POLYCHLORINATED DIBENZO-*p*-DIOXINS, DIBENZOFURANS AND CO-PLANAR BIPHENYLS IN FOODSTUFFS SAMPLES FROM CATALONIA (SPAIN)

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Introduction

After the recent Belgian incident in may 1999 where an important dioxin contamination episode of animal feed was detected, the department of Public Health (Departament de Sanitat i Seguretat Social) of the Catalonia government (Generalitat de Catalunya) initiated the first surveillance programn on polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and co-planar biphenyls (PCBs) determination in foodstuffs. The study consisted in the analyses of 70 different foodstuff samples produced all over the four provinces of Catalonia. This work also constitutes a preliminary approach to achieve accurate data on the evaluation of the daily intake of dioxins and related compounds by the Catalonia population. Thus, on lipid bases, levels of PCDDs/PCDFs as well as co-planar PCBs could be quantified in untreated milk, pure virgin olive oil, butter, chicken (fat and meat), pig (fat and meat). Mussels samples were also determined on whole weigh analysed as indicated in the draft EC Regulation for PCDDs/PCDFs determination in fish samples [1].

Methods and Materials

All food items were collected through the services of the Department of Public Health and they were properly transported to the laboratory between november 1999 and January 2000 for PCDDs/PCDFs and co-planar PCBs (#77, #126 and #169) analyses. Until their processed, the samples remained frozen prior their analysis.

Owing to their lipophilic nature, the analyses of PCDDs/PCDFs as well as co-planar PCBs were carried out on the assumption that major content of these compounds was placed in the lipid fraction. Thus, extraction procedures varied depending on the samples nature: chicken and pork meats were liophilized and the fat was removed by soxhlet for 24 h with toluene:ciclohexane (1:1); the lipid fraction contained in milk samples was extracted using organic solvents (diethyl ether, petroleum ether, etc.); butter, oil and fat samples were directly dissolved in n-hexane.

Afterwhich, the samples were spiked with knwon amounts of a $^{13}C_{12}$-PCDDs/PCDFs and co-planar $^{13}C_{12}$-PCBs mixture. Then, organic matrix was removed by a sulphuric acid treatment, whereas PCDDs/PCDFs and PCBs remained in the n-hexane fraction. Finally, the extracts were rotary concentrated prior to the clean up process.

The clean up was based on the use of multilayer silica, basic alumina and PX-21 carbon adsorbents as described in reference 2. Data acquisition for PCDDs/PCDFs and co-planar PCBs were achieved simultaneosly in a single HRGC/HRMS analysis. Purified extracts were analyzed by HRGC/HRMS/EI(+)-SIM on a GC 8000 series gas chromatograph (Carlo Erba Instruments,

Milan, Italy) coupled to an Autospec Ultima mass spectrometer (Micromass, Manchester, UK) equipped with a CTC A 200S autosampler, at 10000 resolving power (10% valley definition). Chromatographic separation was achieved 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) with helium as the carrier gas at a linear velocity of 35 cm/s (T: 100°C) in the splitless injection mode (1-2 μL). The temperature program was: 140 °C (1min) to 200 °C (1min) at 20 °C/min, then at 3°C/min to 300 °C and held isothermally for 20 min at 300 °C for the DB-5 GC column and 140 °C (1min) to 200 °C (1min) at 20 °C/min, then at 2°C/min to 280 °C. Quantification was carried out by the isotopic dilution method [2]. Relative response factors (RRF) were performed for each individual 2,3,7,8-chlorosubstituted PCDDs/PCDFs and co-planar PCBs congeners by the analysis of fiver different mixtures of labeled and unlabeled standards. The results are expressed in pg I-TEQ/g and pg WHO-TEQ/g [3]. TEQs values were calculated using the limit of detection (LOD) value for non-detects compounds or values below to the LOD as indicated in reference 1.

Results and Discussion

The median values as well as the minimum and the maximum concentrations of PCDDs/PCDFs including co-planar PCBs expressed in pg/g (WHO-TEQ and I-TEQ) on a lipid weight basis are presented in Table 1. Levels in mussels samples are expressed on a whole weight basis.

Milk:

Dioxin content in milk samples ranged from 0.09 to 0.90 pg I-TEQ/g milk fat (median of 0.36 pg I-TEQ/g fat) and between 0.09 to 2.22 including co-planar PCBs (n:19) with a median of 0.98 pg I-TEQ/g. The results expressed in WHO-TEQ ranged from 0.11-1.08 pg/g fat milk (median of 0.43 pg WHO-TEQ/g fat) and 0.11-2.40 pg/g fat milk inlcuding co-planar PCBs (median of 1.05 pg WHO-TEQ/g fat). In general, the dioxin contamination of the milk samples are low and in the range of French and German average [4,5] or some particular sites in USA [6]. So far, all samples analyzed presented a dioxin content below to the limit of 5 pg I-TEQ/g fat established for its commercialization in the previous euorpean countries and below to the limit of 3 pg WHO-TEQ/g proposed in the EC Regulation [1].

Virgin Olive Oil:

The levels of PCDDs/PCDFs in virgin olive oil samples also presented a very low contamination level with a narrow range of concentration ranging between 0.12and 0.38 pg WHO-TEQ/g (n:15) and a mendian value of 0.21 pg WHO-TEQ/g. No remarkable differences were found when co-PCBs were considered, between 0.14and 0.44 pg WHO-TEQ/g (median of 0.27 pg WHO-TEQ/g). The values expressed in pg I-TEQ/g were slighly lower (see table 1).

Butter:

The values determined in butter samples presented also low contamination levels. The findings ranged between 0.27 and 0.65 pg I-TEQ/g fat butter (with a median of 0.47 pg I-TEQ/g fat) and from 0.72 to 1.54 pg I-TEQ/g including PCBs (median of 1.06 pg I-TEQ/g fat). The values expressed in pg WHO-TEQ varied from 0.32 to 0.73 pg/g fat with a median of 0.54 pg/g fat and between 0.76 and 1.63 pg/g fat when co-planar PCBs were included (median of 1.12 pg/g fat). These results are consistent to those reported by Fiedler et al (1999) despite the fact that our values are slighly lower.

Chicken:
A variable content of PCDDs/PCDFs and co-planar were observed in the 12 samples studied. The levels of PCDDs/PCDFs ranged between 0.3-3.5 pg I-TEQ/g fat chicken (0.4-3.6 WHO-TEQ/g fat), where the congener distribution was dominated by a remarkable concentration of OCDD (between 2.7 and 179 pg/g fat chicken). The ranging levels increased from 0.36 to 3.8 pg I-TEQ/g when co-planar PCBs were considered. The congener distribution of PCBs demonstrated an important contribution of PCB #77 (which concentration was up to 36 pg/g fat) followed by PCB #126 (levels up to 6.8 pg/g) and PCBs#169 (levels below 1 pg/g fat). Special attention were focussed in one case where the levels were 34.31 pg WHO-TEQ/g fat. In this particular case, major differences were observed in the congener distribution whereas an increase of all of the 2,3,7,8-PCDDs/PCDFs congener as well as co-planar PCBs was observed overall. The complexity of this pattern revealed an unknown external contamination which will be subsequently evaluated in the next future.

Pork:
Levels of PCDDs/PCDFs varied from 0.12 to 1.97 pg I-TEQ/g fat sample (0.13-2.09 pg WHO-TEQ/g fat) with a median of 0.77 pg I-TEQ/g fat (0.85 pg WHO-TEQ/g fat). The levels ranged between 0.16 to 2.0 pg I-TEQ/g fat (0.17-2.11 pg WHO-TEQ/g fat) when co-planar PCBs were considered. All cases presented levels below to the maximum value proposed in the EC Directive (today in a draft version) which set a dioxin content limit of 2 pg WHO-TEQ/g fat [1].

Mussels:
5 different mussels samples were also analyzed. The dioxin content in mussels varied between 1.08 to 5.55 pg I-TEQg whole product (1.19 to 5.60 pg WHO-TEQ/g whole product) with median of 3.58 pg I-TEQ/g product (3.65 pg/g expressed in WHO-TEQ). The congener distribution was dominated by a remarkable presence of 2,3,7,8-TCDF which levels ranged between 4.28 and 21.81 pg/g whole product. Special attention were the co-planar PCBs, whereas the congener distribution was characterized by a remarkable content of PCB#77 (values between 491.45 and 2647 pg/g whole product) followed by PCB#126 (37.27-334.22 pg/g whole product) and PCB#169 (4.52-75.41 pg/g whole product).

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Table 1. Median values, minimum and maximum concentrations (expressed in pg/g WHO and I-TEQ) of PCDDs/PCDFs and co-plnar PCBs in food samples from Catalonia.

No of samples		PCBs TEQ	PCDD-PCDF I-TEQ	Total I-TEQ	PCDD-PCDF WHO-TEQ	Total WHO-TEQ
Virgin Olive Oil						
n:15	Median	0.06	0.19	0.25	0.21	0.27
	Maximum	0.14	0.36	0.42	0.38	0.44
	Minimum	<0.01	0.11	0.14	0.12	0.15
Mussels						
n:5	Median	16.29	3.58	19.86	3.65	19.93
	Maximum	33.49	5.55	35.09	5.60	35.15
	Minimum	3.86	1.08	7.97	1.19	8.03
Butter						
n:9	Median	0.59	0.47	1.06	0.54	1.12
	Maximum	0.89	0.65	1.54	0.73	1.63
	Minimum	0.37	0.27	0.72	0.32	0.76
Milk						
n:19	Median	0.62	0.36	0.98	0.43	1.05
	Maximum	1.41	0.90	2.22	1.08	2.40
	Minimum	<0.01	0.09	0.09	0.11	0.11
Pork						
n:10	Median	0.09	0.77	0.86	0.85	0.94
	Maximum	0.31	1.97	2.00	2.09	2.11
	Minimum	0.02	0.12	0.16	0.13	0.17
Chicken						
n:13	Median	0.30	3.47	3.77	3.58	3.88
	Maximum	0.70	33.29	33.97	33.63	34.31
	Minimum	0.01	0.36	0.52	0.40	0.56