with 2.25 min extraction time, excluding PCB 180 and 209 since, for these compounds, the highest peaks areas were obtained with 4 min. A compromise was made, and the experimental conditions selected were 2.25 min for extraction time in 16 mL.

3.1.3. Comparison of extraction techniques

To finish, a comparison of general parameters, such as extraction time, solvent consumption and cost, for the different extraction methods were selected. The extraction time for FUSE was about four times shorter (4.4 min) than UASE (20 min). Another important parameter was the solvent consumption for economic reasons; FUSE needs only 16 mL unlike 50 mL of UASE. Neverthe-
less, FUSE demands more sophisticated equipment in comparison with a normal ultrasound bath.

3.2. Analytical system

Analysis of PCBs is routinely done on a semi-specific detector (i.e. ECD), and identification based on retention time. The stability of the relative response factor by ECD is checked during successive calibrations, supporting the stability and robustness of the method. ECDs are sensitive, but do not give unequivocal confirmation, which may be achieved with GC/MS analysis, matching retention time and library spectrum. Nevertheless, full scan or selected ion monitoring GC/MS has sensitivity limitations that prohibit them from confirming ECD “hits” at very low levels. An alternative approach is tandem mass spectrometry (MS/MS), where a target compound is isolated from matrix and then fragmented to generate very unique spectra. GC–ion-trap MS has proven to be an effective analytical technique for both qualitative and quantitative trace analyses. Advances in ion-trap technology allow the use of sophisticated tandem mass spectrometry (MS/MS) techniques in conjunction with GC analyses to enhance selectivity (Malavia, Santos, & Galcerán, 2008).

MS/MS operating conditions and CID parameters such as precursor ion isolation window, excitation voltage, time and energy were optimised in order to maximise the sensitivity and selectivity in the detection of the selected PCBs (Fernández-González et al., 2011). The effect of each parameter upon the MS/MS process was studied by varying only one of the parameters while keeping the others constant. The optimal CID conditions for the analysis of the selected PCBs were: isolation windows of the precursor ions ±1 m/z, isolation time 10 ms and excitation time 15 ms.

3.3. Performance of the analytical method

External standard calibration was chosen to quantify analyte values for GC-ECD and GC–MS/MS techniques using multicompo-

![Fig. 2. Graphics showing the influence of main effects on the extraction of target PCBs with FUSE. The overall recovery measure of effect is represented on the plot as a vertical line. Horizontal line represented values corresponding to the upper (+) and lower (−) selected levels taken by each variable: extraction time (0.5 and 4 min); volume (8 and 16 mL) and cycles (1 and 2).](image-url)
ponent standards with eight calibration standards (0.5; 1; 2.5; 5; 10; 20; 40; 50 µg/L). Linear calibration curves fit reasonably in a concentration scale of two orders of magnitude for all selected compounds. Mandel fitting test \( P = 99\% \) was additionally performed to verify the linearity range. Matrix effects were also evaluated as it is shown at the end of this section.

Accuracy was estimated as the ratio found/added concentration and expressed as percentage. Fig. 1 shows that recoveries obtained range from 70 to 98 with relative standard deviations (RSD%) between 1.0 and 6.0 for UASE, but from 75 to 106 with RSD% between 4.0 and 12 for FUSE. Similar concentrations of the target PCBs were achieved by both techniques but with higher RSD% by FUSE due to its higher extraction capacity, including matrix components. Nevertheless an extraction technique with RSD% around 10% would be a precise method for extracting the selected compounds in feed samples.

Quantification limits (QLs) were defined as the concentration of the analyte that produced a signal-to-noise ratio of 10 (American Chemical Society, 1980) and were then tested experimentally by spiking blank samples at such levels \( n = 6 \). To calculate QLs of overall method, the factor of enrichment and the lower obtained recoveries were used to consider the worst case. Fig. 1 shows the quantification limits obtained by both methods.

Unfortunately, GC-ECD technique does not provide sufficient selectivity. Once a positive sample is encountered, additional confirmation is necessary in order to avoid false positives and in this way, GC–MS/MS was used in the present work. Coeluting undetected matrix components may reduce or enhance the ion intensity.

### Table 2
Concentration (µg/kg) of the selected PCBs in animal feed and feed additives, together with TEQ levels in congener-specific mean concentrations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Animal fats</th>
<th>Vegetable oils</th>
<th>Fatty acids calcium soaps</th>
<th>Without added fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANFA</td>
<td>VEGO</td>
<td>FACS</td>
<td>WAFA</td>
</tr>
<tr>
<td>PCB 11</td>
<td>n.d.</td>
<td>0.46</td>
<td>0.40</td>
<td>n.d.</td>
</tr>
<tr>
<td>PCB 28</td>
<td>n.d.</td>
<td>0.51</td>
<td>0.52</td>
<td>n.d.</td>
</tr>
<tr>
<td>PCB 52</td>
<td>n.d.</td>
<td>0.43</td>
<td>0.42</td>
<td>n.d.</td>
</tr>
<tr>
<td>PCB 101</td>
<td>n.d.</td>
<td>0.35</td>
<td>0.37</td>
<td>n.d.</td>
</tr>
<tr>
<td>PCB 77</td>
<td>n.d.</td>
<td>0.22</td>
<td>0.20</td>
<td>n.d.</td>
</tr>
<tr>
<td>PCB 118</td>
<td>n.d.</td>
<td>0.33</td>
<td>0.33</td>
<td>n.d.</td>
</tr>
<tr>
<td>PCB 138</td>
<td>n.d.</td>
<td>0.14</td>
<td>0.16</td>
<td>n.d.</td>
</tr>
<tr>
<td>PCB 153</td>
<td>n.d.</td>
<td>0.12</td>
<td>0.12</td>
<td>n.d.</td>
</tr>
<tr>
<td>PCB 169</td>
<td>n.d.</td>
<td>0.11</td>
<td>0.11</td>
<td>n.d.</td>
</tr>
<tr>
<td>PCB 209</td>
<td>n.d.</td>
<td>0.12</td>
<td>0.12</td>
<td>n.d.</td>
</tr>
<tr>
<td>ΣPCBs TEQ</td>
<td>3.7</td>
<td>0.72</td>
<td>0.72</td>
<td>n.d.</td>
</tr>
<tr>
<td>% ΣPCBs</td>
<td>3.7</td>
<td>0.72</td>
<td>0.72</td>
<td>n.d.</td>
</tr>
<tr>
<td>% PCB11TEQ</td>
<td>37</td>
<td>12–51</td>
<td>20</td>
<td>n.d.–33</td>
</tr>
</tbody>
</table>

### Table 3
Average concentration of the selected PCBs in animal feed groups, together with TEQ levels in congener-specific mean concentrations.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Concentration (µg/kg)</th>
<th>Animal fats</th>
<th>Vegetable oils</th>
<th>Fatty acids calcium soaps</th>
<th>Without added fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Min–Max</td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>PCB 11</td>
<td>0.46</td>
<td>0.40</td>
<td>&lt;LOQ–0.52</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td>PCB 52</td>
<td>0.51</td>
<td>0.52</td>
<td>0.43–0.61</td>
<td>0.17</td>
<td>0.16</td>
</tr>
<tr>
<td>PCB 101</td>
<td>0.43</td>
<td>0.42</td>
<td>0.25–0.60</td>
<td>0.15–0.12</td>
<td>2.1</td>
</tr>
<tr>
<td>PCB 77</td>
<td>1.7</td>
<td>0.73</td>
<td>0.49–4.0</td>
<td>0.47</td>
<td>0.35</td>
</tr>
<tr>
<td>PCB 118</td>
<td>0.50</td>
<td>0.53</td>
<td>0.48–0.58</td>
<td>0.33–0.72</td>
<td>0.18</td>
</tr>
<tr>
<td>PCB 138</td>
<td>0.22</td>
<td>0.20</td>
<td>0.16–0.30</td>
<td>0.18–0.28</td>
<td>0.24</td>
</tr>
<tr>
<td>PCB 153</td>
<td>0.14</td>
<td>0.16</td>
<td>0.11–0.20</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>PCB 169</td>
<td>0.12</td>
<td>0.13</td>
<td>&lt;LOQ–0.15</td>
<td>0.28</td>
<td>0.23</td>
</tr>
<tr>
<td>PCB 209</td>
<td>0.11</td>
<td>0.10</td>
<td>0.070–0.15</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>ΣPCBs</td>
<td>3.7</td>
<td>0.72</td>
<td>0.40–1.3</td>
<td>0.055–0.79</td>
<td>0.085</td>
</tr>
<tr>
<td>% PCB1</td>
<td>3.7</td>
<td>0.72</td>
<td>0.40–1.3</td>
<td>0.055–0.79</td>
<td>0.085</td>
</tr>
<tr>
<td>% PCB11TEQ</td>
<td>37</td>
<td>12–51</td>
<td>20</td>
<td>15–24</td>
<td>45</td>
</tr>
<tr>
<td>% PCB11</td>
<td>2.8</td>
<td>n.d.–8.4</td>
<td>n.d.–8.4</td>
<td>n.d.–33</td>
<td>n.d.–33</td>
</tr>
</tbody>
</table>
of the analytes. Thus, response factors obtained from standard solution and in matrix-loaded sample may differ significantly and matrix effects must be eliminated and compensated to obtain quantitatively accurate results. In this way, and although quantitatively recoveries were obtained and they evidence that no significant matrix effect take place, a comprehensive evaluation of signal suppression was performed for each analyte in order to assess its effect on the quantification. Two different types of calibration curves were studied: calibration curves prepared using n-hexane and calibration-set solutions prepared in a sample matrix. The data obtained from the analysis of each calibration set were fitted to straight lines by the least squares method and slopes of each calibration curve were compared calculating an F statistic. In this case the slopes were not statistically different and therefore, it could be confirmed that the matrix content does not introduce a systematic bias in the analytical signals.

3.4. Sources and distribution of PCBs in animal feed and feed ingredients

Once the analytical methodology was validated, it was applied to eighteen samples of feed and sixteen feed ingredients. The set of samples analysed each day was processed by UAE together with: the analysis of a blank extract that eliminates a false positive by contamination in the extraction process and the analysis of fortified samples to verify the extraction efficiency of each group of feed samples and ingredients. The concentration of the selected PCBs in animal feed and feed additives is shown in Tables 2 and 3. The increasing order of ∑PCBs in the selected feed samples was: VEGO feeds (0.71 µg/kg) < WAFA feeds (1.2 µg/kg) < FAC feeds (3.0 µg/kg) < ANFA feeds (3.7 µg/kg). With regard to feed ingredients, the highest ∑PCBs were found in the samples of fish oil (67 µg/kg) < in shell powder (56 µg/kg) < in calcium soap (8.3 µg/kg) and in bran (7.1 µg/kg), while in all other samples had ∑PAHs bellow 5.0 µg/kg. Similar results were found by Ábalos et al. (2008) in a Project supported by the European Union. They found that fish oil samples had the highest values for polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and DL-PCBs compared to the levels found in vegetable samples. Kim, Kim, Yun, Kwon, and Son (2007) found that samples with the highest PCDD/F levels were also fish oils followed by fish-meal and shell powder.

The use of the total toxic equivalent quantity (TEQ) approach for risk assessment and management purposes has been formally adopted (Kutz et al., 1990; Storelli, 2008) and in this way, the EU maxima levels have been set for DL-PCBs in ng TEQ/kg for feed and feed ingredients (UE 2006/13/EC) and specify a maximum level of: 1.5 ng WHO-TEQ_{DL-PCBs}/kg for feeds; 0.35 ng WHO-TEQ_{DL-PCBs}/kg for feed materials of plant origin with exception of vegetable oils, which level was set in 0.50 ng WHO-TEQ_{DL-PCBs}/kg; 0.35 ng WHO-TEQ_{DL-PCBs}/kg for feed materials of mineral origin; 0.75 ng WHO-TEQ_{DL-PCBs}/kg animal fat; 14 ng WHO-TEQ_{DL-PCBs}/kg for fish oils and 7.0 ng WHO-TEQ_{DL-PCBs}/kg for fish protein hydrolysates containing more than 20% fat. The TEQ levels in congener-specific mean concentrations are shown in Tables 2 and 3. The increasing order for DL-PCBs in ng TEQ/kg in the selected feed samples was
FAC feeds (0.085 ng WHO-TEQDL-PCBs/kg) < WAFA feeds (0.12 ng WHO-TEQDL-PCBs/kg) < VEGO feeds (0.31 ng WHO-TEQDL-PCBs/kg) < ANFA feeds (0.72 ng WHO-TEQDL-PCBs/kg). As can be seen and although ANFA feeds showed the highest concentrations ranging from 0.40 to 1.3 ng WHO-TEQDL-PCBs/kg feed, only one sample presented similar concentrations to the maximum levels of DL-PCBs allowed by the Commission Directive 2006/13/EC for feeds (1.5 ng WHO-TEQDL-PCBs/kg). The congener ranges of the samples (Table 3) showing high concentrations of DL-PCBs in ANFA, VEGO and WAFA feed samples compared with FAC samples, where indicator PCBs and PCB 11 were around 70%. In the case of feed ingredients, shell powder (123 ng WHO-TEQDL-PCBs/kg), fish oils (17 and 18 ng WHO-TEQDL-PCBs/kg) and fishmeal (1.8 ng WHO-TEQDL-PCBs/kg) presented the highest TEQ. Even, two of the three selected fish oils showed higher TEQ values than the levels allowed by the EU (14 ng WHO-TEQDL-PCBs/kg) for fish oils. Nevertheless, no European legislation regulates the presence of this kind of additives. The congener ranges for feed ingredients were similar to the ranges for feed samples. These results agree with the previous results obtained by Ábalos et al. (2008), which determined the highest concentrations of DL-PCBs in fish oils. Vegetable oils and fatty acids obtained by Ábalos et al. (2008), which determined the highest concentrations of PCB 118. Interpretation of scores plots was more difficult for feed ingredients from vegetal or mineral origin such as cereals, fruits and tubers, vegetable proteins, fibre foods and animal proteins.

In order to determine which PCBs discriminate the pollution level between the selected feeds and ingredients stepwise discriminant function analysis was used. In this way for the four groups of feeds (ANFA, VEGO, FACs and WAFA), three DL-PCBs (PCB 169, 118 and 77) had the highest discriminant power at 5% significance level using two discriminant functions (95% and 0.75% respectively). This result showed that the fat sources play an important role in the distribution of PCBs. Fig. 3B shows a plot of individual data on the plane determined by the two discriminant functions where feeds appear associated, suggesting three groups. One group clustered the FAC feeds, another group clustered the VEGO and WAFA feeds, and the last one the ANFA feeds.

The same procedure was carried out with the selected feed ingredients. Although, eight steps were necessary to complete the discriminating model, the results obtained were good and with only two discriminant functions, 100% of the total variance was explained. It was found that four variables (PCB 77, 138, 156 and 169) were significant predictors. Fig. 3D shows the distribution of the selected ingredients as a function of their PCB content using the first two discriminant functions. Four groups could be distinguished: the first one composed of animal protein (fish meal); the second one of cereals, fruits and tubers, vegetable protein and fibre foods, the third of fish oils and the fourth of shell powder.

4. Conclusions

The optimal conditions established in the experiments for the extraction of the selected PCBs have been validated in different feed samples, in which no differences due to different matrix components on the extraction procedures were observed. Hence, the extraction methods proposed in this work are robust enough to determine the selected PCBs in feed samples. Recoveries ranged from 70% to 98% by UAE and from 75 to 106% by FUSE with estimated quantification limits between 0.11 and 0.30 µg/kg in feeds and ingredients and between 0.20 and 0.75 µg/kg in fats. UAE does not demand sophisticated equipment but on the contrary demands time and solvent. FUSE involves more sophisticated equipment but demands lower time and solvent. Nevertheless, matrix effects were observed by FUSE. Therefore, both techniques were suitable for determining PCBs in feeds and feed ingredients.

Higher levels of PCBs were detected in feed samples as well as in feed ingredients (fish oils and shell powder) of animal origin. Up to 1.3 ng WHO-TEQDL-PCBs/kg were detected in feed samples, 123 ng WHO-TEQDL-PCBs/kg in shell powder and 18 ng WHO-TEQDL-PCBs/kg in fish oil. The congener ranges of these samples showing high presence of DL-PCBs compared with FAC samples, where indicator PCBs and PCB 11 were around 70%. Similar results were found for feed ingredients from vegetal or mineral origin such as vegetable oils or fatty acids calcium soaps, showed higher concentrations in NDL-PCBs. In this way, it should be pointed out the lack of specific categories of substances intended for feedstuff purposes that are regulated in Commission Directive 2006/77/EC is a problem.

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