POLYCHLORINATED BIPHENYL LEVELS IN DAIRY-LIKE SOYBEAN DERIVATIVES FROM SPAIN.

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Introduction

Polychlorinated biphenyls (PCBs) are a group of pollutants wide sparse in the environment due to their ample use in the past. Among the 209 possible isomers, attention is usually focused on those congeners that are stereoisomers of the 2,3,7,8-tetrachlorodibenzo-p-dioxin, which showed a toxicity similar to that of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) [1]. In fact, determination of toxic coplanar PCBs has been proposed as a useful tool for evaluating the potential toxic health risk of samples [2-4]. From this point of view, a significant number of papers have shown that the presence of these pollutants in fatty food can be of special interest due to their direct incidence on humans [3,5,6]. This is cause for concern especifically in the case of dairy products because of their wide consumption by population. [7].

Owing to the nutritional properties of soybean, soybean-based dairy-like products are proposed as one of the most interesting alternatives for people allergic to animal whey proteins. Due to the increase in the consumption of this type of products, an effort has been made in recent years to ascertain their nutritional characteristics [8]. Nevertheless, so far, the information reported concerning organohalogenated pollutant levels such as PCBs in this type of dairy-like products is scarce [5].

In this study, an analytical method previously validated for individual PCB analysis in powdered full-fat milk [9] is adapted and validated for PCB analysis in soybean dairy-like products. Subsequently, the method was applied to the analysis of these pollutants in different dairy-like derivatives, including eight brands of infant formulas, eight brands of liquid soybean milk and four different samples of powdered soybean milk (three brands). In all cases, the toxic tetraequivalents of 2,3,7,8-TCDD (TEQs) due to these pollutants were calculated. These levels were compared to those found in some dairy products or human breast milks from Spain and from other countries.

Matherial and Methods

All solvents were pestipur quality and were purchased from SDS (France). Anhydrous sodium sulphate (Panreac, Spain), Silica gel 60 (Merck, Germany) and Florisil (Sigma, USA) were used as adsorbents.

The 15 PCB congeners studied were selected based on their toxicity and relative abundance in biological and environmental samples according to the World Health Organisation criteria (WHO) [1]. Individual isomers (PCBs IUPAC No. 28, 77, 101, 105, 118, 126, 138, 151, 153, 156, 167, 169, 170, 180 and 194) [10] and Arochlor 1260 were purchased from Ehrenstorfer (Germany). Two individual PCBs (No. 12, 3,4-dichlorobiphenyl and No. 209, 2,2′,3,3′,4,4′,5,5′,6,6′-decachlorobiphenyl) were used as external standards in the instrumental analysis step by high resolution gas chromatography equipped with an electron capture as detector (HRGC-ECD).

The soybean dairy products investigated were selected among the best well-known brands in Spain. They were purchased from supermarkets in Alcalá de Henares (Madrid, Spain).

Briefly, the analytical method consisted of a solid-liquid extraction of a 20 g sample mixed with 5g of silica gel and 20 g of anhydrous sodium sulphate with 300 ml of an acetone:hexane mixture (1:1, v:v). No special sample preparation was required, except for liquid samples that were previously freeze dried. The clean-up of the fatty extracts and the final isolation of the PCB fraction was developed by using SiO_2-H_2SO_4 and Florisil columns according with previously validated methodologies [9,10]. A blank sample was runned every 4 soybean samples in order to check any contamination throughout the analytical procedure. The identification and quantification of the individual PCB congeners were performed by HRGC-ECD using a HP 5890 Series II gas chromatograph. A fused silica capillary BPX.5 column (60 m, 0.25 mm i.d., 0.25 mm film thickness) was used (SGE, Australia). Confirmation of the individual PCBs was developed by HRGC-LRMS using a HP 5890 Series II gas chromatograph coupled to a HP 5971 A mass spectrometer in selected ion monitoring (SIM) mode.

Results and discussion

In the experimental conditions finally proposed in this work, an unambiguous separation of PCBs 77 and 126 from PCBs 110, 126, and 178 was achieved. No chromatographic coelution problems were detected when analysing PCB 77 and PCB 126 levels in the Arochlor 1260 by HRGC-LRMS under the proposed conditions.

The methodology was validated for the analysis of PCBs in soybean infant formulas. Mean recoveries for the spiked coplanar congeners studied were in the 88-114% range, with coefficients of variation (CV) lower than 9.8%. The CV related to the determination of endogenous PCBs were in the 1.5-10.0% range. In general, these results agree with those found in literature for spiked breast milk samples [3,11]. Blank samples showed that no background interferences was introduced by the analytical procedure used.

In all soybean infant formulas analysed PCBs 28, 77, 101, 118, 126, 138, 153, and 170 were detected at quantifiable levels, while congener 169 was below the limit of detection (Table 1). PCB 194 was found at quantifiable level only in two samples. Among the congeners studied, PCBs 101, 77, 153, 28, and 138 were the most abundant, with mean concentrations of 2.14, 1.65, 1.56, 1.38, and 1.04 ng/g of lipids, respectively. Schecter el

all. [5] reported levels of PCBs 28, 138, 153, and 180 in one soybean infant formula below the detection limits (2 ng/g of lipids).

In the liquid soybean milks analysed PCBs 28, 77, 101, 118, 126, 138, 153, 167, 170, and 180 were found at quantifiable levels, whereas congeners 169 and 194 were below their limits of detection (Table 1). PCBs 105, 151, and 156 were found at quantifiable levels only in some of the investigated brands. Among the individual isomers studied, the most abundant were PCBs 101, 77, 153, 28, and 138, with 0.78, 0.62, 0.46, 0.37, and 0.25 ng/g of lipids as mean concentrations, respectively. Thereby, the most apparent difference between cow's milks in Spain [9] and liquid soybean milks were the largest contributions found in these latter for PCBs 77 and 126 (Table 1). Nevertheless, this result agreed with those found when analysing soybean infant formulas.

All the investigated congeners were detected at quantifiable levels in any of the powdered soybean milks analysed, except for PCBs 105, 169 and 194, which were in all cases below their limits of detection (Table 1). PCBs 28, 101, and 77 were found to be the most abundant in powdered soybean milks, with 2.41,1.48, and 1.26 ng/g of lipids as mean concentrations, respectively. In general, the following more abundant congeners were 153, 138, and 118, with mean levels of 0.50, 0.28, and 0.21 ng/g of lipids; whereas the lowest mean concentrations corresponded to PCBs 156, 170, 151 and 167, (0.01, 0.05, 0.06, and 0.06 ng/g of lipids as average, respectively).

In order to evaluate the toxicity of each sample studied, TEQs from PCBs were calculated following the toxic equivalent factor model proposed by the WHO [1]. Similar values were calculated for the different investigated brands of the three types of soybean dairy-like products studied. The total average for the 8 soybean infant formula brands analysed was 0.043 ng TEQ/g of lipids. A lower total average was calculated for the 8 liquid soybean milks investigated (0.012 ng TEQ/g fat weight). This value was also lower than those usually found in literature for cow's milk [9], but was very similar to that calculated for powdered-like milks (0.014 ng TEQ/g of lipids).

In this study, discriminant analysis was used for the characterisation and classification of PCB profiles from different dairy-like products investigated. In fact, the two canonical discriminant functions calculated by the fitted model enabled a total differentiation among the profiles calculated from soybean-based liquid, powdered and infant formulas. The fitted model could be used for predicting which group an unknown sample is most likely to fall into.

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Table 1. PCB levels (ng/g of lipids) in the different soybean products investigated.

PCB	Infant formulas		Liquid milks		Powdered milks	
	Average	SD[a]	Average	SD	Average	SD
28	1.38	1.56	0.37	0.27	2.41	0.72
101	2.14	2.06	0.78	0.47	1.48	0.73
77	1.65	1.68	0.62	0.34	1.26	0.53
151	0.22	0.30	0.06	0.08	0.06	0.13
118	0.53	0.44	0.15	0.11	0.21	0.05
153	1.56	1.14	0.46	0.19	0.50	0.13
105	0.13	0.13	0.13	0.24	ND	-
138	1.04	0.85	0.25	0.11	0.28	0.06
126	0.41	0.36	0.11	0.05	0.13	0.03
167	0.12	0.14	0.09	0.04	0.06	0.05
156	0.05	0.08	0.02	0.02	0.01	0.02
180	0.71	0.89	0.14	0.05	0.11	0.08
169	ND[b]	-	ND	-	ND	-
170	0.28	0.26	0.03	0.01	0.05	0.03
194	0.03	0.07	ND	-	ND	-

[a]SD: Standard Deviation
[b]ND: Not Detected