Evaluation of PCDD/F and DL-PCB presence in feeding fats obtained as co-products or by-products derived from the food chain

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1. Introduction

Animal nutrition constitutes an important issue for the animal production industry. The composition of the feeds may have a direct effect on both, the animal health and the quality and safety of the final products for human consumption.

The FEEDING FATS SAFETY is a project included in the 6th EC Framework Programme which started on January 2005 with the participation of nine European research groups. In general terms, the aim of this project is to fit animal nutrition requirements with the production of safe and good quality meat products, on the basis of using fats (by-products or co-products derived from the food chain) as ingredients of the feeds. In this sense, one of the first objectives of this project was to asses the presence in these fats of different pollutants, such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/Fs) as well as the so-called "dioxin-like" polychlorinated biphenyls (DL-PCBs). Fat samples were classified taking into account their origin and/or the industrial processes involved in their obtention. Thus, the following groups of fats were considered: fish oils (FISH), animal fats (ANFA), acid oils from chemical refining (AOCHE), acid oils from physical refining (AOPHY), lecithins (LECI), recycled cooking oils (RECY), oils extracted from exhausted bleaching earths (EBE), hydrogenated fats from by-products (HYBY), fatty acids calcium soaps (FACS) and a group of miscellaneous fats (MIX).

This study summarizes the PCDD/F and DL-PCB concentrations found in a total of 80 samples belonging to the different categories of fats and oils mentioned above. Special attention was paid to the comparison of the levels observed with the maximum levels established in the European Directive for these contaminants in feedstuffs[1].

2. Materials and Methods

All fat and oil items were collected during 2005 from different countries (i.e. Belgium, France, Germany, Hungary, Italy, Malaysia, Morocco, Norway, Poland, Portugal, Romania, Spain, Sweden or UK).

After the fats sampling and classification processes, samples were send to the Dioxin Laboratory of the IIQAB-CSIC for PCDD/F and DL-PCB determination. The extraction steps varied depending on the sample nature, but as a general procedure, samples were directly dissolved in n-hexane and spiked with known amounts of a $^{13}C_{12}$-PCDD/F and DL-$^{13}C_{12}$-PCB mixture. Then, organic matrix was removed by a sulphuric acid treatment, whereas PCDDs/Fs

and PCBs remained in the n-hexane fraction. In some cases (i.e. high density fats), solid-liquid adsorption chromatography in open columns was employed using silica modified with sulphuric acid as an adsorbent instead of preparing a n-hexane solution. The samples were directly added on the top of the column and analytes were eluted from the matrix using n-hexane as a solvent. Finally, the extracts were rotary concentrated prior to the clean up process.

Purification was accomplished by automated clean up (Power Prep TM, FMS Inc.) based on the use of multilayer silica, basic alumina and PX-21 carbon adsorbents. n-Hexane extracts were loaded and pumped through individual sets of multilayer silica followed by a basic alumina column with n-hexane. Interferences were eliminated with n-hexane:dichloromethane (98:2). Next, PCDDs/Fs were eluted from the alumina column and transferred to the PX-21 carbon column with n-hexane:dichloromethane (1:1). The interferences were eluted on carbon column using ethyl acetate:toluene (1:1) in the forward direction, and PCDDs/Fs were collected from the carbon column in the reverse direction with toluene.

Instrumental analysis was based on the use of high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS). All analyses were performed on a Agilent gas chromatograph fitted with a 40m x 0.18 mm i.d. x 0.18µm film thickness DB-5ms fused silica column (J&W Scientific, CA, USA) connected to a Micromass Ultima NT high resolution mass spectrometer (EBE geometry) controlled by a Masslynx data system. All sample injections, as solutions in nonane, were carried out by a PAL automated system under data control system. Analytes were separately analysed in three different acquisitions, one for PCDDs/Fs, a second for non-ortho DL-PCBs and a third fraction for mono-ortho DL-PCBs. Chromatograms of GC effluents were achieved using a EI+ source and operating in the SIM mode at 10000 resolving power.

Quantification was carried out by the isotopic dilution method. Relative response factors were performed for each individual analyte by the analysis of six different calibration solutions for PCDDs/Fs and seven in the case of DL-PCBs. The results are expressed in TEQ values using WHO-TEFs and calculated in '*upperbound*'[2].

3. Results and discussion

In this study a total of 80 fats or oils currently employed as feed ingredients or considered as potential candidates for feedstuff components were selected for a PCDD/F and DL-PCB analysis. The different categories of fats, together with the corresponding acronyms, number of samples analyzed in each case and the obtained results expressed in pg WHO-TEQ/g of sample are summarized in Table 1.

In general, it has to be remarked that most of these samples are co-products or by- products of the food chain obtained from the application of different production processes (i.e. hot or cold rendering, esterification, hydrogenation). This constitutes an important drawback in terms of accurate knowledge of both, the fat composition and the complexity of the matrix, in comparison with other so-called pure fats or oils. From a regulatory point of view, this aspect becomes particularly important since, in some cases, the final products analyzed could be mixtures of two or more fats with different origin. Therefore, these samples can not be related

to one of the specific categories regulated in the present legislation[1]. Moreover, from an analytical point of view, great efforts had to be performed for the analysis of these fat mixtures due to the complexity of the matrices, formed in many cases by substances with large differences among their physicochemical properties.

Table 1. Sample category, acronym, number of samples analyzed and total results (PCDDs/Fs + DL-PCBs), expressed in pg WHO-TEQ/g.

Category	Acronym	n	Min	Max	Average	Median
Fish oils	FISH	9	1.87	42.47	13.61	9.40
Animal fats	ANFA	22	0.13	1.60	0.74	0.71
Lecithins	LECI	8	0.04	0.17	0.09	0.07
Acid oils from chemical refining	AOCHE	15	0.21	5.35	1.02	0.62
Acid oils from physical refining	AOPHY	10	0.35	6.92	2.36	1.68
Oils extracted from exhausted bleaching earths	EBE	2	1.01	1.75	1.38	1.38
Recycled cooking oils	RECY	8	0.16	0.86	0.55	0.58
Hydrogenated fats	HYBY	4	0.53	2.13	1.31	1.30
Fatty acids calcium soaps	FACS	1*	0.37	0.37	0.37	0.37
Miscellaneous	MIX	1*	0.30	0.30	0.30	0.30

* Pooled sample

As expected, the highest concentration of PCDDs/Fs and DL-PCBs were obtained for samples of fish origin. A total of nine FISH samples were analyzed. The contamination levels varied between 1.87 to 42.47 pg WHO-$TEQ_{PCDDs/Fs+DL-PCBs}$/g oil, with a mean value of 13.61 and a median of 9.40 pg $WHO_{PCDDs/Fs+DL-PCBs}$-TEQ/g oil, respectively. It has to be remarked that some samples presented concentrations equal or above the maximum levels of PCDD/Fs and DL-PCBs allowed by the COMMISSION DIRECTIVE 2006/13/EC for fish oil intended to be used in the production of feedstuffs, which are set at 6 pg WHO-$TEQ_{PCDDs/Fs}$/g oil and 24 pg $WHO_{PCDDs/Fs+DL-PCBs}$-TEQ/g oil, respectively. In terms of PCDD/F congener distribution two different profiles were observed in all the FISH oil samples, independently of the contamination level. Both profiles were in good agreement with data usually reported in the literature for fish and fish oil samples. In one case, there was a predominance of 2,3,7,8-TCDF followed by 2,3,4,7,8-PeCDF; on the contrary, in the second case, despite that these congeners still have an important contribution to the total concentration, the largest contribution was given by the highest chlorinated compounds (OCDF, 1,2,3,4,6,7,8-HpCDD and particularly OCDD).

Twenty two animal fat samples (ANFA) were also selected for PCDD/F and DL-PCB analysis, including fat from poultry, ruminants, pork and mixed fats. In this case, levels of PCDD/Fs and DL-PCBs ranged from 0.13 to 1.60 pg WHO-$TEQ_{PCDDs/Fs+DL-PCBs}$/g fat with an average value of 0.74 and a median value of 0.71 pg WHO-$TEQ_{PCDDs/Fs+DL-PCBs}$/g fat, respectively. Therefore, the results for all ANFA samples indicate that the levels were below the maximum level of 3 pg WHO-$TEQ_{PCDDs/Fs+DL-PCBs}$/g fat set by the EU in this kind of

matrices[1]. On the contrary to the fact observed for FISH samples, the PCDD/F congener distribution profile was very similar in all the ANFA samples, being 1,2,3,4,6,7,8-HpCDD and especially OCDD the most relevant congeners.

The lowest concentrations were achieved for samples of vegetal origin like LECI, with values between 0.04 and 0.17 pg WHO-$TEQ_{PCDDs/Fs+DL-PCBs}$/g fat, with an average and a median of 0.09 and 0.07 pg WHO-$TEQ_{PCDDs/Fs+DL-PCBs}$/g fat, respectively.

Particular attention was paid to the origin of AOCHE, AOPHY and HYBY samples. Despite they usually are complex matrices that can not be related to a single origin, sometimes a clear dependence between the former origin of the fat and the PCDD/F congener profile and levels of PCDD/Fs and DL-PCBs was observed. Thus, for instance, in the case of some AOCHE samples from animal fat and AOCHE samples from fish oil, the results were very similar to those reported for the ANFA and FISH samples, respectively.

EBE and RECY materials were also evaluated. In this case, though the levels obtained were lower in comparison with other samples belonging to the ANFA or FISH categories, it has to be pointed out that the use of these materials is restricted or forbidden for feed production purposes. Finally, three and ten samples, belonging to the FACS and MIX categories respectively, were analyzed in two separated pools, since their used is very scarce in feed production. In both cases, low levels of PCDDs/Fs and DL-PCBs were observed.

Acknowledgments

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4. References

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