LEVELS OF POLYCHLORINATED DIBENZO-*P*-DIOXINS AND DIBENZOFURANS IN FEED IN PORTUGAL

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Introduction

Food is the main source of human daily intake of PCDDs/Fs (over 90% of the total). The levels of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in food of animal origin are mainly due to bioaccumulation and biomagnification along the food chain.[1]

Since the dioxin contamination of feed in Belgium (in 1999), public concern about PCDD/PCDF levels in animals and food has been raised. Many studies have found high levels of PCDDs and PCDFs in animal and food resulting from the use of contaminated animal feed. Since feed can contribute considerably to the contamination of food, it is important to monitor the dioxin contamination of feeds and feed ingredients.[2]

The dioxin level in food originating from farmed animals is widely the result of dioxin in feed. Thus the control of dioxin level in feed is a decisive tool to reduce the human dioxin intake.

The main scope of this work was to obtain data on concentration of PCDDs and PCDFs in feed consumed by farmed animals in Portugal, in order to verify whether they are in accordance with the EC Directive No. 57/2003.[4] Different types of feed samples were analysed: f*eed material of plant origin, feedingstuffs for fish, compound feedingstuffs, minerals and trace elements.*

Material and Methods

Sampling

All feed samples were collected from the producers in different regions of the country and appropriately transported to the laboratory during the years 2004-2005.

Materials

Prior to use, all the solvents and reagents were checked for the absence of dioxins by GC-HRMS after 10 fold concentration. Internal standards of $^{13}C_{12}$-labeled analogs were obtained from CIL (Cambridge Isotope Laboratories, Woburn, USA). Alumina Basic Super I B was purchased from ICN (Promochem, Barcelona, Spain). Carbosphere 80/100 mesh was purchased from Altech (I.L.C., Lisbon).

Method

Samples were extracted with a mixture of CH_2Cl_2/n-hexane (1:1) in order to obtain the fat fraction that contains the PCDDs and PCDFs.[1] For quantification by the isotope dilution method, internal standards of $^{13}C_{12}$ analogs were added to the samples prior to the extraction. The extracts were evaporated to dryness and the amount of fat was weighed to obtain the fat content of the sample.

The extracted lipids were redissolved and brought onto the top of a Carbosphere column (carbon chromatography) which was placed in a reflux unit and refluxed for 2 h with CH_2Cl_2. Then, the column was rinsed with toluene and refluxed with toluene for 1 h. After cooling to room temperature, the column was inverted in the reflux unit and the PCDDs/PCDFs were eluted from the column by refluxing with toluene for at least 20 hours. This fraction was carefully evaporated to dryness.[1,5]

This residue was dissolved in hexane and the mixture was brought onto a column containing 1 g of 44% H_2SO_4-silica gel, and 5 g of alumina. The alumina column (alumina chromatography) was rinsed twice with hexane and then washed with a mixture of hexane/dichloromethane (98:2 v/v). This eluate was discarded. The PCDDs/PCDFs were obtained with an hexane/ dichloromethane mixture (60:40 v/v). Finally, the eluate was evaporated to dryness in nonane containing injection standard $^{13}C_6$ 1,2,3,4-TCDD.[5]

Instrumental Analysis

The quantification of PCDDs/PCDFs was performed by HRGC-HRMS (EI) in MID mode on a Trace GC gas chromatograph coupled to a MAT-95 XL mass spectrometer (ThermoFinnigan, Bremen, Germany) equipped with a AS2000 autosampler. Gas Chromatographic separations were carried out using a DB-5 MS capillary column (60 m x 0,25 mm i.d. from J&W Scientific, USA) using helium as carrier gas. Instrumental conditions and purity control criteria are according to EPA 1613B method.[6]

Results and Discussion

The average concentration values for individual congeners, as well as the average concentration sums calculated as upperbound and the average upperbound WHO-TEQ[7] (calculated multiplying the concentrations with the corresponding WHO-TEFs for each congener) values for PCDDs/PCDFs are given in tables 1 (*feed material of plant origin*, *feedingstuffs for fish* and *compound feedingstuffs*) and 2 (*minerals* and *trace elements*). Concentration and TEQ values of all compounds are reported on a dry matter basis (ng/kg). Upperbound TEQ values are calculated assuming that the non-detected individual congener concentrations are equal to their corresponding limits of detection.

PCDDs/PCDFs TEQ values for *feed material of plant origin* (ranged from 0.048 ng TEQ/kg to 0.22 ng TEQ/kg, with a mean value of 0.10 ng TEQ/kg), *feedingstuffs for fish* 0.2 ng TEQ/kg and *compound feedingstuffs* (ranged from 0.069 ng TEQ/kg to 0.29 ng TEQ/kg, with a mean value of 0.15 ng TEQ/kg), are lower compared with those obtain by Lund K. H.[3]

PCDDs/PCDFs values for mineral -*dicalcium phosphate* (ranged from 0.049 ng TEQ/kg to 0.091 ng TEQ/kg, with a mean value of 0.07 ng TEQ/kg) and *calcium carbonate* 0.07 ng TEQ/kg - are close to those reported in other European countries[3] and lower than those obtained by Kim D-G.[8]

For the *zinc oxide* samples, the CDDs/PCDFs values obtained ranged from 0.11 ng TEQ/kg to 0.37 ng TEQ/kg, with a mean value of 0.24 ng TEQ/kg.

In conclusion, the levels of PCDDs/PCDFs in the f*eed material of plant origin, feedingstuffs for fish, compound feedingstuffs, minerals and trace elements* presented here are comparable to levels found in other EU countries and are, in each case, below the EC Regulation limits.[4]

Table 1 - Average results of PCDDs/PCDFs in feed material of plant origin, feedingstuffs for fish and compound feedingstuffs.

	feed material of plant origin (n=6)	feedingtuffs for fish (n=1)	compound feedingstuffs (n=10)
2,3,7,8-TCDD	0.02	0.012	0.02
1,2,3,7,8-PeCDD	0.03	0.021	0.05
1,2,3,4,7,8-HxCDD	0.04	0.019	0.06
1,2,3,6,7,8-HxCDD	0.04	0.12	0.07
1,2,3,7,8,9-HxCDD	0.16	0.061	0.07
1,2,3,4,6,7,8-HpCDD	0.65	2.67	0.48
1,2,3,4,6,7,8,9-OCDD	17.29	12.95	8.61
2,3,7,8-TCDF	0.02	0.39	0.03
1,2,3,7,8-PeCDF	0.02	0.07	0.03
2,3,4,7,8-PeCDF	0.02	0.12	0.04
1,2,3,4,7,8-HxCDF	0.04	0.013	0.04
1,2,3,6,7,8-HxCDF	0.05	0.06	0.06
2,3,4,6,7,8-HxCDF	0.05	0.072	0.05
1,2,3,7,8,9-HxCDF	0.05	0.015	0.06
1,2,3,4,6,7,8-HpCDF	0.04	0.15	0.47
1,2,3,4,7,8,9-HpCDF	0.06	0.065	0.08
1,2,3,4,6,7,8,9-OCDF	0.16	0.43	0.32
Sum PCDDs/Fs	18.75 (4.45-84.52)	17.24	10.55 (3.1-21.23)
TEQ PCDDs/Fs	0.10 (0.048-0.22)	0.2	0.15 (0.069-0.29)

Table 2 - Average results of PCDDs/PCDFs in minerals (dicalcium phosphate, calcium carbonate) and trace elements (zinc oxide).

	dicalcium phosphate (n=2)	calcium carbonate (n=1)	zinc oxide (n=2)
2,3,7,8-TCDD	0.01	0.0079	0.01
1,2,3,7,8-PeCDD	0.02	0.018	0.02
1,2,3,4,7,8-HxCDD	0.03	0.22	0.01
1,2,3,6,7,8-HxCDD	0.03	0.031	0.02
1,2,3,7,8,9-HxCDD	0.05	0.029	0.03
1,2,3,4,6,7,8-HpCDD	1.89	0.064	0.13
1,2,3,4,6,7,8,9-OCDD	39.55	26.48	1.22
2,3,7,8-TCDF	0.01	0.0095	0.10
1,2,3,7,8-PeCDF	0.01	0.013	0.11
2,3,4,7,8-PeCDF	0.05	0.0085	0.22
1,2,3,4,7,8-HxCDF	0.02	0.0172	0.20
1,2,3,6,7,8-HxCDF	0.01	0.0172	0.16
2,3,4,6,7,8-HxCDF	0.02	0.0188	0.25
1,2,3,7,8,9-HxCDF	0.01	0.023	0.09
1,2,3,4,6,7,8-HpCDF	0.11	0.021	0.63
1,2,3,4,7,8,9-HpCDF	0.02	0.026	0.13
1,2,3,4,6,7,8,9-OCDF	0.46	0.032	0.79
Sum PCDDs/Fs	47.27 (2.93-81.6)	27.03	4.13 (1.12-7.15)
TEQ PCDDs/Fs	0.07 (0.049-0.091)	0.07	0.24 (0.11-0.37)

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