PRESENCE OF POLYCHLORINATED DIBENZO-p-DIOXINS (PCDDs), DIBENZOFURANS (PCDFs) AND BIPHENYLS (PCBs) IN SELECTED FOOD SAMPLES FROM HUELVA (SPAIN)

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Introduction

The main objective of this study was to obtain representative data on levels of dioxin and related compounds in food consumed by the general population in Huelva, a city located in the south-east part of Spain situated in an area affected by heavy industries. The analytical programme included determinations of PCDDs, PCDFs and an extensive list of PCBs including non-*ortho* and mono-*ortho* congeners.

Since the main way of exposure to PCDD/Fs and PCBs for humans is food, the analysis of these chemicals in food samples has increased in the last times. With the establishment of new European directives[1] regulating the presence of these compounds in foodstuff, efforts are being done to determine the levels of these compounds in different food samples since 90 % of human exposure is through the diet and mainly due to foodstuffs of animal origin[2].

Materials and Methods

Sampling

Food products were purchased from different markets in Huelva City (Spain) between February and July 2001. The products were selected depending on their consumption by the population living in the area. The food products were stored at –20 ºC and transported to the laboratory. Once at the laboratory, they were stored in stable conditions, either lyophilised or frozen, until analysis. For fish, molluscs and crustaceans, just the edible part was taken for the analysis

Sample preparation

The extraction of PCBs and PCDDs/Fs involved a matrix solid phase dispersion. Samples were ground in a mortar with anhydrous sodium sulphate and silica gel. After addition of a mixture of the $^{13}C_{12}$ labelled non-*ortho* PCBs and $^{13}C_{12}$ labelled 2,3,7,8-PCDD/Fs standards, the samples were extracted with a mixture acetone:hexane (1:1,V/V). Further clean-up and lipid removal was achieved by using acid and basic modified silica gel multilayer columns[3] using hexane as elution solvent.

Fractionation and instrumental analysis

Final fractionation among the studied compounds was achieved by using Supelclean™ Supelco ENVI™-Carb tubes as described elsewhere[4]. Three fractions were eluted containing ortho-substituted PCBs (including mono-*ortho* PCBs), non-*ortho* PCBs and PCDDs/Fs congeners, respectively.

Ortho substituted PCBs instrumental analysis

Congeners number 28, 52, 95, 101, 132, 138, 149, 153, 170, 180, 183, 194, 105, 114, 118, 123, 156, 157, 167 and 189 were analysed by GC-µECD (Agilent 6890 Series II- equipped with a ^{63}Ni electron capture µ-detector) as described by Gómara et al. (2002)[5]. TCN and PCB 209 were used as internal standards.

Non-ortho substituted PCBs (PCB 77, 126 and 169) and PCDDs/Fs instrumental analysis

The quantification of non-*ortho* PCBs and PCDDs/Fs was performed by GC-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). Instrumental conditions are described in Abad et al.(2002)[6]. The quantification was carried out by the isotopic dilution method.

Results and Discussion

Concentration values of all compounds in ng/g or pg/g are reported in table 1 on a fat weight basis (f.w.). The human-TEFs proposed by the WHO in 1998[7] have been used to calculate the TEQs, and assuming that nondetects are equal to their corresponding limit of detection (LOD), since the maximum levels of these compound in foodstuff settled by the EU are referred to the upperbound level.

A. Milk and dairy products

A total of five commercial pasteurised whole milk samples were individually analysed. For dairy products, one butter, one cream and four yoghurt samples were taken to determine the levels of chlorinated contaminants. Milk presented a mean *ortho* PCB value of 20.22 ng/g. For the dairy products analysed, the levels were in the same range of milk samples, but yoghurt exhibited the highest levels. Milk samples presented a mean value of 1.92 pg/g WHO-TEQ, representing the contribution of PCDD/Fs approximately a 50 % of the total value. The same was observed for the rest of dairy products analysed, although in cream and butter the contribution of non-*ortho* PCBs was remarkable. PCBs and PCDD/Fs showed similar accumulation patterns in both milk and dairy products, confirming the findings from Fürst et al (1992)[8] that congener patterns of dioxins and PCBs in dairy products relate closely to those in milk.

All the milk and dairy products analysed in this study are far below the limit established by the EC Directive[1] for this kind of products (3 pg WHO-TEQ PCDD/F / g fat weight).

B. Fatty sea fish, lean sea fish and canned fish samples

Different fish species typical from the city market were sampled and further pooled into two groups. The first group, fatty sea fish, was composited by sardine (*Sardina sp.*) (n=30), mackerel (*Scomber sp.*) (n=2), and tuna fish (*Thunnus sp.*) (n=1). The second group, lean sea fish, was composited by little sole (*Dicologlossa sp.*) (n=17), tope (*Galeorhinus sp.*) (n=2) and common two-banded seabream (*Diplodus sp.*) (n=17). Fatty sea fish showed lower levels of *ortho* PCBs than lean sea fish. Total WHO-TEQ values ranged between 15.09 and 19.88, pg/g for lean and fatty sea fish respectively. In this type of samples, non-*ortho* PCBs WHO-TEQ accounted for more than 50 % to the total WHO-TEQ value.

A sample of canned tuna fish and canned sardine were also analysed. The PCB levels were lower than those found for pooled fish samples. Canned sardine showed the highest WHO-TEQ level in the same order as fish samples, while canned tuna presented lower levels than the fish samples. Contrary to fish samples, the contribution of non-*ortho* PCBs to the total WHO-TEQ (11 and 21 %, respectively) is lower than the contribution of dioxins and furans (86 and 73 %).

C. Molluscs and crustaceans

Bivalves results are the mean value between coquina clam (*Donax sp.*) and a pooled sample of frequently consumed clams in the area (*Venus sp.* and *Tapes sp*.). Cuttlefish (*Sepia sp.*) (n=6) and prawns (n=30) are very appreciated species in the area and therefore they were included in the study. Samples of bivalves showed the highest *ortho*-PCB levels, followed by prawns and cuttlefish. WHO-TEQ levels, ranging between 4.88 and 10.94 pg/g, were lower than those found for fish species, presenting bivalves the highest values. In this case, dioxins and furans were the family that contributed in a higher percentage to the total WHO-TEQ value, except for cuttlefish, where they accounted in a similar percentage.

Neither the two pooled fish samples nor the mollusc and crustaceans samples analysed presented values above the limit set by the EU for this kind of products expressed as fresh weight (4 pg WHO-TEQ PCDD/Fs fresh weight)[1]. The canned sardine sample did present values above the limit set by this directive.

D. Meat and meat products

The following meat and meat products were analysed: chicken (two single samples), chicken entrails, pork meat, cured ham (sample composited by 2 types of cured ham) and pork products (mean value between hard pork sausage, loin and a pooled sample composited by different pork sausages (hard pork sausage, blood sausage and salami-type sausage)). Chicken entrails presented the highest *ortho* PCB levels, followed by chicken, pork and pork products. WHO-TEQ levels ranged from 2.03 to 8.34 pg/g, showing the highest levels the chicken sample. PCDD/Fs accounted in a higher percentage to the total WHO-TEQ value (54-73 %) than non-*ortho* PCBs, except for pork sample where the contribution was similar (42 and 49 %, for PCDD/Fs and non-*ortho* PCBs, respectively). In this case, all samples presented PCDD/Fs WHO-TEQ levels over the limit set by the EC Directive[1], except chicken entrails.

E. Oil samples, eggs and bakery products

Olive oil and sunflower oil were analysed in this study. The olive oil sample was a pooled sample composited by 3 different types of commercial olive oil. The levels of *ortho* PCBs were higher for sunflower oil. Nevertheless these levels are low compared to other food samples analysed in this study. WHO-TEQ values ranged from 0.71 to 2.04 pg/g and PCDD/Fs WHO-TEQ values between 0.29 and 0.34 pg/g. In this case non-*ortho* PCBs also accounted in a higher percentage to the total than dioxins and furans, representing a 49 and a 71 % of the total content, for olive and sunflower oil, respectively. None of the oil samples analysed presented PCDD/Fs WHO-TEQ levels above the ones set by the EC Directive[1].

The egg sample was a pooled sample composited by eggs from two different farms located in the area (6 eggs from each farm). The total *ortho* PCB level found was 14.56 ng/g. The total WHO-TEQ level was 1.43 pg/g (data on non-*ortho* PCBs are not reported), in the same order as those found for whole milk samples and dairy products analysed. The WHO-TEQ PCDD/Fs level found was 1 pg/g, value not exceeding the maximum set by the EC Directive[1].

For bakery products, the *ortho* PCB level found was 10.91 ng/g. The total WHO-TEQ was 0.85 pg/g, even lower to the reported for dairy products and eggs. WHO-TEQ PCDD/Fs level was 0.42 pg/g accounting in fast 50 % to the total WHO-TEQ value.

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Table 1. Levels and mean levels (min – max) of PCDD/Fs, non-*ortho* PCBs (pg/g f.w.) and *ortho*-PCBs (ng/g f.w.) in the food samples analysed. WHO-TEQs are expressed as pg/g f.w. considering non-detected values equal to the limit of detection

	Milk (N=5)	Yogurt	Cream	Butter	Lean sea fish	Fatty sea fish
ortho -PCBs	20,22 (6,79 - 51,09)	49,02	11,15	9,58	126,6	60,15
Non-*ortho* PCBs	21,47 (NQ - 61,1)	NQ	32,66	24,27	244,9	482,1
2378 PCDDs	5,27 (2,71 - 10,74)	4,35	3,30	2,28	6,10	5,30
2378 PCDFs	3,32 (1,19 - 6,28)	2,54	2,05	2,67	10,50	9,64
Total PCDD/Fs	35,27 (9,82 - 55,61)	9,94	64,80	26,23	20,29	20,89
WHO-TEQ mono-*ortho* PCBs	0,62 (0,12 - 1,68)	1,35	0,18	0,24	3,16	0,68
WHO-TEQ non-*ortho* PCBs	0,30 (NQ - 0,55)	NQ	0,59	0,87	7,82	15,82
WHO-TEQ PCDDs	0,49 (0,32 - 0,67)	0,73	0,43	0,36	1,85	1,34
WHO-TEQ PCDFs	0,52 (0,53 - 1,50)	0,56	0,34	0,66	2,26	2,04
Total WHO-TEQ	1,92 (0,88 - 3,18)	2,65	1,54	2,12	15,09	19,88

	Bivalves (N=2)	Prawns	Cuttle fish	Canned tuna	Canned sardine	Bakery	Eggs
ortho -PCBs	121,8 (113,1 - 130,5)	58,66	31,24	3,84	34,36	10,91	14,56
Non-*ortho* PCBs	66,15 (NQ - 132,3)	258,8	89,50	26,99	85,05	1,79	/
2378 PCDDs	8,49 (7,47 - 9,50)	17,06	4,36	10,46	181,5	23,29	6,60
2378 PCDFs	4,47 (3,94 - 5,00)	12,54	6,06	4,06	17,12	2,34	2,93
Total PCDD/Fs	37,31 (21,45 - 53,18)	167,19	17,88	16,09	285,9	27,73	49,27
WHO-TEQ mono-*ortho* PCBs	3,00 (1,83 - 4,16)	2,07	0,41	0,07	1,01	0,36	0,42
WHO-TEQ non-*ortho* PCBs	0,88 (NQ - 1,77)	3,47	2,34	0,36	3,79	0,07	/
WHO-TEQ PCDDs	4,14 (0,48 - 7,79)	2,72	1,20	2,16	8,90	0,30	0,59
WHO-TEQ PCDFs	2,04 (0,66 - 3,42)	1,46	0,94	0,55	4,19	0,12	0,41
Total WHO-TEQ	10,94 (4,74 - 15,37)	9,72	4,88	3,14	17,90	0,85	1,43

	Meat prod. (N=3)	Pork	Cured ham	Chicken (N=2)	Entrails	Olive oil	Sunflower oil
ortho -PCBs	20,74 (5,55 - 46,38)	31,08	5,29	46,17 (40,68 - 51,67)	68,19	2,63	6,2
Non-*ortho* PCBs	78,31 (30,88 - 120,0)	291,8	24,65	288,5 (116,8 - 460,1)	166,5	20,25	59,63
2378 PCDDs	45,10 (20,77 - 66,00)	8,96	15,21	60,59 (34,77 - 86,41)	19,98	3,04	1,25
2378 PCDFs	4,47 (2,65 - 6,00)	2,60	11,56	17,33 (3,51 - 31,14)	9,76	0,86	0,65
Total PCDD/Fs	69,416 (50,29 - 81,90)	18,25	29,09	285,1 (241,9 - 328,2)	106,7	15,76	11,42
WHO-TEQ mono-*ortho* PCBs	0,29 (0,13 - 0,52)	0,26	0,14	1,05 (0,80 - 1,30)	2,16	0,08	0,26
WHO-TEQ non-*ortho* PCBs	0,64 (0,33 - 0,75)	1,49	0,30	2,15 (0,56 - 3,74)	1,53	0,34	1,44
WHO-TEQ PCDDs	0,69 (0,36 - 1,24)	0,96	1,73	3,61 (0,91 - 6,30)	2,04	0,15	0,21
WHO-TEQ PCDFs	0,41 (0,23 - 0,50)	0,32	1,17	1,54 (0,45 - 2,62)	1,21	0,14	0,13
Total WHO-TEQ	2,03 (1,04 - 3,00)	3,03	3,34	8,34 (2,79 - 13,97)	6,94	0,71	2,04

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