CONCENTRATIONS OF PCDD/Fs, PCBs, AND PBDEs IN BREAST MILK OF WOMEN FROM CATALONIA, SPAIN. A FOLLOW-UP STUDY

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Introduction

Persistent organic pollutants (POPs) are characterized not only by their persistence but also by their toxicity, potential for long-range transport, and potential for bioaccumulation. The combination of these properties means that these chemicals are particularly hazardous in the environment.

Incineration has demonstrated to be a commercially available technology for hazardous waste (HW) disposal. However, the stack emission from HW incinerators (HWI) of a number of inorganic and organic substances has raised an important concern about the environmental and health consequences of this process of treating HW. In 1996, the construction of the first and to date the only HWI in Spain was initiated in Constantí (Tarragona County, Catalonia, Spain). Regular operations in the facility started in 1999. In the same area, significant industrial activities with a notable number of potential focuses of environmental pollutants, which include two oil refineries, an important complex of petrochemical industries, and a municipal solid waste incinerator among others, are also operating.

In order to establish the potential health risks of exposure of the general population living near the HWI, a biological monitoring program was designed[1-5]. To establish baseline levels of polychlorinated dibenzo-*p*-dioxins (PCDD/Fs) and polychlorinated biphenyls (PCBs) in breast milk, samples of women living in the neighborhood of the facility were collected in 1996, before starting regular operations[2]. After four years of operations at the HWI (2002), a new monitoring survey was carried out, and the concentrations of PCDD/Fs and PCBs, as well as those of polybrominated diphenyl ethers (PBDEs) were measured in breast milk[4, 6]. Human milk is an ideal biomonitor for assessing exposure to POPs such as PCDD/Fs, PCBs, and PBDEs. These contaminants reach the human body through environmental and dietary exposure. They sequester in adipose tissue, serum, and milk, and equilibrate at similar levels on a fat weight basis[7]. With long (5–10 years) half-lives, POPs are persistent in humans, as they do in the environment. The purpose of the present study was to determine the current concentrations of PCDD/Fs, PCBs and PBDEs in milk of women living in the vicinity of the HWI, and to compare the results with levels found in the baseline study, as well as with those obtained after 4 years of regular operation of the facility.

Materials and methods

During 2007, breast milk samples were collected from women living in Tarragona County (Spain) in zones under potential influence of the HWI. The participants in the study were 25–35 years old, who had lived in Tarragona County for at least the last 5 years. Only healthy primiparae mothers were included in this survey.

For analytical determinations, fat from milk sample was extracted with a mixture of diethyl ether and hexane after addition of sodium oxalate and ethanol. Fat content was determined gravimetrically. An amount of 1.5 g of the fat was spiked with internal standards: sixteen ^{13}C-labeled PCDD/Fs congeners, four ^{13}C-labeled non-*ortho* PCBs (PCB 77, 81, 126, and 169), fifteen ^{13}C-labeled other PCBs (PCB 30 [^{12}C-labeled], 52, 80, 101, 105, 118, 123, 138, 153, 156, 157, 170, 180, 194, and 209), and eight ^{13}C-labeled PBDEs (PBDE 28, 47, 77, 99, 100, 153, 154, and 183). The sample was defatted in a silica gel column impregnated with sulfuric acid and further clean-up of the sample was achieved with an activated alumina column. Fractionation of PCDD/Fs and non-*ortho*-PCBs from other PCBs and PBDEs was performed with an activated carbon column.

The quantification of analytes was performed by selective ion recording using a VG 70-250 SE (VG Analytical) and Autospec Ultima (Micromass) mass spectrometer (resolution 10,000) both equipped with a HP 6890 gas chromatograph with fused silica capillary columns (DB-DIOXIN, 60 m, 0.25 mm, 0.15 µm for PCDD/Fs and PCBs and DB-5 MS, 60 m, 0.25 mm, 0.25 µm for PBDEs). Limits of quantification (LOQ) for the different PCDD/F, PCB, and PBDE congeners ranged between 0.08 and 1.0 pg/g fat, 0.1 pg/g and 0.8 ng/g fat, and 0.002 and 3.1 ng/g fat, respectively. Recoveries for all internal standards were more than 60%. The calculation of the toxic native compounds was done using the corresponding $^{13}C_{12}$-labeled internal standards, automatically correcting for potential losses occurred during the extraction, clean-up and analysis processes.

For calculations, when a concentration was under its quantification limit (LOQ), the level was assumed to be one-half of that LOQ (ND = 1/2 LOQ). Statistical significance was performed by an analysis of variance (ANOVA) or a Kruskal-Wallis test, depending on whether or not data followed a normal distribution. A probability of 0.05 ($p<0.05$) was considered as significant. The statistical software SPSS version 15.0 was used for data analyses. Toxic equivalents (TEQ) were calculated according to the 2005 WHO-TEFs[8].

Results and discussion

The individual concentrations of PCDD/Fs, PCBs and PBDEs in breast milk of 15 women living in the vicinity of the HWI are shown in Table 1. In the present study, PCDD/Fs ranged from 45.4 to 143 pg/g fat (2.9 to 11.2 pg WHO-TEQ/g fat) with a mean value of 92.8 pg/g fat (7.6 pg WHO-TEQ/g fat). The most toxic PCDD congener (TCDD) showed mean concentrations of 0.99 pg/g fat, while the maximum concentration corresponded to OCDD, with a mean value of 50.3 pg/g fat. Total PCBs ranged from 89.2 to 481 ng/g fat (1.5 to 9.4 pg WHO-TEQ/g fat) with a mean value of 210 ng/g fat (4.8 pg WHO-TEQ/g fat). Regarding the most toxic PCB congeners, PCBs 126 and 169, showed mean concentrations of 34.8 pg/g fat and 26.1 pg/g fat, respectively. With respect to the total PCBs, congeners 153 and 180 reached the highest levels (53.7 ng/g fat and 48.0 ng/g fat, respectively) (data not shown). In turn, ΣPBDE concentrations (sum of 15 congeners) ranged from 1.2 ng/g fat to 7.3 ng/g fat with a mean value of 3.2 ng/g fat.

A summary of the results of the present and previous studies (baseline and 2002) is presented in Table 2. A general decrease of the concentrations in breast milk was noted for PCDD/Fs and both planar and total PCBs. This reduction is more remarkable between 1996 and 2002, being the differences statistically significant ($p<0.001$). For PCDD/Fs a 51% decrease between 1998 and 2002, and a 16% decrease between 2002 and the current survey were observed (mean concentrations of 241, 118, and 92.8 pg/g fat, respectively). Regarding ∑PCBs, a 46% decrease between the baseline and the 2002 survey, and a 18% decrease between the 2002 and the current survey was observed (471, 255 and 210 ng/g fat, respectively). However, PBDE concentration in breast milk showed a slight increase (33%), although not significant, between 2002 and 2007 (2.4 and 3.2 ng/g fat, respectively).

On the other hand, PCDD/F, PCB and PBDE concentrations in milk samples were also evaluated according to the specific place of residence of the participants (urban and industrial zones). The results for the baseline (1996), 2002 and current surveys are shown in Table 3. In the current survey, PCDD/F concentrations in milk of women living in urban areas were 38% higher than the corresponding to the industrial zones (9.0 and 5.6 pg WHO-TEQ/g fat, respectively). Total PCBs were also higher (40%) in the urban than in the industrial area (5.7 and 3.4 pg WHO-TEQ/g fat). For PBDEs, the difference (20%) between the urban an industrial zones was more limited, 3.5 and 2.8 ng/g fat, respectively. Comparing the current results with those obtained in previous studies (baseline and 2002), it can be noted that the decrease found in the levels of PCDD/Fs and PCBs in breast milk has been more important in women living in the industrial than in the urban zones. Because dietary intake is the main route of exposure to PCDD/Fs, PCBs and PBDEs for the general population[9], the different dietary habits between both groups could be the reason of these differences. The decrease observed in PCDD/Fs and PCB concentrations in the current study in relation to those of the baseline survey is probably due to the reduction of PCDD/F and PCB emissions. In turn, this reduction would be mainly due to regulations and prevention to the introduction of these pollutants in the environment. The stringent treatment and disposal requirements imposed on industrial wastes highly contaminated with POPs (PCDD/Fs and PCBs) would be another reason for this reduction.

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Table 1: Concentrations of PCDD/Fs, PCBs, and PBDEs in breast milk (2007) of 15 women living in the vicinity of a HWI in Catalonia, Spain

	PCDD/Fs			PCBs		PBDEs
Sample	∑PCDD/Fs[1] (pg/g fat)	WHO-TEQ (pg/g fat)	Sum of planar PCBs (pg/g fat)	∑PCBs[2] (ng/g fat)	Total WHO-TEQ (pg/g fat)	∑PBDEs[3] (ng/g fat)
1	47.1	4.1	34.3	96.8	2.5	2.0
2	45.4	5.4	92.1	318	6.7	1.2
3	56.0	7.4	49.6	137	3.2	1.4
4	90.2	6.7	45.8	122	3.0	2.3
5	97.8	7.0	51.2	131	3.3	5.7
6	46.4	2.9	24.7	89.2	1.5	4.1
7	92.1	7.9	89.5	229	5.4	2.5
8	113	10.8	136	481	9.4	3.2
9	90.5	9.6	99.6	263	6.0	3.5
10	129	11.2	85.9	233	6.6	3.8
11	103	8.4	70.9	159	5.0	3.5
12	143	10.7	112	329	8.6	7.3
13	103	7.0	42.6	177	2.7	2.8
14	135	8.6	67.4	236	4.3	2.6
15	101	6.7	46.5	149	3.7	2.6
Mean	92.8	7.6	70.3	210	4.8	3.2
Stan Dev	31.8	2.4	31.3	106	2.3	1.6
Minimum	45.4	2.9	24.7	89.2	1.5	1.2
Maximum	143	11.2	134	481	9.4	7.3

[1] 17-PCDD/F congeners, [2] 37-PCB congeners, [3] 15-PBDE congeners

Table 2: Mean levels and standard deviations of PCDD/Fs, planar PCBs, ∑PCBs (33) and PBDEs in samples of milk from women living in the vicinity of a HWI in Catalonia, Spain

	1996	2002	2007	% Variation 1996-02	% Variation 2002-07
PCDD/Fs					
Sum PCDD/Fs (pg/g fat)	241 ± 72	118 ± 72.2	92.8 ± 31.8	51	16
pg WHO-TEQ/g fat	12.1 ± 2.7	8.3 ± 3.1	7.6 ± 2.4	31	8
Planar PCBs					
Sum of planar PCBs (pg/g fat)	128 ± 37	74.0 ± 24	70.3 ± 31	42	5
pg WHO-TEQ/g fat	8.2 ± 2.7	3.8 ± 1.5	4.3 ± 2.0	54	-13
Total PCBs					
∑ PCBs (33) (ng/g fat)	471 ± 147	255 ± 97	210 ± 106	46	18
pg WHO-TEQ/g fat	9.4 ± 3.1	5.0 ± 1.7	4.8 ± 2.3	47	4
PCDD/Fs + PCBs					
pg WHO-TEQ/g fat	21.4 ± 5.9	13.2 ± 4.4	12.4 ± 4.4	38	6
PBDEs					
∑ PBDEs (15) (ng/g fat)	*	2.4 ± 1.7	3.2 ± 1.6	-	-33

* PBDE concentrations were not measured in the baseline survey (1996). PBDE calculations were carried out by considering that the concentration of non-detected PBDE congeners was ND = 0, in 2002, and ND = 1/2 LOQ, in 2007.

Table 3: Concentrations (mean and standard deviation) in breast milk samples according to the specific place of residence

	Baseline (1996)		2002		Current study	
	Urban (n = 9)	Industrial (n = 6)	Urban (n = 7)	Industrial (n = 8)	Urban (n = 9)	Industrial (n = 6)
PCDD/Fs	11.4 ± 2.9	12.9 ± 2.8	8.9 ± 2.3	7.8 ± 3.5	9.0 ± 1.7	5.6 ± 1.8
Planar PCBs	7.5 ± 2.6	9.3 ± 2.7	5.1 ± 1.2	3.7 ± 1.4	5.1 ± 2.0	3.0 ± 1.6
Total PCBs	8.5 ± 2.9	10.7 ± 3.1	5.9 ± 1.4	4.3 ± 1.6	5.7 ± 2.2	3.4 ± 1.8
∑ PBDEs	*	*	2.3 ± 1.9	2.5 ± 1.5	3.5 ± 1.5	2.8 ± 1.8

PCDD/F and PCB concentrations are expressed in pg WHO TEQ/g fat. Levels of PBDEs are expressed in ng/g fat. * PBDE concentrations were not measured in the baseline survey (1996).