A CASE OF POULTRY CONTAMINATION BY POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN PORTUGAL: IDENTIFICATION OF LEVELS AND SOURCE OF CONTAMINATION

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are two groups of toxic and persistent chemical substances. They are mainly by-products of industrial processes waste incineration processes but can also occur from natural processes like forest fires or volcanic activity.[1] PCDDs and PCDFs can induce many toxic responses, like immunotoxicity, carcinogenicity and adverse effects on reproduction, development, and endocrine functions.[2]

Food is the main source of human intake of PCDDs/Fs (more than 90% of the total daily intake comes from food). 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]

In the framework of the 2004 dioxin surveillance plan in foodstuffs, PCDD/Fs levels exceeding the European Union (EU) tolerance limit were detected in five samples from a poultry farm located at Central region of Portugal.

In order to identify the source of contamination different samples were collected in the farm under investigation, biological samples (poultry meat), feed and litters (poultry beds – wood shavings). The Analytical results obtained confirm the exceeding levels in the poultry meat. The results relative to feed and feeding material reveal background levels on PCDD/Fs. The litters (poultry beds – wood shavings) were highly contaminated.

Material and Methods

Sampling

All food items were collected from the farmers in many regions of the country and were appropriately transported to the laboratory. The food samples were kept at -20 ºC until they were processed.

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). Carbosphere 80/100 mesh was purchased from Altech (I.L.C., Lisbon). The alumina Basic Super I B was purchased from ICN (Promochem, Barcelona, Spain).

Method

Samples were extracted with organic solvents 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 extraction. The extracts were evaporated to dryness and the fat content determined by weighing.

Extracted lipids were redissolved and brought onto the top of a Carbosphere column 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 24 hours. This fraction was carefully evaporated to dryness.[1,2,4]

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 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 and the residue dissolved in nonane containing injection standard $^{13}C_6$ 1,2,3,4-TCDD.[2,4]

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.[2,5]

Results and Discussion
The dioxins levels found in the firsts five samples studied (Table 1) shows levels between 5,7 – 13,32 pg TEQ-WHO/g fat, with a prevalence of PCDDs (87.4-90.0%), particularly 1,2,3,4,6,7,8,9-OCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8-PeCDD relative to the PCDFs (10-12.0%) 1,2,3,4,6,7,8,9-OCDF, 1,2,3,4,6,7,8-HpCDF and 2,3,4,6,7,8-HxCDF.

Table 1
Results of PCDDs/PCDFs in poultry meat (pg/g fat).

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
2,3,7,8-TCDD	0.099	0.32	0.098	0.069	0.048
1,2,3,7,8-PeCDD	1.87	2.65	2.07	3.33	1.68
1,2,3,4,7,8-HxCDD	1.99	2.36	1.36	3.47	1.44
1,2,3,6,7,8-HxCDD	15.73	23.97	15.68	35.77	12.89
1,2,3,7,8,9-HxCDD	4.09	4.56	3.01	5.42	2.95
1,2,3,4,6,7,8-HpCDD	115.85	174.35	125.89	230.16	92.93
1,2,3,4,6,7,8,9-OCDD	255.35	473.26	426.45	859.41	194.37
2,3,7,8-TCDF	0.095	0.1	0.11	0.076	0.21
1,2,3,7,8-PeCDF	1.02	1.43	0.92	1.67	0.87
2,3,4,7,8-PeCDF	0.75	0.92	0.85	1.48	0.72
1,2,3,4,7,8-HxCDF	2.65	3.31	2.31	4.5	1.75
1,2,3,6,7,8-HxCDF	2.98	4.87	2.8	5.98	2.45
2,3,4,6,7,8-HxCDF	3.46	4.82	3.04	6.22	2.47
1,2,3,7,8,9-HxCDF	0.2	0.27	0.22	0.25	0.13
1,2,3,4,6,7,8-HpCDF	19.29	39.51	25.03	49.28	17.81
1,2,3,4,7,8,9-HpCDF	1.98	3.61	2.76	4.33	1.66
1,2,3,4,6,7,8,9-OCDF	18.06	34.34	28.78	53.11	16.09
g TEQ-WHO/g fat	6.91	10.15	7.08	13.32	5.7

Further samples collected in the farm under investigation gave the followings results:

- Levels of 7.42-24.28 pg TEQ-WHO/g fat in poultry meat, confirming the contamination found in the previously collected poultry samples;
- Feed (0.094 to 0.11 pg TEQ-WHO/ g) and feedings (0.11 to 0.42 pg/ TEQ-WHO/g) samples levels were very low and close to the detection limits.
- Poultry litters (wood shavings) were highly contaminated: 29.27 to 111.11 pg TEQ-WHO/g.

The PCDDs/PCDFs profiles found in the poultry meat and litters are showed in Figure 1 and Figure 2 respectively. The similar congener profiles in both types of samples suggest clearly the litters as their contamination source for PCDDs/PCDFs.

The congener profile was identical in all contaminated samples. The OCDD was the most abundant congener among dioxins, followed by 1,2,3,4,6,7,8-HpCDD and 1,2,3,6,7,8-HxCDD. The OCDF was predominant amount furans, followed by 1,2,3,4,6,7,8-HpCDF.

From a toxicological point of view, the congeners which most contributed to TEQ values in poultry meat were 1,2,3,4,6,7,8-HpCDD (22.2-28.3%), 1,2,3,6,7,8-HxCDD (20.8-22.0%) and 1,2,3,7,8-PeCDD (18.7-28.3%). In the litters samples were 1,2,3,4,6,7,8-HpCDD (38.13-60.25%), 1,2,3,6,7,8-HxCDD (10.63-24.53%) and

1,2,3,7,8-PeCDD (8.15-12.47%). This congeners profile is similar to those obtained in identical incidents elsewhere (Scortichini and J.J. Llerena).[6,7]

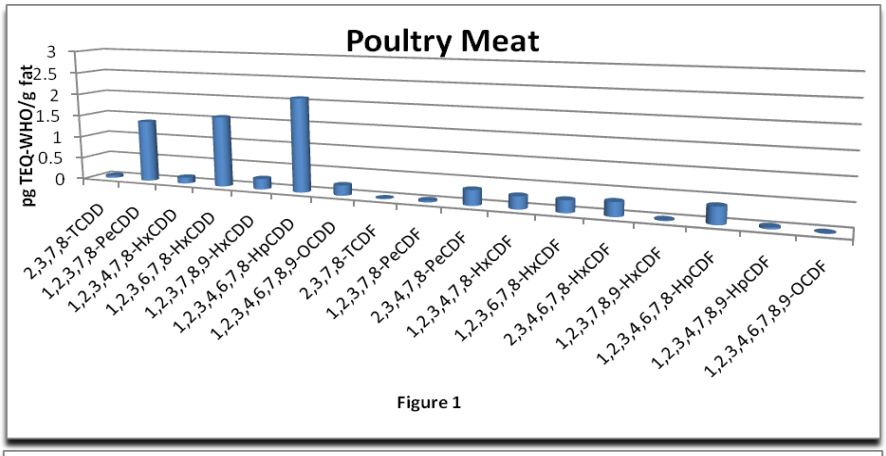

Figure 1

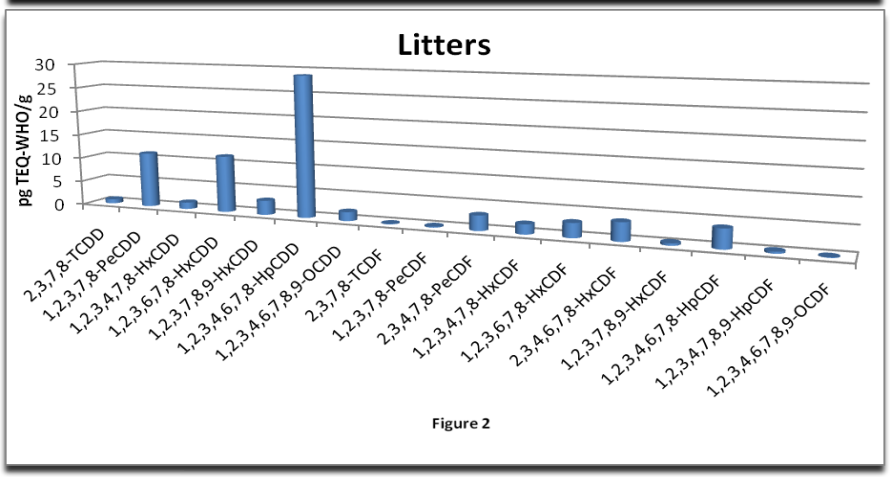

Figure 2

The HpCDD/Fs and OCDD/Fs congener abundance was apparently caused by the use of wood treated with pentachlorophenol (PCP), a biocide highly persistent in the environment used in paintings for wood preservation, in pulp, textile and leathers industries.[6]

The present values clearly reveal the problem of contaminated wood shavings used by unaware breeders in chicken litters: even very low amounts of particles ingested by chicken could cause serious contamination of food for human consumption, such eggs and meat.[6]

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