

## Monitoring dioxins and furans in subjects living in the vicinity of a hazardous waste incinerator after 4 years of operation

Ana Bocio<sup>1</sup>, Jose Luis Domingo<sup>1</sup>, Francisco Garcia<sup>1</sup>, Marta Schuhmacher<sup>1</sup>, Joan Maria Llobet<sup>1</sup>

<sup>1</sup>Laboratory of Toxicology and Environmental Health, Reus

### Introduction

In 1996, construction of a hazardous waste incinerator (HWI) was initiated in Constantí, Tarragona, Spain. Because this facility was the first, and up to date, the only HWI in Spain, the concern about its potential environmental impact and health risks has been considerable in the public opinion. Regular operations in the facility started in 1999. In order to establish the potential health risks of PCDD/PCDF exposure on the general population living near the new HWI, a biological monitoring program was designed. Samples of blood, breast milk and adipose tissue were obtained during the construction period of the HWI from subjects living in the neighborhood of the facility and analyzed for baseline levels of PCDD/PCDF<sup>1,2,3</sup>. Moreover, taking into account that food is the main route of exposure to PCDD/Fs, the assessment of the dietary intake of these pollutants by the population living in the area under potential influence of the HWI was also included in the monitoring program<sup>4</sup>.

Approximately 4 years after the new HWI began regular operations (2003), biological samples from individuals living in the same area were again collected, and PCDD/PCDF concentrations determined. The update of PCDD/PCDF intake from food is essential in order to establish clearly if any potential increase in the levels of these compounds in biological tissues of individuals living in the area under influence of the HWI could be due to environmental emissions of PCDD/PCDF by the facility or to increases of the levels of these pollutants in food.

In this paper, we present the concentrations of PCDD/PCDF in biological human samples from subjects living in the vicinity of the HWI. The dietary intake of these compounds by the population in this area, after 4 years of regular operations, is also shown. Results were compared with baseline PCDD/PCDF levels obtained in 1999.

## Materials and Methods

### *Sample collection*

***Adipose tissue:*** During 2002, 15 abdominal adipose tissue samples were obtained from autopsies of individuals (4 women and 11 men) who at the time of death had lived in the city of Tarragona or surroundings at least during the last 10 years.

***Breast milk:*** Fifteen breast milk samples were collected during 2002 from healthy primiparae mothers, aged between 25-35 years old, and living in Tarragona County in zones under potential influence of the HWI for at least the last five years.

***Blood:*** Blood samples were obtained from 20 donors in a blood bank (10 males and 10 females), who had been living in the area under evaluation at least during the last 10 years.

***Food:*** In September 2002, food samples were randomly acquired in local markets, large supermarkets and grocery stores from different locations of Tarragona County. A total of 35 samples composed by subsamples were analyzed. More detail about sampling and kind of food was recently reported<sup>5</sup>.

### **Analytical procedure:**

***Adipose tissue:*** Lipid content determinations were performed by extraction of 1 g of the homogenate with hexane/MeCl<sub>2</sub>. An internal standard consisting of <sup>13</sup>C-labeled PCDD/Fs at each chlorination level was added before extraction. The cleaned extracts were analyzed for PCDD/Fs using an Agilent 6890 gas chromatograph coupled to a Micromass Autospec mass spectrometer (SIR/EI HRGC/HRMS). Chromatographic separation was achieved by splitless injection of 1 µl on a non-polar DB-5MS column. More details about extraction and fractionating of adipose tissue samples were reported by Schuhmacher et al<sup>6</sup>.

***Breast milk:*** Samples were extracted with a mixture of diethyl ether and hexane after addition of sodium oxalate and ethanol. An amount of 1.5 g of the fat was spiked with <sup>13</sup>C-labeled-PCDD/Fs. Lipids were removed in a silica gel column with sulfuric acid initially purified on an activated carbon column containing Celite. Further clean-up of the sample was achieved with an activated aluminum column. The quantification of PCDD/PCDFs was performed by selective ion recording using a VG 70-250 SE (VG Analytical, Manchester, UK) mass spectrometer (resolution 10,000) equipped with a HP 6890 gas chromatograph with fused silica capillary column. Additional details about analytical procedure was reported by Schuhmacher et al.<sup>7</sup>

***Blood:*** Lipids were extracted with isopropanol/hexane through a solid phase. The clean-up procedure and fractionation of the crude extract of each whole sample was carried out by adsorption chromatography as a multilayer clean-up with silica gel, florisil, and alumina columns. The cleaned extracts were analyzed by HRGC/HRMS using a Fisons CE 8000 GC coupled with a VG Autospec Ultima system (EI and multiple ion detection mode, resolution > 10,000). The isomer specific separation was done on a DB-5 MS capillary column. Additional information about treatment of blood samples and analytical determination of PCDD/Fs was previously reported<sup>8</sup>.

***Food:*** Samples were mixed with a small amount of Na<sub>2</sub>SO<sub>4</sub>, spiked with a mixture of <sup>13</sup>C<sub>12</sub>-PCDD/PCDF standards and extracted over 24 h with toluene. The clean-up procedure and fractionation of the sample aliquot were carried out by adsorption chromatography as a multi-step-clean-up, using multilayer silica column and alumina columns. Measurements and quantifications were performed by HRGC/HRMS (Fisons CE 8000 GC coupled with a VG Autospec Ultima system (EI and multiple ion determination mode resolution > 10,000)).

PCDD/PCDF analysis was performed using non-polar columns of DB5-type. Analytical determination of PCDD/Fs in food samples was previously reported by Bocio and Domingo<sup>5</sup>.

**Calculations:** Toxic equivalents (TEQ) were calculated using the toxicity equivalent factors established by the WHO in 1998 (WHO-TEF), and International TEF established by the NATO (I-TEF). For calculations, it was assumed that non-detected congener concentrations were equal to half of the respective limit of detection (ND= ½ LOD). Estimates of average daily food consumption were obtained from a recent nutritional study carried out in the same area.

**Results and Discussion**

Table 1 summarizes individual PCDD/F concentrations of the 15 adipose tissue samples collected during 2002. Age and sex are also shown. Values ranged from 1.5 to 41 pg WHO-TEQ/g lipid, with mean and median values of 11 and 7.4 pg WHO-TEQ/g lipid, respectively. In turn, PCDD/F concentrations in the 15 breast milk samples collected in 2002 are presented in Table 2. Values are between 5.1 and 46.8 pg WHO-TEQ/g lipid, with mean and median levels of 11.9 and 9 pg WHO-TEQ/g lipid, respectively. Table 3 shows PCDD/F concentrations in plasma samples analyzed in 2003. Levels ranged between 5.4 and 32 pg WHO-TEQ/g lipid, with a mean value of 17.8 and a median value of 17.7 pg WHO-TEQ/g lipid. Finally, PCDD/F concentrations in food samples collected in Tarragona County in 2002 are shown in Table 4. The highest value (fresh weight) corresponds to blue fish (0.61 pg WHO-TEQ/g), followed by tinned fish and seafood.

**Table 1.** Individual PCDD/F levels (pg/g lipid) in adipose tissue from subjects living in Tarragona County (Spain)

Sample	Age (years)	Sex	I-TEQ	WHO-TEQ
1	42	M	4.9	5.8
2	55	M	7.6	9.5
3	85	F	34	41
4	19	M	6.2	7.4
5	62	M	4.1	4.2
6	60	F	7.6	8.0
7	84	M	13	15
8	68	M	11	13
9	75	F	1.2	1.5
10	36	M	5.8	7.3
11	31	M	3.6	4.2
12	28	M	2.7	3.2
13	64	M	5.3	6.4
14	70	M	9.0	11
15	94	F	24	29

M: male; F: female.

**Table 2.** Individual PCDD/F levels (pg/g lipid) in breast milk from mothers living in Tarragona County (Spain)

Sample	I-TEQ	WHO-TEQ
1	6.5	7.7
2	4.3	5.1
3	13.7	16.3
4	10.4	12.5
5	9.4	11.1
6	5.5	6.5
7	7.5	8.8
8	39.9	46.8
9	8.4	10.0
10	7.7	9.0
11	4.9	5.8
12	6.6	7.9
13	4.8	5.6
14	7.9	9.6
15	13.2	15.6

In general terms, in the present survey PCDD/F concentrations in biological tissues of subjects living in Tarragona County are lower in the present survey than those found in the baseline study (Table 5). The decreases in PCDD/F levels in adipose tissue, from 36.3<sup>1</sup> to 11 pg WHO-TEQ/g lipid (70% of reduction), and in breast milk, from 11.8 to 10 pg I-TEQ/g lipid<sup>2</sup>, are in accordance with the reductions observed in plasma of non occupationally exposed subjects during the same period. Mean PCDD/F concentration in plasma diminished from 27 pg I-TEQ/g lipid in 1998<sup>2</sup> to 16 pg I-TEQ/g lipid in 2003<sup>8</sup>, which means a reduction of 41%.

The decreases in the levels of PCDD/Fs in biological tissues are also in agreement with the very important reduction observed in the dietary intake of PCDD/Fs by the population of the area, which diminished from 210 pg I-TEQ/day in 1998<sup>4</sup> to the current 59.6 pg I-TEQ/day<sup>5</sup> (Table 6). The reductions between baseline survey and the current study in PCDD/PCDF concentrations in adipose tissue, breast milk, and plasma samples of non-occupationally exposed people living in the neighborhood of the HWI, are also in accordance with the decreases in the levels of PCDD/Fs in soil and herbage samples collected in the vicinity of the facility<sup>9,10</sup>. These results indicate that PCDD/F exposure for the population living in the vicinity of the HWI is not significantly increased by PCDD/F emissions from the stack.

**Table 3.** Individual PCDD/F levels (pg/g lipid) in plasma from subjects living near a HWI in Tarragona, Spain. Data for 2002

Sample	Age	Sex	I-TEQ	WHO-TEQ
1	40	M	17.0	20.7
2	37	M	20.7	24.2
3	37	F	13.5	14.1
4	42	F	14.5	16.9
5	21	F	4.7	5.4
6	41	F	12.8	13.8
7	59	M	5.1	5.4
8	36	M	14.3	15.8
9	19	F	7.9	8.4
10	40	F	21.3	23.8
11	62	F	23.2	27.0
12	32	F	11.3	14.0
13	23	F	15.4	17.7
14	48	M	14.7	17.7
15	46	M	18.3	21.2
16	53	M	18.5	20.7
17	58	F	18.4	21.7
18	53	M	19.0	20.3
19	20	M	13.6	15.8
20	59	M	29.3	32.0

M: male, F: female.

**Table 4.** PCDD/F levels (mean, values) in food groups collected in Tarragona County, in 2002

Food group	pg WHO-TEQ/g fat	pg WHO-TEQ/g fresh weight
Vegetables	-	0.01
Pulses	-	0.01
Cereals	-	0.04
Fruits	-	0.01
White fish	4.9	0.07
Seafood	11.6	0.12
Blue fish	5.1	0.61
Tinned fish	1.0	0.13
Pork	0.3	0.02
Chicken	0.8	0.03
Beef	0.7	0.03
Lamb	0.7	0.04
Eggs	0.5	0.04
Whole milk	0.7	0.02
Semiskim. milk	0.9	0.01
Dairy products	0.6	0.08
Oil	0.2	-
Margarine	0.3	-

**Table 5.** PCDD/PCDF concentrations (mean and median values) in biological tissues in 1998 and 2003 (pg I-TEQ/g lipid).

	Plasma		Adipose tissue		Breast milk	
	Mean	Median	Mean	Median	Mean	Median
1998	27.0	26.8	31	26.3	11.8	11.7
2003	16	15	9.2	6.2	10	7.7
% reduction	41	44	70	76	15	34

**Table 6.** Estimated daily intake of PCDD/PCDF by the general population living near a hazardous waste incinerator (HWI) in Tarragona County, Catalonia (Spain)

Food group	pg TEQ/day <sup>a</sup>	
	1998	2002
Vegetables	17.1	1.3 (1.4)
Pulses	3.0	0.2 (0.3)
Cereals	48.5	8.3 (9.2)
Fruits	24.2	1.8 (2.0)
Fish and seafood	30.4	20.2 (21.5)
Meat and meat products	20.8	5.4 (5.6)
Eggs	3.5	1.3 (1.3)
Milk	32.0	3.9 (4.1)
Dairy products	1.8	8.3 (8.8)
Oils and fats	28.8	8.8 (9.7)
Total intake	210.1	59.6 (63.8)

<sup>a</sup>Values expressed in I-TEQ. In parenthesis, values expressed in WHO-TEQ.

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