Levels of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Food Samples in Portugal

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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 (*e.g.*, metallurgy, bleaching of paper pulp and manufacturing of some herbicides and pesticides), waste incineration processes (medical waste, urban waste, burning wires and tires), 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.[2,3]

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.[1,3]

Until the Dioxins Analysis Laboratory, operating since 2003 in the Laboratory of Analytical Organic Chemistry and Synthesis (LAQAS) of INETI in Lisbon, implemented the analytical methodologies for evaluation of the levels of PCDDs/PCDFs in Portuguese food, no data has been available. Collaboration was established with Portuguese Authorities in food and feed for determination of PCDDs and PCDFs in many matrices in order to comply with the recommendations of the European Commission on the monitoring of background levels of dioxins.

The main scope of this work was to obtain data on concentration of PCDDs and PCDFs in food consumed by the Portuguese population, in order to verify whether they are in accordance with the EEC regulation (EC No. 2375/2001, 2001).[4] Different types of food samples were analysed: milk, eggs, cheese, meat and edible mixed vegetable oils.

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 during the year of 2004. The samples of edible mixed vegetable oils were collected from commercial sources. The food samples were kept at -20 :C 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 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 (milk, cheese and eggs) and 2 (meat and vegetal oils). Concentration and TEQ values of all compounds are reported on a fat basis (pg/g fat). Upperbound TEQ values are calculated assuming that the non-detected individual congener concentrations are equal to their corresponding limits of detection. The average values for lipid contend were 3.34% for milk, 17.3% for cheese, 10.12% for eggs, 9.79% for beef, 8.25% for pork, 16.82% for lamb.

Table 1

Average results of PCDDs/PCDFs in milk, cheese and eggs.

	Milk (n=3)	Cheese (n=3)	Eggs (n=3)
2,3,7,8-TCDD	0.12	0.01	0.02
1,2,3,7,8-PeCDD	0.28	0.02	0.09
1,2,3,4,7,8-HxCDD	0.24	0.03	0.11
1,2,3,6,7,8-HxCDD	1.33	0.11	0.15
1,2,3,7,8,9-HxCDD	0.82	0.03	0.22
1,2,3,4,6,7,8-HpCDD	1.91	0.08	1.92
1,2,3,4,6,7,8,9-OCDD	37.25	5.53	8.84
2,3,7,8-TCDF	0.31	0.02	0.03
1,2,3,7,8-PeCDF	0.23	0.03	0.10
2,3,4,7,8-PeCDF	0.20	0.12	0.07
1,2,3,4,7,8-HxCDF	0.27	0.06	0.07
1,2,3,6,7,8-HxCDF	0.28	0.05	0.04
2,3,4,6,7,8-HxCDF	0.27	0.07	0.14
1,2,3,7,8,9-HxCDF	0.24	0.02	0.21
1,2,3,4,6,7,8-HpCDF	0.32	0.06	0.080
1,2,3,4,7,8,9-HpCDF	0.42	0.02	0.08
1,2,3,4,6,7,8,9-OCDF	0.56	0.16	0.12
Sum PCDDs/Fs	45.05 (11.9-87.5)	6.42 (4.53-9.87)	12.28 (3.74-17.72)
TEQ PCDDs/Fs	0.93 (0.57-1.23)	0.14 (0.085-0.22)	0.27 (0.20-0.36)

Table 2

Average results of PCDDs/PCDFs in Meat (Beef, Pork and Lamb) and vegetable oils.

	Beef (n=2)	Pork (n=2)	Lamb (n=2)	Edible Mixed Vegetable Oils (n=3)
2,3,7,8-TCDD	0.19	0.08	0.41	0.18
1,2,3,7,8-PeCDD	0.29	0.11	0.43	1.05
1,2,3,4,7,8-HxCDD	0.12	0.11	0.32	0.36
1,2,3,6,7,8-HxCDD	0.65	0.18	0.45	3.37
1,2,3,7,8,9-HxCDD	0.33	0.10	0.25	0.36
1,2,3,4,6,7,8-HpCDD	2.48	1.20	1.05	0.48
1,2,3,4,6,7,8,9-OCDD	12.15	13.30	11.20	7.04
2,3,7,8-TCDF	0.19	0.09	0.39	0.95
1,2,3,7,8-PeCDF	0.10	0.10	0.22	0.50
2,3,4,7,8-PeCDF	0.42	0.28	0.36	5.01
1,2,3,4,7,8-HxCDF	0.11	0.16	0.22	0.32
1,2,3,6,7,8-HxCDF	0.27	0.28	0.24	0.43
2,3,4,6,7,8-HxCDF	0.19	0.08	0.32	5.01
1,2,3,7,8,9-HxCDF	0.11	0.10	0.27	0.37
1,2,3,4,6,7,8-HpCDF	0.51	0.54	0.49	0.71
1,2,3,4,7,8,9-HpCDF	0.27	0.22	0.49	0.62
1,2,3,4,6,7,8,9-OCDF	1.23	1.14	1.13	1.03
Sum PCDDs/Fs	19.6 (12.4-26.8)	18.1 (15.9-20.3)	18.2 (10.4-26.1)	27.79 (14.15-46.55)

TEQ PCDDs/Fs	0.92 (0.91-0.93)	0.47 (0.24-0.69)	1.30 (0.6-1.97)	0.33 (0.03-0.63)

PCDDs/PCDFs TEQ values for cow milk ranged from 0.57 to 1.23, with a mean value of 0.93, are lower compared with those obtain by Chovancova *et al*.[3] and are similar to those monitored in other European Mediterranean countries (0.28 to 1.33 pg TEQ /g for cow milk).[2]

The values obtained for cheese and eggs ranged from 0.0085 to 0.22 (mean value of 0.14 pg TEQ /g) and (0.20 to 0.36 pg TEQ/pg) respectively are lower than those obtained by Focant *et al*. [9] in 2002 (1.67 pg TEQ/g and 2.76 pg TEQ/pg).

PCDDs/PCDFs values of beef and pork are lower than those reported in other European and Mediterranean countries (0.89 to 2.26 pg TEQ/pg for beef and pork)[2] and of lamb meat are close to those obtained by Focant *et al*. (1.55 pg TEQ/pg).[8]

For the edible mixed vegetable oils samples, all PCDDs/PCDFs congeners are below the maximum limit (0.75 pg TEQ/pg).[4]

In conclusion, the levels of PCDDs/PCDFs in the food samples presented here are comparable to levels found in other EU countries and are, in each case, below the EC Regulation limits.[4]

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