DETERMINATION OF PCDD/F AND NON-*ORTHO* PCB LEVELS IN TWO ENVIRONMENTS OF RIVER EBRO (SPAIN).

M. Cabes[1,2], J. Díaz-Ferrero[1,2], R. Martí[1], F. Broto-Puig[2], L. Comellas[2], M.C. Rodríguez-Larena[1]
[1] Environmental Laboratory [2] Section of Chromatography
Institut Químic de Sarrià (Ramon Llull University), Via Agusta 390, 08017 Barcelona (SPAIN)

INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are ubiquitous pollutants of great concern due to their characteristics of environmental persistence, tendency to bioaccumulate through the foodchain and toxicity (endocrine disruption, carcinogenic effects, etc.). They have been never produced for industrial purposes, but they are introduced in the environment as by-products of several processes: waste incineration, metal smelting, bleaching of pulp using chlorine, synthesis of halogenated chemicals, etc. Ebro River, which is 928 km long and drains a total area of 85,000 km^2 approximately, is one of the most important rivers of Spain. Previous studies from different authors [1-3] have detected high levels of organochlorine pesticides and polychlorinated biphenyls (PCB) in some especially polluted areas of it. One of them, Ribera d'Ebre region (Catalonia, Spain), is the aim of this work. In our study, we have compared the PCDD/F and non-*ortho* PCB levels in two places of this region, which are ca. 6 km from each to other. A chemical plant that manufactured in the past organochlorine compounds (like PCB, HCB, etc.) is located in the bank of the river between the two sampling points. In this study, we have focused on levels of dioxin-like compounds (PCDD/F and PCB) in fish from the river, since it is well known that this kind of pollutants bioaccumulates in lipid tissue of living organisms and are suspected to produce a wide spectrum of abnormalities in wildlife [4, 5]. In addition, samples of river water and soil and pine needles in the proximity of the river were analysed to better characterise both environments.

EXPERIMENTAL

Samples: In place A (before the factory) two carps (*Cyprinus carpio*) were collected and in place B (after the factory) two carps, two *Scardinius erythrophthalmus* and fifteen *Alburnus alburnus* were collected. Characteristics of length and weight of fish are in Table 1.

Table 1. Characteristics of fish

Fish	Length (cm)	Weight (g)
Carp A1	32	522
Carp A2	39	793
Carp B1	37	764
Carp B2	30	511
Scardinus B1	27	363
Scardinus B2	26.5	----
Alburnus B	8.5-15.5	6-41

After cleaning the fish skins with ethanol, fillets were cut and skins removed following the method proposed by UNEP [6]. Due to their small size, the fillets of the fifteen *Alburnus alburnus* were pooled and a single sample of this kind of fish was analysed.
In both places, one sample of soil and one sample of superficial water of the river were collected. In addition, green pine needles from several trees were sampled in each location.
The analyses of the different matrices were carried out according to the previously validated procedures summarised in Table 2. With each series of samples a complete procedure blank was performed to check the lack of contamination by solvents, material, etc.

Table 2. Description of analytical methods used for soils, pine needles, water and fish.

Matrix	**Soil**	**Pine needles**	**Water**	**Fish**
Amount	30 g	50 g	10 L	20 g
Pre-treatment	Grinding and sieving (2 mm)	Fine cutting	Acidification pH 5-7	Homogenisation of fillet
Addition of internal standards: $^{13}C_{12}$-2,3,7,8-PCDD/F, $^{13}C_{12}$-non-*ortho* PCB				
Extraction	Soxhlet; toluene 20 h	1) Digestion HCl 1N 2) Soxhlet, toluene 24 h	SPE C_{18} Speedisk	Soxhlet Hx:DCM (1/1, v/v) 24 h
Purification	Multilayer silica column	1) Sulphuric silica column 2) Multilayer silica column	Multilayer silica column	1) Fat content determination 2) Sulphuric silica column 3) Multilayer silica column
Fractionation	SPE carbon tubes	Florisil column and purification in SPE carbon tubes	SPE carbon tubes	SPE carbon tubes
Concentration under nitrogen stream up to 15 µL				
Addition of syringe standards ($^{13}C_{12}$-1234-TCDD and $^{13}C_{12}$-123789-HxCDD for PCDD/F and $^{13}C_{12}$-PCB 123 for PCB)				
Analysis by HRGC-HRMS (SPB5 and DB-DIOXIN capillary columns)				

RESULTS AND DISCUSSION

The results obtained in the two sampling points are shown in Table 3. The results are expressed in TEQ and they have been calculated using the new toxic equivalency factors (TEF) for fish recommended by WHO [7].

Table 3. Concentration of PCDD/F and non-*ortho* PCB in samples from Ebro River.

Sampling location	Sample	PCDD/F		Non-ortho PCB		Total ITEQ
		Conc.*	ITEQ	Conc.*	ITEQ	
A	Carp A1 (pg/g lipid)	92.4	2.68	2840	3.77	6.45
	Carp A2 (pg/g lipid)	144	2.25	3010	1.85	4.11
	Water A (pg/L)	5.39	0.001	2.00	0.0002	0.001
	Soil A (pg/g d.w.)	8.06	0.15	10.2	0.01	0.16
	Pine needles A (pg/g d.w.)	12.0	0.60	33.3	0.003	0.61
B	Carp B1 (pg/g lipid)	305	85.0	21900	41.3	126
	Carp B2 (pg/g lipid)	444	107	62000	32.4	139
	Scardinus B1 (pg/g lipid)	314	48.2	10800	5.2	53.3
	Scardinus B2 (pg/g lipid)	150	21.7	78200	15.4	37.2
	Alburnus (pg/g lipid)	167	19.8	112000	26.4	46.3
	Water B (pg/L)	4.89	0.03	194	0.04	0.07
	Soil B (pg/g d.w.)	1600	58.5	9780	5.37	63.8
	Pine needles B (pg/g d.w.)	2240	23.0	1150	0.93	23.9

* *Sum of 2,3,7,8-congeners excluding those with concentrations lower than the limit of detection*

<u>*Fish samples*</u>: Concentrations in carps A1 and A2 were similar to those reported in bibliography for fish from non-polluted areas[1] [8]. However, concentrations in fish from place B were higher and more similar to those described for polluted industrialised areas [10, 11]. In all cases, the concentrations of non-*ortho* PCB were higher than those of PCDD/F and the contribution of PCB to ITEQ ranged from 10 % to 58 %. The most concentrated PCB was PCB 77; however PCB 126 was that with a higher influence in TEQ. The profile of PCDD/F was different between the fish of place A and place B: the most important congeners in carps A1 and A2 were OCDD 75, OCDF 135, HpCDD 73 and TCDF 83, while in fish from place B, PeCDF 114 and TCDF 83 were the congeners with the highest levels.

<u>*Water, soil and pine needle samples*</u>: Water samples in both places showed similar concentration of PCDD/F but higher levels of non-*ortho* PCB were detected in place B (after the factory). The levels of dioxin-like compounds (both PCDD/F and non-*ortho* PCB) in soils and pine needles were much higher in place B than in place A (before the factory). The higher ITEQ detected in place B

[1] In order to compare the results of this study to those reported in bibliography, TEQ have been recalculated using former TEF [9]

were due to the increase in the level of furans (especially high concentration of OCDF 135 was detected) and non-*ortho* PCB. This increase in furan concentrations is coincident with that observed in fish samples. In fact, PCDF are by-products of the synthesis of PCB and the factory mentioned above manufactured technical mixtures of PCB similar to Aroclor 1260 and Aroclor 1268. This profile of PCB has been detected in samples of the same zone in previous studies [3]. In addition, in previous works, we had detected OCDF 135 as the most important 2,3,7,8-PCDF (in concentration) in this kind of technical mixtures [12].

We can conclude that the levels of dioxin-like compounds (PCDD/F and non-ortho PCB) in fish and also in environmental samples are higher in the river area after the factory.

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