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RELACIONADOS**

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## TEMPORAL EVOLUTION OF PCDD/Fs IN BREAST MILK OF WOMEN LIVING NEAR A HAZARDOUS WASTE INCINERATOR

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### Introduction

Mature breast milk may be used for non-invasive monitoring of environmental toxicants.<sup>1</sup> This is an ideal matrix for measurement of the body burden of lipophilic persistent organic pollutants (POPs) in nursing women, due to their relatively high lipid content.<sup>2</sup> In 1999, the first and to date the only Spanish hazardous waste incinerator (HWI) started to operate in Constantí (Tarragona County). Before the HWI started to operate, a wide surveillance program, consisting on biological and environmental monitoring studies, was initiated. In the baseline study (1998), samples of breast milk were collected from individuals living in the vicinity of the facility.<sup>3</sup> In 2002 and 2007, new surveys were again carried out using the same biological monitor.<sup>4, 5</sup> In the present study, we report the results of a new sampling campaign, where the levels of PCDD/Fs were again determined in breast milk of primiparae mothers living in the area under direct influence of the HWI. In addition, the current levels were compared with the concentrations obtained in previous surveys.

### Materials and Methods

#### *Breast milk sampling*

In 2012, 20 samples of breast milk were collected from women (age: 23-44) who had lived in Tarragona County (Spain) at least for the last 5 years. Fifteen samples corresponded to women living in urban zones (Tarragona downtown), while the remaining five samples belonged to women living near an important industrial area. The time elapsed between delivery and sampling was between one and two months. Milk samples of each participant were pooled (50–100 mL) and stored frozen (–20 °C) until PCDD/F analysis. The study protocol was reviewed and approved by the Ethical Committee for Human Studies of the Hospital de la Vall d'Hebrón, Barcelona, Spain. An informed written consent was obtained from each participating woman.

#### *PCDD/F determination*

Details about the analysis of the samples were previously reported.<sup>5</sup> Briefly, an amount of 1.5 g of the fat, whose content had been determined gravimetrically, was spiked with <sup>13</sup>C-labelled internal standards in order to analyze the 17 toxic 2,3,7,8-chlorinated PCDD/Fs. Extraction solvent was exchanged to hexane only. The sample was defatted in a multilayer silica gel (MN-Kieselgel 60, 0.063-0.2/70-230 mesh ASTM, prewashed and activated) column containing acidic and neutral layers of silica and sodium sulfate (Merck, p.a., activated), and analytes were eluted with hexane. PCDD/Fs were purified on an aluminum oxide column (Merck, 90 1097) and on an activated carbon column (Carbopack C, 60/80 mesh) connected to aluminum oxide column. The first fraction was eluted with dichloromethane:hexane (20%/80 % v/v), and the second fraction, which contained PCDD/Fs, with toluene. Recovery standards (<sup>13</sup>C-1,2,3,4-TCDD and <sup>13</sup>C-1,2,3,7,8,9-HxCDD) were added before eluent was replaced by 10–15 µL of nonane. Quantification was performed by selective ion recording using an Autospec Ultima (Micromass Ltd., Manchester, UK) high-resolution mass spectrometer (resolution 10,000) equipped with a HP 6890 gas chromatograph. A fused silica capillary column DB-5MS (60 m, 0.25 mm, 0.25 µm) was used. Two µL were injected into a split-splitless injector. Limits of quantification (LOQ) varied between 0.01-4.9 pg/g in fat, depending on each individual congener. Recoveries for internal standards were higher than 60% for all congeners.

#### *Data analysis*

For calculations, non-quantified congener concentrations were assumed to be equal to one-half of the respective limit of quantification (ND=1/2 LOQ). Statistical significance was performed by analysis of variance (ANOVA) or a Kruskal–Wallis test. A probability of 0.05 (p<0.05) was considered as significant. The statistical software SPSS version 19.0 was used for data

analyses. Toxic equivalents (TEQ) were calculated according to the 2005 WHO-TEFs.<sup>6</sup> Furthermore, in order to compare TEQ values of the current study to those of our previous surveys, the latter were recalculated by applying the same 2005 WHO-TEFs.

### Results and Discussion

The individual concentrations of PCDD/F in breast milk of 20 women living in the vicinity of the HWI are shown in Table 1. In the present study, PCDD/Fs ranged from 18.0 to 126 pg/g fat (1.06 to 12.3 pg WHO-TEQ/g fat), with a mean value of 48.9 pg/g fat (4.79 pg WHO-TEQ/g fat). The congener 2,3,7,8-TCDD showed a mean concentration of 0.66 pg/g fat, being detected in all samples. The maximum concentration corresponded to OCDD, with a mean value of 24.1 pg/g fat. Regarding furans, the highest mean value corresponded to 2,3,4,7,8-PeCDF (3.7 pg/g fat). In contrast, OCDF was under the LOQ in all samples.

Table 1. Individual concentrations of PCDD/Fs (in pg/g fat) in breast milk of 20 women living in the vicinity of a HWI in Catalonia, Spain.

Sample	$\Sigma$ PCDD/Fs <sup>a</sup>	WHO-TEQ
1	29.4	2.93
2	110	8.36
3	29.1	3.31
4	58.5	4.73
5	35.3	4.53
6	89.8	9.96
7	47.0	4.33
8	52.1	6.16
9	18.0	1.59
10	25.7	2.82
11	38.6	4.53
12	37.1	1.83
13	40.1	5.55
14	20.7	1.06
15	126	12.3
16	56.1	5.54
17	55.4	4.33
18	49.4	4.40
19	36.9	4.13
20	22.6	3.35
Mean	48.9	4.79
SD	28.9	2.76
Min	18.0	1.06
Max	126	12.3

The results of the previous and current studies (baseline: 1998, 2002, 2008, and 2012) are summarized in Table 2. A general decrease of the PCDD/F concentrations in breast milk can be seen. Between 2007 and 2012, a 47% statistically significant reduction ( $p < 0.001$ ) was observed in the total PCDD/F concentrations, while the decrease percentage reached 80% ( $p < 0.001$ ) with respect to the baseline study (1998). When assessing PCDD/F concentrations as WHO-TEQs, a similar decrease was noted between 1998 and 2002 (61% of reduction), while a 37% decrease between the 2007 and the current survey was observed. However, this decrease did not reach a level of statistical significance ( $p > 0.05$ ).

For the general population, dietary intake is the main route of exposure to PCDD/Fs. In the present study, the decrease observed in PCDD/F concentrations in relation to those of the baseline survey, is probably due to the general reduction of PCDD/F emissions, as well as the continued decrease in the dietary exposure to these contaminants of the population living in the neighborhood of the HWI.<sup>7</sup> As a result of the 2001 Stockholm Convention, stringent treatment and disposal requirements have been imposed on industrial wastes potentially involved in contamination with POPs, such as PCDD/Fs.

Table 2. Concentrations (mean  $\pm$  standard deviation) of PCDD/Fs samples of breast milk from women living in the vicinity of the HWI in Catalonia, and differences according to the specific place of residence.

	Baseline				% Variation	
	1998 (n=15)	2002 (n=15)	2007 (n=15)	2012 (n=20)	1998- 2012	2007- 2012
<b>Total PCDD/Fs</b>						
Sum PCDD/Fs (pg/g fat)	242 $\pm$ 69	118 $\pm$ 72.3	92.2 $\pm$ 31.8	48.9 $\pm$ 28.9	-80**	-47**
(pg WHO -TEQ/g fat)	12.2 $\pm$ 2.8	10.6 $\pm$ 9.6	7.6 $\pm$ 2.4	4.8 $\pm$ 2.8	-61**	-37
<b>Area of residence:</b>						
Urban (pg WHO -TEQ/g fat)	11.5 $\pm$ 2.8	13.9 $\pm$ 13.4	9.0 $\pm$ 1.7	5.3 $\pm$ 2.9	-54**	-41*
Industrial (pg WHO -TEQ/g fat)	13.1 $\pm$ 2.7	7.8 $\pm$ 3.5	5.5 $\pm$ 1.8	3.1 $\pm$ 1.4	-76**	-44

Differences are statistically significant at \*p<0.01, \*\*p<0.001.

PCDD/F levels in human milk samples were also evaluated according to the specific place of residence of the participants (urban or industrial zones). The results for the baseline 1998, 2002, 2007 and current surveys are shown in Table 2. In the present survey, the mean concentration of PCDD/Fs in milk of women living in urban areas was 69% higher than that corresponding to the industrial zones (5.3 vs. 3.1 pg WHO-TEQ/g fat). This difference was similar to that observed in our previous surveys (2002 and 2007). However, in the baseline survey women living in industrial areas presented higher levels than those residing in urban areas.

Comparative data on the concentrations of POPs in breast milk from different regions of the world have been generated in collaborative studies coordinated by the World Health Organization. The results of these studies have shown a consistent decline in the concentrations of PCDD/Fs in breast milk over the last 20 years.<sup>8, 9</sup> In addition, other studies found a time-related decrease in the concentrations of PCDD/Fs in breast milk.<sup>10-12</sup>

### Conclusions

The results of the present study show that, in terms of exposure to PCDD/Fs, the HWI of Constantí (Catalonia, Spain) does not pose a significant impact on the population living in the neighborhood. The results of the current biological surveillance program are in agreement with the significant reduction of PCDD/F concentrations in breast milk of non-occupationally exposed people observed in recent years in most developed countries. In turn, this decrease is also in accordance with the reduction in the dietary intake of PCDD/Fs, reduction that has been observed in concurrent studies carried out in the same area of study.<sup>7</sup>

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