LEVELS OF POPs IN SPANISH COMMERCIAL FISH SPECIES

Parera J[1], Ábalos M[1], Martrat MG[1], Morales L[1], Pérez-Pozo A[2], Flores C[1], Caixach J[1], Rivera J[1], Abad E[1]
[1] Laboratory of Dioxins, Environmental Chemistry Dept., IDÆA-CSIC. C/ Jordi Girona 18-26, 08014 Barcelona, Spain.
[2] Subdirección General de Economía Pesquera. Dirección General de Ordenación Pesquera. Ministerio de Medio Ambiente, Medio Rural yMarino . C/ Velázquez, 144, 28002 Madrid, Spain

Abstract
The occurrence of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), polybrominated biphenyl ethers (PBDEs), perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA) was studied in 32 samples, belonging to 8 different fish species usually consumed in Spain. Mean concentrations ranged from 0,044 to 4,09 pg/g fresh weight (fw) for total PCDD/Fs, while non-ortho PCBs showed mean concentrations ranging from 0,24 to 100,6 pg/g fw. Mean WHO-TEQ concentrations ranged from 0,025 to 0,92 pg WHO-$TEQ_{PCDD/Fs}$/g fw, being these values well below the maximum concentrations established by the EU. When non-ortho PCBs were also included the values increased to a maximum of 4,07 pg WHO-$TEQ_{PCDD/Fs+non\text{-}ortho\ PCBs}$/ g fw. Good correlations were observed for PCDD/Fs *vs.* non-ortho PCBs, in terms of WHO-TEQ concentration, and between total WHO-TEQ concentration (PCDD/Fs+non-ortho PCBs) and total concentration of marker PCBs. Mean values for marker PCBs (as sum of 7 congeners) were between 0,13 ng/g fw and 41,6 ng/g fw. PCN mean values ranged from 2,30 pg/g fw to 9,69 pg/g fw, while mean values of PBDEs were found between 106,3 pg/g fw and 3665,7 pg/g fw. Finally, PFOS mean values were between non-detected levels to 41,7 pg/g fw, while PFOA could not be detected or was below the limit of detection (LOD) in all the analyzed samples.

Introduction
The aim of this study was to determine the levels of several persistent organic pollutants (POPs), such as polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), polybrominated biphenyl ethers (PBDEs), perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA), in the muscle meat of commercial fish species consumed in Spain. The study was carried out in the framework of a surveillance program conducted by the *"Subdirección General de Economía Pesquera. Dirección General de Ordenación Pesquera. Ministerio de Medio Ambiente, Medio Rural y Marino"* of the Spanish Government in collaboration with the Laboratory of Dioxins of the CSIC in Barcelona.

Due to the stable structure and lipophilic character of the abovementioned chlorinated and brominated POPs, they tend to bioconcentrate and biomagnify in the food chain. Nowadays, it is well accepted that food consumption is the main route of non-occupational human exposure to these contaminants. The ingestion of food contributes more than 90% to the total exposure and foodstuffs of animal origin, such as fish and seafood, are recognized as one of the main contributors[1,2]. More recently, scientific interest has also been focused on new POPs, such as the perfluorinated compounds (PFCs). These substances are characterised by their chemical and thermal stability and their surface properties. Due to these characteristics, they are used in a wide variety of industrial and consumer applications including adhesives, cosmetics, cleaners, coatings and electronics[3]. A wide range of PFCs have been detected in various environmental and biological matrices, however little is known about the distribution and accumulation of these materials in the environment. Among PFCs, PFOS and PFOA are the two most often studied substances, since they are also the ones usually present at higher concentrations in the different matrices. A limited number of studies have documented the presence of PFCs in fish liver[4], but information about levels in muscle meat is still scarce in the literature.

Materials and Methods
A total of 32 fish samples were collected during 2007. Sampling included a large group of marine fishes (red mullet, mackerel, anchovy, sardine, yellowfin tuna, bigeye tuna, bluefin tuna and bonito) caught at different fishing areas (e.g. Atlantic Ocean and Mediterranean Sea). Once at the laboratory, the non-edible parts of fish were removed and the muscle meat, skin excluded, was freeze-dried and re-homogenized as a pretreatment steps to the extraction of the analytes.

For PCDD/F and non-ortho PCB analysis, samples were extracted in a Soxhlet for ~24h with toluene:cyclohexane (1:1) after being spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (EPA-1613LCS, Wellington Lab., Guelp, Canada) and $^{13}C_{12}$-DL-PCBs (WP-LCS, Wellington Lab., Guelp, Canada). Next, the extracts were rotary evaporated and kept in the oven overnight (105 ºC) in order to eliminate the solvents prior to gravimetrical fat determination. Afterwards, fat residues were dissolved again in *n*-hexane. Organic components, fat and other interfering substances were removed by treating the *n*-hexane extracts with silica gel modified with sulphuric acid (44%). The extracts were then rotary concentrated and filtered prior to the next clean-up step. Further sample purification and instrumental analysis by high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS) are described elsewhere5. All analyses were performed on a 6890N Network GC System Agilent gas chromatograph (Agilent Technologies Inc., Palo Alto, USA) fitted with a DB-5 ms fused silica column (J&W Scientific, Folsom, USA) and connected through a heated transfer line kept at 280 ºC to an Auto- Spec Ultima NT high resolution mass spectrometer with an EBE geometry (Waters, Manchester, UK).

For marker PCB, PCN and PBDE analysis, the extraction and purification methodology was similar to that previously described for PCDD/Fs and non-ortho PCBs. Briefly, freeze dried samples were spiked with known amounts of $^{13}C_{12}$-PCBs (MBP-MXE, Wellington Lab., Guelp, Canada) and $^{13}C_{12}$-PBDEs (MBDE-MXFS, Wellington Lab., Guelp, Canada) and then extracted in a Soxhlet for ~24h using *n*-hexane:dichloromethane (1:1). After that, the extracts were rotary concentrated and transferred to *n*-hexane. Next, purification and fractionation of these extracts were carried out using a silica gel column modified with sulphuric acid (44%) and a basic alumina column. Instrumental conditions for marker PCB and PBDE analysis by HRGC-HRMS were similar to those for PCDD/Fs and non-ortho PCBs. Electron ionization (EI+) mode was used, operating in the selected ion monitoring (SIM) mode at a resolving power of 10000 (10% valley definition). The ion source and transfer line were set at 250 and 280 ºC respectively. The two most abundant ions of the molecular cluster ions of each homologue group were monitored. Chromatographic separations were performed using DB-XLB (60m x 0.25mm i.d. x 0.25µm film thickness) and DB-5MS (13m x 0.18mm ID x 0.18µm film thickness) columns from J&W Scientific (Folsom, USA), for marker PCBs and PBDEs, respectively. PCN instrumental analysis was also performed by HRGC-HRMS and conditions are described elsewhere[6].

For PFOS and PFOA, approximately 2g of freeze dried fish sample were weighed in a polypropylene vessel and spiked with known amounts of 1,2,3,4-$^{13}C_4$-PFOS and 1,2,3,4-$^{13}C_4$-PFOA (Wellington Lab., Guelph, Canada). Then, sample was extracted in an ultrasonic bath for 30 minutes with 7g of acetonitrile. After that, clean-up was carried out using ENVI-carb and glacial acetic acid. Finally, extracts were evaporated and reconstructed with methanol and analysed by high performance liquid chromatography tandem mass spectrometry (HPLC/ESI-MS/MS) using electrospray ionization (ESI) operating in negative mode. The extracts (10 µL injection volume) were chromatographed on a C_{18} column (1.9 µm, 50x 2.1mm i.d.) (ThermoFinnigan, Milan, Italy) using an Surveyor MS Pump Plus (ThermoFinnigan, Milan, Italy). The gradient operated at a flow rate of 0.2 ml/min starting from 30% MeOH (B) and 70% H_2O (A) to 75% MeOH in 7 min. The HPLC was interfaced to a triple quadrupole TSQ QUANTUM Discovery (ThermoFinnigan, Milan, Italy) equipped with a Ion MAX source operating in negative ion mode. The source temperature was maintained at 300ºC and the spray voltage at -3500 V. The analyses were performed with a selected reaction monitoring (SRM) method that monitored two mass transitions (parent ion/product ion) for each analyte. Quantification was carried out by isotopic dilution.

Results and Discussion

PCDD/F and PCB levels

Mean concentrations of individual PCDD/F and PCB congeners, as well as of the total WHO-TEQs, are shown in Table 1. For PCDD/Fs, the highest levels, expressed as the sum of the 17 toxic congeners, were found in red mullet with a mean value of 4,09 pg/g fresh weight (fw) followed by mackerel, sardine, anchovy, bonito and bluefin tuna; while the lowest levels were found in bigeye and yellowfin tuna. In addition, in terms of total WHO-TEQ concentrations, red mullet also presented the highest mean value (0,92 pg WHO-TEQ/g fw) and the lower levels were those of bigeye and yellowfin tuna, mean values of 0,025 pg WHO-TEQ/g fw. For the case of the non-ortho PCBs, a similar trend was also observed, being again red mullet the specie that showed the highest concentration for these compounds, expressed as the sum of the 4 PCB congeners; although sardine presented

the highest concentration in WHO-TEQ (3,43 pg WHO-TEQ/g fw). Nevertheless, all the calculated WHO-TEQ levels were below the limits established by the EU Regulation for this kind of food products (4 pg WHO-$TEQ_{PCDD/Fs}$/g fw and 8 pg WHO-$TEQ_{PCDD/Fs+DL-PCBs}$ /g fw)[7]. Total marker PCB concentrations (as the sum of the 7 congeners analyzed) in the samples considered in this study also presented large variations depending on the fish specie, with mean values ranging from 41,6 to 0,13 ng/g fw. Thus, red mullet, followed by sardine and mackerel showed the highest concentrations, while bigeye and yellowfin tuna showed the lowest levels.

Taking into account all these results, different correlations between the concentrations of the different groups of pollutants were evaluated. As an example, Figure 1 shows the correlation obtained, from the values of the 32 fish samples, for PCDD/Fs *vs.* non-ortho PCBs, in terms of WHO-TEQ concentrations (A); and, for total WHO-TEQ levels (PCDD/Fs + non-ortho PCBs) *vs.* total concentrations of marker PCBs (B). Good correlation factors were obtained: R^2=0,81 for PCDD/Fs *vs.* non-ortho PCBs, in terms of WHO-TEQ concentrations, and R^2=0,91 for total WHO-TEQ levels (PCDD/Fs + non-ortho PCBs) *vs.* total concentrations of marker PCBs.

In general, the PCDD/F and PCB levels obtained in the present study were comparable to those previously reported in fish species from the Spanish markets[8,9]. However, it has to be mentioned that all fatty fish samples (red mullet, sardine, anchovy and mackerel) were caught in the Mediterranean Sea. In contrast, for the case of tuna species, bigeye and yellowfin tuna samples were from the Atlantic, Pacific and Indian Oceans, while bonito samples were obtained from both, the Atlantic Ocean and the Mediterranean Sea, and the 3 samples of bluefin tuna were caught in the Mediterranean Sea. When PCDD/F and PCB concentrations are compared taking into account the fishing area from where the samples come from, it could be concluded that there is a tendency to find higher WHO-$TEQ_{PCDD/Fs+non\text{-}ortho\ PCBs}$ concentrations in the animals from fishing areas of the Mediterranean Sea than in those fish caught in the Atlantic, Pacific or Indian Oceans.

PBDE and PCN levels
Levels of PBDEs and PCNs in the 8 analyzed fish species are summarized in Table 2. PBDEs were detected in the edible parts of all fish species at concentrations ranging from 106,3 to 3665,7 pg/g fw, in terms of the sum of 9 different PBDE congeners (BDE-28, 47, 66, 85, 99, 100, 153, 154 and 183). Bonito exhibited the highest levels (Mean value: 3665,7 pg/g fw), followed by mackerel, bluefin tuna and sardine with values between 1597,2 and 1242,8 pg/g fw. In contrast, the lowest values were found for yellowfin tuna. PBDE results reported in this study are in concordance with those from different fish samples obtained in Spanish markets[10].

On the other hand, the total PCN levels determined in this study ranged between 2,30 pg/g fw for yellowfin tuna and 9,69 pg/g fw for mackerel (as the sum of tetra- to octa-CN). PCN concentrations were low or near the limit of quantification (LOQ) in most of the cases. In addition, the PCN profiles were characterized by decreasing levels with an increasing degree of chlorination of the congeners from tetra- through octa-CN.

PFOS and PFOA levels
Concentrations for PFOS and PFOA are shown in Table 3. Individual levels for PFOS ranged from non-detected values or below the limit of detection (LOD) to a maximum of 103,0 ng/g fw, while PFOA could not be detected or was below the LOD in all the analyzed samples. The highest levels of PFOS were found in fatty fish species. On the contrary, for the different tuna species, in general PFOS and PFOA were both found below the LOD or not detected. Nowadays, most of the studies performed have measured PFC concentrations in the liver tissue and blood, making comparisons to the current study difficult. Results of PFOS and PFOA were comparable with previously reported contamination of whole fish homogenates from Ohio and Mississipi Rivers, USA; with concentrations that were in the range from 24,4 to 53,9 ng/g fw for PFOS[11]. In a further study, based on Mediterranean Sea fish caught in Italy, levels of PFOS and PFOA in muscle fish were between <2 and 40 ng/g fw and between <1,5 and 43 ng/g fw, respectively[12]. On the other hand, levels of PFOS in fish collected in localities in the neighbourhood of a fluoro chemical factory in Belgium were as high as 9030 ng/g fw[13].

Acknowledgements
This work was supported by the *"Ministerio de Medio Ambiente, Medio Rural y Marino"* of the Spanish Government. M. Ábalos acknowledges an I3PDR contract from CSIC in the framework of the I3P project.

References

1. EU-SCOOP European Commission, Task 3.2.5, June 2000. Brussels, Belgium.
2. Hays S.M. and Aylward L.L. *Regul. Toxicol. Pharmacol.* 2003; 37: 202.
3. Kissa E. 2nd ed. *Marcel Dekker, Inc*.: NewYork. 2001.
4. Houde M., Martin J.W., Letcher R.J., Solomon K.R. and Muir D.C.G. *Environ. Sci. Technol* 2006; 40: 3463.
5. Abad E., Caixach J. and Rivera J. *J. Chromatogr. A* 1997; 786: 125.
6. Abad E., Caixach J. and Rivera J. *Chemosphere* 1999; 38: 109.
7. Commission Regulation (EU) nº 1881/2006, 19 December 2006.
8. Gomara B., Bordajandi L.R., Fernandez M.A., Herrero L., Abad E., Abalos M., Rivera J. and Gonzalez M.J. *Journal Agric Food Chem* 2005; 53: 8406.
9. Bordajandi L.R., Martín I., Abad E., Rivera J. and González M.J. *Chemosphere* 2006; 64: 1450.
10. Gomara B., Herrero L. and Gonzalez M.J. *Environ Sci. Technol* 2006; 40: 7541.
11. Ye X., Strynar M.J., Nakayama S.F., Varns J., Helfant L., Lazorchak J. and Lindstrom A.B. *Environ. Pollution* 2008; 156: 1227.
12. Nania V., Pellegrini G.E., Fabrizi L., Sesta G., Sanctis P., Lucchetti D., Pasquale M. and Coni E. *Food Chem.* 2009; 115: 951.
13. Hoff P. T., Van Campenhout K., Van de Vijver K., Covaci A., Bervoets L., Moens L., Huyskens G., Goemans G., Belpaire C., Blust R. and De Coen W. *Environ. Pollution* 2005; 137: 324.

Table 1. Mean concentrations of individual PCDD/F and PCB congeners and total WHO-TEQ values (upperbound), expressed in pg/g fw, in different fish species consumed in Spain.

	Red mullet n=4	Mackerel n=4	Anchovy n=4	Sardine n=6	Yellowfin tuna n=3	Bigeye tuna n=2	Bluefin tuna n=3	Bonito n=6
2,3,7,8 - TCDD	0,11	0,041	0,026	0,073	<LOQ	<LOQ	0,024	0,032
1,2,3,7,8 - PeCDD	0,20	0,055	0,047	0,13	<LOQ	<LOQ	0,045	0,078
1,2,3,4,7,8 - HxCDD	0,026	0,0087	0,011	0,023	<LOQ	<LOQ	<LOQ	0,0076
1,2,3,6,7,8 - HxCDD	0,17	0,023	0,051	0,081	0,002	<LOQ	0,018	0,034
1,2,3,7,8,9 - HxCDD	0,029	0,0012	0,014	0,026	<LOQ	<LOQ	<LOQ	0,0056
1,2,3,4,6,7,8 - HpCDD	0,12	0,055	0,059	0,082	<LOQ	0,014	0,010	0,032
OCDD	0,56	0,57	0,81	0,34	0,035	0,089	0,061	0,083
2,3,7,8 - TCDF	0,87	1,57	0,22	0,95	<LOQ	<LOQ	0,49	0,76
1,2,3,7,8 - PeCDF	0,30	0,055	0,087	0,15	<LOQ	<LOQ	0,054	0,070
2,3,4,7,8 - PeCDF	0,84	0,26	0,21	0,61	<LOQ	<LOQ	0,14	0,41
1,2,3,4,7,8 - HxCDF	0,16	0,010	0,025	0,037	<LOQ	<LOQ	0,0091	0,013
1,2,3,6,7,8 - HxCDF	0,22	0,018	0,027	0,083	<LOQ	<LOQ	0,015	0,016
1,2,3,7,8,9 - HxCDF	0,0045	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2,3,4,6,7,8 - HxCDF	0,16	0,031	0,026	0,067	<LOQ	<LOQ	0,012	0,012
1,2,3,4,6,7,8 - HpCDF	0,24	0,017	0,013	0,051	<LOQ	<LOQ	0,0082	0,0049
1,2,3,4,7,8,9 - HpCDF	0,015	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
OCDF	0,076	<LOQ	0,021	0,019	0,0072	0,011	0,017	0,0074
∑-PCDD/Fs	4,09	2,72	1,64	2,72	0,044	0,11	0,90	1,56
WHO-TEQ (PCDD/Fs)	0,92	0,40	0,22	0,64	0,025	0,025	0,20	0,41
PCB-77	69,38	60,88	17,09	58,89	<LOQ	<LOQ	13,11	35,31
PCB-81	4,07	2,70	0,23	0,69	<LOQ	<LOQ	0,48	1,94
PCB-126	22,67	24,82	13,77	33,78	0,15	0,17	12,40	25,82
PCB-169	4,48	1,74	2,30	4,64	0,11	0,069	2,45	3,86
∑-non-ortho PCBs	100,6	90,1	33,4	98,0	0,25	0,24	28,4	66,9
WHO-TEQ (non-ortho PCBs)	2,32	2,51	1,40	3,43	0,016	0,018	1,27	2,62
PCB-28	370,2	173,0	75,2	296,7	22,2	17,9	88,7	157,9
PCB-52	415,0	598,9	216,5	748,6	21,1	16,6	325,1	499,9
PCB-101	1199,3	3529,6	1569,8	1197,1	22,6	15,7	1832,8	2247,9
PCB-118	3635,3	2968,5	1253,7	2958,7	13,5	7,8	1960,1	2065,6
PCB-153	19346,1	13512,9	7387,0	14719,5	37,2	33,0	6789,2	11242,7
PCB-138	8691,1	7311,2	4440,2	8467,1	18,3	21,0	5134,1	6034,8
PCB-180	7946,5	6402,5	4254,2	6674,6	20,3	19,0	3803,1	5646,1
∑-PCBs	41603,5	34496,6	19196,6	35062,3	155,2	130,9	19933,2	27894,8

Table 2. Mean concentrations of PBDEs and PCNs, expressed in pg/g fw, in different fish species consumed in Spain.

	Red mullet n=4	Mackerel n=4	Anchovy n=4	Sardine n=6	Yellowfin tuna n=3	Bigeye tuna n=2	Bluefin tuna n=3	Bonito n=6
BDE-28	5,6	50,1	17,7	59,7	1,7	23,5	94,3	97,3
BDE-47	71,8	674,4	181,9	481,5	20,5	435,8	799,6	731,9
BDE-66	0,64	87,2	17,2	15,0	0,94	9,0	115,5	65,8
BDE-100	28,3	201,0	64,4	131,6	2,4	123,6	243,6	204,8
BDE-99	52,9	295,5	43,9	58,8	5,8	31,9	94,1	136,4
BDE-85	3,2	14,4	0,51	1,3	0,13	1,2	12,9	9,7
BDE-154	67,4	157,9	43,2	37,3	5,0	18,2	150,5	152,5
BDE-153	33,4	68,1	22,0	57,0	14,4	20,0	41,0	402,4
BDE-183	84,1	48,6	89,8	400,6	55,5	19,7	31,8	1865,0
∑-PBDEs	347,4	1597,2	480,6	1242,8	106,3	682,9	1583,2	3665,7
tetra-CN	1,40	4,26	0,95	1,73	0,68	1,46	1,64	0,91
penta-CN	4,33	4,33	1,06	3,69	0,19	0,53	3,61	4,57
hexa-CN	2,06	0,79	0,88	1,68	0,37	1,04	0,99	0,99
hepta-CN	0,13	0,16	0,53	0,47	0,76	0,90	0,87	<LOQ
octa-CN	<LOQ	<LOQ	<LOQ	<LOQ	0,18	<LOQ	<LOQ	<LOQ
∑-PCNs	8,09	9,69	3,57	7,82	2,30	4,53	7,22	8,15

Table 3. Concentrations of PFOS and PFOA, expressed in ng/g fw, in different fish species consumed in Spain. Mean values together with maximum and minimum levels (in parentheses) are included.

Fish specie	PFOS ng/g fw	PFOA ng/g fw
Red mullet n=4	15,2 (6,3 – 24,4)	<LOD
Mackerel n=4	2,0 (<LOD – 4,7)	n.d.
Anchovy n=4	41,7 (9,1– 103,0)	<LOD
Sardine n=6	10,0 (<LOD – 36,7)	<LOD
Yellowfin tuna n=3	n.d.	<LOD
Bigeye tuna n=2	n.d.	<LOD
Bluefin tuna n=3	(n.d. – 4,2)	<LOD
Bonito n=6	(n.d. – 1,3)	n.d.

n.d.: not detected

<LOD: PFOS: < 0.8 ng/g fw; PFOA: <0.2 ng/g fw

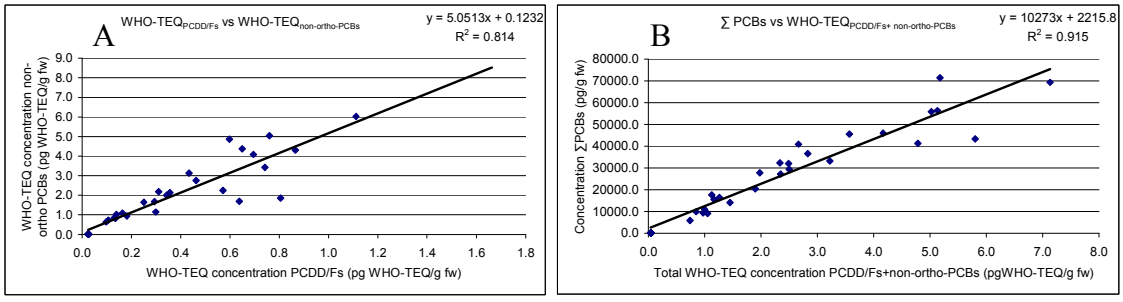

Figure 1. Correlation obtained, from the 32 fish samples, for PCDD/Fs *vs.* non-ortho PCBs, in terms of WHO-TEQ concentrations (A); and, for total WHO-TEQ levels (PCDD/Fs + non-ortho PCBs) *vs.* total concentration of marker PCBs (B).