DETERMINATION OF PCDDs AND PCDFs IN DIFFERENT ANIMAL FEED INGREDIENTS

Ethel Eljarrat, Josep Caixach and Josep Rivera

Mass Spectrometry Laboratory, Ecotechnologies Department, I.I.Q.A.B., C.S.I.C., Jordi Girona 18-26, 08034 Barcelona, Spain

Introduction

Since the dioxin contamination of animal feed in Belgium in 1999, public concern about PCDD and PCDF levels in animals and food has been raised. Several studies have found high levels of PCDDs and PCDFs in animals and food resulting from the use of contaminated animal feed[1-5]. Since feed can contribute considerably to the contamination of food, it is important to monitor the dioxin contamination of feeds and feed ingredients. The aim of this research was to evaluate the contamination caused by PCDDs and PCDFs in different animal feed ingredients. Two different groups of ingredients were analysed: one of animal origin, and the other of mineral origin.

Materials and Methods

Thirty two samples were selected for this study. These samples were classified in two different groups:

- Seventeen samples of animal origin, including samples of hemoglobin, animal fat, fish oil, fish meal and meat and bone meal.
- Fifteen samples of mineral origin, including samples of bentonite, damoline, kaolin, magnesite, sepiolite and zeolite.

Extraction and clean-up

Samples of animal origin were liophilized and manually ground before extraction. For samples with a fat content, the protocol consisted in fat extraction, followed by fat elimination and finally purification of the extract. The fat extraction was carried out in a Randall extractor for two hours with toluene[6]. After extraction, the samples were spiked with a mixture of fifteen $^{13}C_{12}$-labeled 2378-substituted isomers. The fat elimination consisted in an acid attack using a separatory funnel with H_2SO_4 conc. and in a subsequent washing with H_2O Millipore. The clean-up of the extracts was carrried out in an automated system, using multilayer silica, alumina and carbon columns. Samples of mineral origin were manually ground before extraction. The extraction was carried out in a Soxhlet apparatus for 24 hours with toluene. After extraction, the crude extracts were subjected to the same automated clean-up described above. All the samples were finally concentrated to incipient dryness prior to the addition of a mixture of $^{13}C_{12}$-1234-TCDD and $^{13}C_{12}$-123789-HxCDD as the recovery standard.

Instrumental analysis
Purified PCDD / PCDF extracts were analysed by HRGC-HRMS on a Fisons 8060 gas chromatograph fitted with a DB-5 (J&W Scientific, CA, USA) fused-silica capillary column (60 m x 0.25 mm ID, 0.25 μm film thickness) coupled to an AutoSpec-Ultima (Micromass, Manchester, UK) mass spectrometer operating in the electron impact ionization (electron energy 38 eV) at 10.000 resolving power. Quantitative determination was performed by the isotope dilution method based on the relative response factors (RRFs) previously obtained from five standard solutions. The acceptance criteria for data include: chlorine isotope ratio within ± 15% of the correct ratio, peak maxima retention time within two seconds and peak responses at least three times the background noise level.

Results and discussion
Table 1 and 2 summarize the PCDD and PCDF concentrations obtained for the samples of animal origin and of mineral origin, respectively. We present the total I-TEQ and the total WHO-TEQ values. Total TEQ values reported were calculated assuming that all values less than the limit of detection (LOD) are equal to the LOD. The ratio $R_{\text{WHO-TEQ PCDDs/WHO-TEQ PCDFs}}$ was also calculated to compare the PCDD and PCDF contribution in the total toxicity of the samples.

Samples of animal origin
PCDDs and PCDFs were quantified in all samples. The range based on the fat content was 0.48 to 8.50 pg I-TEQ/g fat and 0.52 to 9.08 pg WHO-TEQ/g fat. The meat and bone meal and fish meal were the ingredients that presented the highest I-TEQ and WHO-TEQ values. The $R_{\text{WHO-TEQ PCDDs/WHO-TEQ PCDFs}}$ ranged between 0.64 and 2.68, the highest values being those obtained for meat and bone meal samples. The OCDD was the dominating congener in all the samples.

The literature of dioxin levels in feed ingredients is very scant. Rappe *et al.*[1] reported levels in meat and bone meal and fish meal, and their findings were similar to those obtained in our study. The values were 2.06 pg I-TEQ/g fat and 2.11 pg WHO-TEQ/g fat for the meat and bone meal sample, and 9.87 pg I-TEQ/g fat and 11.8 pg WHO-TEQ/g fat for the fish meal sample. The $R_{\text{WHO-TEQ PCDDs/WHO-TEQ PCDFs}}$ was 2.66 for meat and bone meal and 2.16 for fish meal. The OCDD was also the dominating congener in both samples.

European regulation for dioxin levels in this kind of feed ingredients is being discussed.

Samples of mineral origin
PCDDs and PCDFs were quantified in all samples. The range was 0.05 to 388.21 pg I-TEQ/g and 0.05 to 460.59 pg WHO-TEQ/g. The kaolin was the mineral product with the highest I-TEQ and WHO-TEQ values. The other mineral products presented levels below the background values normally founded in non contaminated soils. The $R_{\text{WHO-TEQ PCDDs/WHO-TEQ PCDFs}}$ ranged between 0.67 and 6486.20, and were higher than those obtained for samples of animal origin. It should be pointed out that the contamination found in kaolin samples is characterized by a practically total contribution of PCDDs. However, the PCDF values resembled those obtained in the other mineral products. The source of dioxins detected in kaolin samples was not established.

Table 1 PCDD and PCDF concentrations in samples of animal origin.

	WHO-TEQ	I-TEQ	$R_{WHO\text{-}TEQ\ PCDDs/WHO\text{-}TEQ\ PCDFs}$
Hemoglobin (pg/g)			
H-1	0.07	0.07	0.89
H-2	0.07	0.06	0.76
H-3	0.03	0.02	0.73
Animal fat (pg/g fat)			
AF-1	0.72	0.75	1.82
AF-2	0.66	0.69	2.11
AF-3	0.52	0.48	0.77
AF-4	0.59	0.54	1.17
Fish oil (pg/g fat)			
FO-1	2.29	2.02	0.64
FO-2	2.49	2.96	1.18
FO-3	2.65	2.35	0.80
Fish meal (pg/g fat)			
FM-1	3.21	3.28	0.86
FM-2	2.82	2.77	0.76
FM-3	1.46	1.42	1.09
FM-4	2.47	2.62	0.89
FM-5	9.08	8.00	0.68
Meat and bone meal (pg/g fat)			
MBM-1	2.82	2.72	2.13
MBM-2	6.77	8.50	2.68

The literature of dioxin levels in these mineral products is also very scant. Holcomb *et al.*[7] reported the dioxin level of 22.44 pg WHO-TEQ/g in a bentonite sample, which was much higher than our findings.

It is interesting to compare our results with the limits proposed by the regulation on the conditions for the authorisation of some additives in feedingstuffs[8]. A limit of 0.5 pg WHO-TEQ/g was established for some mineral products used in the manufacturing of animal feed ingredients. For all the samples studied, the values obtained were below this limit, with the exception of the kaolin samples, where the levels obtained showed a high level of contamination.

Acknowledgements
The authors are indebted to M.A.Adrados, M.G.Martrat and J.Sauló for their collaboration in this study.

Table 2 PCDD and PCDF concentrations in samples of mineral origin.

	WHO-TEQ	I-TEQ	$R_{WHO\text{-}TEQ\ PCDDs/WHO\text{-}TEQ\ PCDFs}$
Bentonite (pg/g)			
B-1	0.20	0.19	1.05
Damoline (pg/g)			
D-1	0.14	0.14	1.40
Kaolin (pg/g)			
K-1	232.31	193.24	2130.32
K-2	460.59	388.21	6486.20
Magnesite (pg/g)			
M-1	0.10	0.08	1.67
M-2	0.05	0.05	1.52
M-3	0.06	0.05	1.29
M-4	0.05	0.05	1.35
Zeolite (pg/g)			
Z-1	0.05	0.05	0.67
Sepiolite (pg/g)			
S-1	0.27	0.21	4.38
S-2	0.33	0.25	3.14
S-3	0.29	0.22	10.23
S-4	0.23	0.17	6.03
S-5	0.16	0.11	4.64
S-6	0.23	0.18	2.95

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