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A PBPK model to estimate PCDD/F levels in adipose tissue: Comparison with experimental values of residents near a hazardous waste incinerator

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ABSTRACT

This study was aimed at determining the concentrations of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) in 15 samples of adipose tissue from subjects who had been living in the vicinity of a hazardous waste incinerator (HWI). The results were compared with levels obtained in previous surveys carried out in 1998 (baseline study), 2002 and 2007. The current (2013) concentrations of PCDD/Fs in adipose tissue ranged from 2.8 to 46.3 pg WHO-TEQ/g fat (mean and median concentrations: 11.5 and 7.4 pg WHO-TEQ/g fat, respectively), being significantly lower (64%) than those observed in 1998. In contrast, no significant differences in the mean PCDD/F concentrations were noted in the period 2002–2013. The significant decrease of the PCDD/F content in fat, also noted in other biological monitors such as plasma and breast milk, is in agreement with the reduction in the dietary intake of PCDD/Fs found in the same area of study. Similarly to other investigations across Europe, an increase of PCDD/F levels in adipose tissue in relation to age was observed, while no significant differences were noted according to gender. A multicompartmental physiologically-based pharmacokinetic (PBPK) model was also applied to estimate the levels of PCDD/Fs in adipose tissue. When comparing the modeled and experimental concentrations of PCDD/Fs in that tissue, very similar values were obtained for the four surveys, which indicates this can be a reliable tool to predict the internal dose of PCDD/Fs.

1. Introduction

Due to their toxic potential for humans and wildlife, as well as bioaccumulation and persistence capacity, contamination by polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) is an environmental problem of global concern. PCDD/Fs are released to the environment from a number of sources, including traffic, chemical manufacturing, iron and steelmaking, as well as open burning of materials in forest fires, accidental fires and unintentional landfill fires (Schuhmacher and Domingo, 2006; Estrellan and Iino, 2010; Ooi and Lu, 2011). In the past, incinerators were catalogued as important sources of toxic emissions, particularly PCDD/Fs and heavy metals (Hu et al., 2004). However, the installation of modern cleaning technologies to comply with maximum emission levels, according to European standards (0.1 ng I-TEQ/Nm^3), has substantially reduced the environmental impact of these facilities, not only in terms of PCDD/Fs, but also other air pollutants (Glorennec et al., 2005; Vilavert et al., 2010).

Incineration, also known as waste-to-energy, has become one of the most widely used alternatives for waste management, being considered as a serious option for the disposal of municipal solid, hazardous and medical wastes. In comparison to other waste treatments, incineration has a wide range of advantages, of which volume reduction, energetic recovery and microbial elimination are the most evident. Moreover, the incineration of hazardous waste is often selected as the most desirable disposal method, when these cannot be properly recycled (Ferré-Huguet et al., 2006; Mari et al., 2013). Unfortunately, this process may release a wide range of chemicals to air, therefore contaminating water, soil and biota by pollutant deposition, and ultimately affecting the human health of residents living in the surrounding of hazardous waste incinerators (HWIs). Despite of the heterogeneity of stack emissions, which include heavy metals, semivolatile and volatile organic compounds, special attention has been paid to PCDD/Fs (Kulkarni et al., 2008; Mari and Domingo, 2010), becoming one of the chemicals of most scientific and social interest. Because of this, the European Union (EU) imposed strict operating conditions and technical requirements on waste incineration plants and co-incineration plants, according to the EU Directive 2000/76/EC. After application of this regulatory measures and implementation of Best Available Techniques (BAT), a

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gradual decrease in the concentrations of PCDD/Fs in human biological tissues has been noted worldwide (Hagmar et al., 2006; Nadal et al., 2013), with plasma and breast milk being two of the most visible monitors. This fact has been associated to the parallel reduction in the dietary intake of these pollutants (Domingo et al., 2012). Notwithstanding, the potential health risks derived from HWI stack emissions still generate a considerable concern among the population (Liu et al., 2012; Bunsan et al., 2013). PCDD/Fs accumulate in human adipose tissues over lifetime. These contaminants are slowly metabolized in the human body and elicit adverse effects including developmental and reproductive toxicity, cancer, and endocrine disruption (Mocarelli et al., 2008; White and Birnbaum, 2009). The levels of contaminant residues in adipose tissue can provide valuable information on steady-state concentrations, as a way to integrate the body burdens of lipophilic chemicals accumulated overtime. Although ambient air, breast milk and human blood are used as core media for the sampling and analysis of persistent organic pollutants (POPs), according to the Global Monitoring Plan (GMP) under the Stockholm Convention (Fiedler et al., 2013), other biomonitors may complementarily prove the achievement of GMP quantitative objectives.

The objective of this study was to determine the concentrations of PCDD/Fs in samples of adipose tissue of individuals living in the neighborhood of a HWI in Catalonia (Spain), 14 years after the facility started its regular operations. The results of this survey were compared with data from previous campaigns carried out before (1998) and after (2002 and 2007) the plant began to operate. Data from the biological monitoring study was also used to validate a multicompartment physiologically-based pharmacokinetic (PBPK) model to estimate the levels of PCDD/Fs in adipose tissue.

2. Materials and methods

2.1. Area of study

The HWI herein studied is located in Constantí (Tarragona County, Catalonia, Spain), relatively close to other potential industrial sources of environmental pollutants, such as a big oil refinery, an important complex of chemical industries, and a municipal solid waste incinerator (MWSI), among others. In addition, an intense traffic is present in the zone, which is crossed by a highway, a motorway and a number of roads. The facility is located 5 km from the closest populated nuclei (Constantí and Reus), and 10 km from Tarragona downtown. Information about the HWI, as well as more characteristics of the surrounding area were described in detail elsewhere (Schuhmacher et al., 1999a,b,c).

2.2. Sampling

During 2012–2013, adipose tissue samples of 15 autopsied subjects living in zones of Tarragona County (Catalonia, Spain) under potential impact of the HWI were collected. At the time of death, all the individuals had lived for at least the last 10 years in the area under evaluation. All samples were obtained from the same body compartment (abdominal adipose tissue). Samples were stored in polyethylene containers and kept at −20 °C until analysis. Samples were classified according to sex (9 men and 6 women) and age (mean age of the subjects was 52 years, ranging from 30 to 74). Individual and medical information of the participants were collected by passing a questionnaire to the relatives. No occupational exposure to PCDD/F was found for any of the subjects. Sampling was conducted in collaboration with forensic physicians of the Institute of Legal Medicine of Catalonia (Tarragona Division), whose Research Committee evaluated and approved the study.

2.3. Analytical procedure

Analysis of PCDD/Fs was done according to a procedure derived from the US EPA methods 1613 and 8290A. Samples were extracted by Soxhlet during 20 h by using a mixture of hexane:acetone (3:1 v/v) (both pesticide grade). Samples were dissolved in hexane, and a mixture of $^{13}C_{12}$-PCDD/F standards was spiked in order to control potential losses during the extraction and clean-up processes. For lipids removal, 1 g tissue in 30 mL of n-hexane (pesticide grade) was shaken in 5 mL of high purity acid silica (44% H_2SO_4) for 1 min. The clean-up procedure and fractionation of the crude extract was carried out by adsorption chromatography as multi-step clean-up using silica and alumina columns. Firstly, each ample was cleaned over a multilayer column containing, from bottom to top, 2 g of silica, 5 g of basic silica (33% NaOH 1 N), 2 g of silica, 10 g of acid silica (44% H_2SO_4), 2 g of silica, and 1 g of Na_2SO_4. The extract was then transferred to the column, and eluted with 130 mL n-hexane (pesticide grade). Secondly, the eluate was concentrated, transferred to an alumina column containing 5 g of Al_2O_3 (basic Woelm I) and 1 g of Na_2SO_4, and eluted with 40 mL n-hexane:dichloromethane (98:2 v/v) (pesticide grade). This fraction was discarded, and PCDD/Fs were collected by eluting 75 mL of an n-hexane:dichloromethane mixture (50:50 v/v). The PCDD/F fraction was collected and concentrated to near dryness with a nitrogen flux. Finally, 25 μL of $^{13}C_{12}$-PCDD/F injection standards were added.

The analysis of PCDD/Fs was carried out by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The extract obtained after extraction and clean-up was injected on an Agilent 6890 gas chromatograph equipped with a ZB5-MS capillary column and coupled to a Waters Autospec Ultima mass spectrometer. The chromatographic process separated the 17 toxic 2,3,7,8-substituted congeners from each other. The mass spectrometer measured (via "selected ion recording" at a resolution of >10000) two selected ions per congener group for both the native and labeled compounds. The calculation of the concentrations was done by using the corresponding ^{13}C congener level, automatically correcting according to the recovery percentage specific for each congener. In addition, the relative standard deviation (RSD) was calculated as a measure of the uncertainty. In all cases, the RSD of the control sample was lower than 10%. A quality control/quality assurance (QC/QA) protocol was carefully followed. There were minimum requirements for: 1) daily calibration check as to sensitivity (signal/noise), chromatographic separation and maximum deviation from calibration curve (20%); 2) identification such as isotope ratio of the ions monitored, retention time deviation, and signal to noise ratio of the signal; 3) quantification as to number of data points, chromatographic separation, minimum recovery of ^{13}C-labeled extraction-standards, and linear range; 4) procedure blanks, whose levels should be generally lower than reporting limits of the analytical procedures; 5) control samples, which should meet the criteria outlined in the procedure; and 6) calibration drift by analyzing the middle calibration standard at the end of the analysis sequence.

2.4. Data treatment

The SPSS 19.0 statistical software package was used for data analysis. Total PCDD/F concentrations were calculated according to the 2005 WHO-TEFs (van den Berg et al., 2006). PCDD/F levels obtained in previous campaigns (1998, 2002 and 2007) were also recalculated according to the 2005 WHO-TEFs. The Levene test was applied to study the equality of variances. Furthermore, the ANOVA or Kruskal–Wallis tests were executed. A probability lower than 0.05 ($p < 0.05$) was considered as statistically significant. For calculations, when a PCDD/F congener presented a level below the respective limit of detection (LOD), the concentration was assumed to be one-half of that limit (ND = 1/2 LOD).

3. Results and discussion

3.1. Concentrations of PCDD/Fs in adipose tissue

The individual PCDD/F concentrations in adipose tissue samples from 15 subjects from Tarragona County, are summarized in Table 1.

Table 1
Individual PCDD/F concentrations (pg WHO-TEQ/g fat) in adipose tissue samples of individuals who had been living for at least the last 10 years in Tarragona County, Spain.

Sample	Gender	Age (years)	pg WHO-TEQ/g fat
1	M	56	10.2
2	M	53	7.4
3	F	74	21.4
4	M	37	2.9
5	F	40	6.7
6	M	50	18.2
7	M	59	11.3
8	M	46	2.8
9	M	56	12.8
10	F	72	46.3
11	F	40	5.1
12	M	64	6.0
13	M	36	2.9
14	F	73	13.9
15	F	30	5.1

M: Male; F: Female

Age and sex of the individuals are also given. The mean levels of the 17 substituted-PCDD/F congeners, as well as the total PCDD/F concentration, in adipose tissue samples collected in the baseline (1998), previous (2002 and 2007) and current (2013) surveys, are summarized in Table 2. In the baseline (1998) study, a mean PCDD/F concentration in adipose tissue of 32.1 pg WHO-TEQ/g fat was found (median: 26.7 pg WHO-TEQ/g fat; range: 14.2–70.1 pg WHO-TEQ/g fat) (Schuhmacher et al., 1999b). In the 2002 survey, the mean and median levels of PCDD/Fs were 9.9 and 6.8 pg WHO-TEQ/g fat, with minimum and maximum values of 1.4 and 36.1 pg WHO-TEQ/g fat, respectively (Schuhmacher et al., 2004). In the immediately previous campaign (2007), the mean PCDD/F concentration in adipose tissue was 14.6 pg WHO-TEQ/g fat (median: 7.5 WHO-TEQ/g), with values ranging from 3.3 to 55.4 pg WHO-TEQ/g fat (Nadal et al., 2008). In the current (2013) survey, mean and median PCDD/F concentrations of 11.5 and 7.4 pg WHO-TEQ/g fat, respectively, were observed, while minimum and maximum concentrations were 2.8 and 46.3 pg WHO-TEQ/g fat, respectively. Comparing the mean PCDD/F concentrations of the baseline (1998) study with those of the current (2013) survey, an important significant reduction was found (64%). However, during the period 2002–2013 no significant differences in the mean PCDD/F concentrations were noted, being median levels very similar. This temporal trend is in agreement with the significant reduction of PCDD/Fs in other biological tissue samples from non-occupationally exposed people of the same area. Between 1998 and 2012, a significant decrease of mean PCDD/F concentrations in plasma from residents in Tarragona County was found. Levels were reduced from 27.0 to 6.18 pg I-TEQ/g lipid (77%; $p < 0.001$) (Nadal et al., 2013). Similarly, the concentrations of PCDD/Fs in breast milk of women living in the same area were significantly lower in 2012 than those obtained in the 1998 survey (12.2 and 4.8 pg WHO-TEQ/g fat, respectively; $p < 0.001$). Although the overall assessment of the data indicate a similar decreasing trend of PCDD/F burdens in the 3 biomonitors (plasma, breast milk, and adipose tissue), the profile of this latter is slightly different from the remaining two. The temporal decline of PCDD/Fs in plasma and breast milk has been progressive, being the values of the intermediate sampling campaigns lower than that of the baseline survey (1998), but higher than that of the most recent study (2012–2013). Thus, mean plasma concentrations of 15.7 and 9.36 pg I-TEQ/g lipid were found in 2002 and 2007, respectively, while PCDD/F levels in breast milk in 2002 and 2007 were 10.6 and 7.6 pg WHO-TEQ/g fat, respectively. On the other hand, the most important reduction of PCDD/Fs in fat tissue was only detected in the period 1998–2002, while no significant changes have been found since then. This would indicate that fat tissue acts more as a storage compartment, when compared with plasma and breast milk. Anyhow, the significant reduction in the levels of PCDD/Fs in fat tissue is consistent with the decreasing trend in the dietary intake of PCDD/Fs observed in recent years. The intake of PCDD/Fs through food consumption for the adult population of Tarragona County was 210.1 pg I-TEQ/day in the baseline survey (1998) (Domingo et al., 1999), while in 2012 the dietary intake dropped to 33.1 pg WHO-TEQ/day (Domingo et al., 2012), being this intake similar to that previously found in 2006 (Martí-Cid et al., 2008). Although human exposure to PCDD/Fs may occur through a number of routes such as inhalation, dermal contact, and ingestion of soils and dust, it is well known that the diet is quantitatively the main exposure pathway for non-occupationally exposed individuals (Nadal et al., 2004a; Passuello et al., 2010; Windal et al., 2010).

The current concentrations of PCDD/Fs in samples of adipose tissue from individuals of Tarragona County are within the range of those reported in the scientific literature. Takenaka et al. (2002) found mean PCDD/F concentrations of 49.0 pg WHO-TEQ/g fat in fat tissue of Japanese people, collected in 1999, being this level similar to the results of our baseline survey, conducted only one year before. In Turkey, Çok

Table 2
Mean levels of PCDD/F congeners (in pg/g fat) in adipose tissue samples of residents near a HWI in Tarragona County (Catalonia, Spain).

Congener	1998	2002	2007	2013
2,3,7,8-TCDD	4.13 ± 3.03[a]	1.39 ± 1.53[b]	1.68 ± 1.86[b]	1.24 ± 1.14[b]
1,2,3,7,8-PeCDD	11.37 ± 4.74[a]	3.73 ± 3.51[b]	5.28 ± 4.80[b]	4.11 ± 3.48[b]
1,2,3,4,7,8-HxCDD	5.61 ± 2.86[a]	2.78 ± 1.73[b]	3.30 ± 3.61[a,b]	2.26 ± 2.26[b]
1,2,3,6,7,8-HxCDD	59.4 ± 30.2[a]	19.2 ± 18.9[b]	28.1 ± 29.3[b]	25.3 ± 26.6[b]
1,2,3,7,8,9-HxCDD	8.12 ± 6.45[a]	2.08 ± 2.03[b]	3.55 ± 4.47[b]	2.63 ± 4.37[b]
1,2,3,4,6,7,8-HpCDD	84.9 ± 60.9[a]	10.2 ± 8.0[b]	20.0 ± 28.9[b]	20.3 ± 52.9[b]
OCDD	478 ± 320[a]	53.6 ± 51.0[b]	152 ± 188[b]	113 ± 196[b]
2,3,7,8-TCDF	0.94 ± 0.58[a]	0.34 ± 0.4[b]	0.40 ± 0.40[b]	0.35 ± 0.27[b]
1,2,3,7,8-PeCDF	0.92 ± 0.47[a,b]	0.5 ± 0.45[b]	1.4 ± 1.33[a]	0.31 ± 0.26[b]
2,3,4,7,8-PeCDF	21.1 ± 11.5[a]	5.71 ± 5.95[b]	9.94 ± 9.43[b]	7.43 ± 6.93[b]
1,2,3,4,7,8-HxCDF	7.02 ± 3.33[a]	2.32 ± 1.75[b]	3.29 ± 3.25[b]	2.83 ± 2.97[b]
1,2,3,6,7,8-HxCDF	8.22 ± 3.99[a]	2.03 ± 1.86[b]	3.31 ± 3.47[b]	2.69 ± 2.86[b]
1,2,3,7,8,9-HxCDF	0.62 ± 0.35[a]	0.39 ± 0.41[b]	0.06 ± 0.05[b]	0.07 ± 0.05[b]
2,3,4,6,7,8-HxCDF	2.2 ± 1.28[a]	0.38 ± 0.44[a]	0.88 ± 0.73[a]	0.69 ± 1.15[a]
1,2,3,4,6,7,8-HpCDF	4.81 ± 2.17[a]	2.06 ± 0.65[a]	2.99 ± 2.54[a,b]	2.34 ± 3.13[b]
1,2,3,4,7,8,9-HpCDF	0.39 ± 0.10[a]	0.31 ± 0.52[a,b]	0.10 ± 0.06[b]	0.11 ± 0.05[b]
OCDF	0.72 ± 0.27[a]	2.59 ± 1.27[b]	0.49 ± 0.30[a]	0.31 ± 0.15[a]
Total WHO-TEQ	32.1 ± 15.3[a]	9.9 ± 9.3[b]	14.6 ± 14.2[b]	11.5 ± 11.1[b]
	(26.7)	(6.8)	(7.5)	(7.4)

[a,b]Different superscripts indicate statistically significant differences at $p < 0.05$.
In parenthesis, median values.

et al. (2007) observed that PCDD/F concentrations ranged from 3.2 to 19.7 pg WHO-TEQ/g fat (mean: 9.2 pg WHO-TEQ/g fat) in 23 adipose tissue samples collected from men in 2004. In turn, Shen et al. (2009) reported that the mean PCDD/F level in 24 adipose tissue samples collected in 2006 in the Zhejiang Province (China) was 19 pg TEQ/g fat (range: 2.5–56 pg TEQ/g fat). In general terms, these levels are quite similar to those found in the present survey. Furthermore, our results indicate that the incineration of hazardous wastes did not result in any detectable exposure of PCDD/Fs to the population surrounding the facility, as indicated by the TEQ levels in adipose tissue. The PCDD/F congener profile showed that OCDD was the predominant congener, followed by 1,2,3,6,7,8-HxCDD and 1,2,3,4,6,7,8-HpCDD (Table 2). On the other hand, all samples showed considerably higher levels of dioxins (PCDDs) than furans (PCDFs), which is in agreement with the results of our previous studies (Schuhmacher et al., 1999b, 2004). Table 3 summarizes the PCDD/F concentrations in adipose tissue of each one of the sampling campaigns, classified according to gender. Similarly to previous surveys, in the current (2013) study women showed notable higher levels of PCDD/Fs than men (16.4 vs. 8.3 pg WHO-TEQ/g fat). However, the difference was not significant in any of the four studies.

An increase of PCDD/F levels in adipose tissue with age was observed ($R^2 = 0.4389$) not only in the current (2013) survey but also in previous (1998, 2002 and 2007) studies. The concentrations of PCDD/Fs in 60 samples of adipose tissue collected between 1998 and 2012, according to the age at the time of death, are depicted in Fig. 1. Additionally, in order to analyze the potential differences of the PCDD/F concentration in adipose tissue in the younger and older populations, samples were classified into two groups, according to the median age: ≤53 years and >53 years. In 2013, PCDD/F concentrations of 5.85 and 17.40 pg WHO-TEQ/g fat were observed in the group of under and over 53 years, respectively, reaching this difference the level of statistical significance ($p < 0.05$). Taking into account the global number of samples from the 4 campaigns ($n = 60$), mean PCDD/F levels in adipose tissue of people who had died being ≤53 years and >53 years were 11.47 and 25.52 pg WHO-TEQ/g fat, respectively ($p < 0.01$). In Korea, Moon et al. (2011) found a mean concentration of PCDD/Fs of 3.4 pg TEQ/g fat in human adipose tissue from women (aged between 40 and 68). No correlations were observed between the age of the subject and the burdens of dioxin-like contaminants. Similarly, no significant correlations between age and PCDD/F levels in human adipose tissue were found in China (Shen et al., 2009). In contrast, some European investigators have detected a positive association between PCDD/F levels in adipose tissue and age, in 420 individuals living in southern Finland (Kiviranta et al., 2005), and in 20 women living in southern Spain (Lopez-Espinosa et al., 2008). Although notable differences in the PCDD/F body burdens have been noted in different geographical areas, mainly linked to fish consumption (Nadal et al., 2004b), the reasons of this should be studied in more detail. Age is likely an indicator of past cumulative exposures. People born in earlier times experienced higher environmental PCDD/F levels and thus they carry a higher body burden through life. There is evidence from human and animal studies that blood dioxin half-lives, in the absence of weight change or fat redistribution, increase with age. However, this effect is relatively minor compared to the birth cohort effect of historical exposures (Lorber and Phillips, 2002).

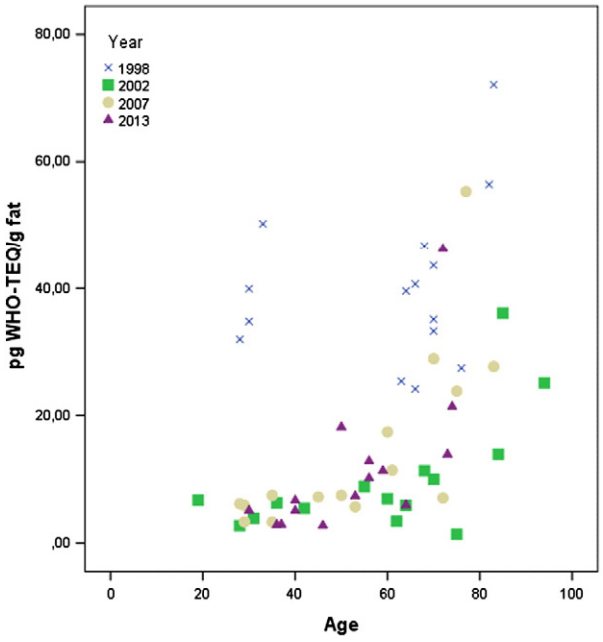

Fig. 1. PCDD/F concentrations in samples of adipose tissue from individuals of Tarragona County in 1998, 2002, 2007 and 2013.

The accumulation of PCDD/Fs according to gender has also been assessed in a number of investigations. However, most of them have focused on blood and plasma. Fromme et al. (2009) did not find any significant difference in the blood concentration of PCDD/Fs between men and women. However, they observed a significant increase in the WHO-TEQ levels with age. In the city of Mataró (Catalonia, Spain), Parera et al. (2013) recently found that PCDD/F levels in blood were higher in women than in men, showing both gender groups a slight reduction in comparison to precedent campaigns. Reis et al. (2007) also noted slightly higher PCDD/F levels in blood of Portuguese women, although differences in dioxin blood levels between males and females were not statistically significant. These authors also found that age was also associated with total TEQs in blood, with a significant correlation in a way that elder individuals owned higher PCDD/F values.

In recent years, the evaluation of several NHANES data sets have shown that a number of POPs are related to either body mass index (BMI) or waist circumference (Lee et al., 2007; Elobeid et al., 2010). BMI is physiologically related to the body's capacity to eliminate PCDD/Fs (Schildkraut et al., 1999). The elimination capacity of PCDD/Fs is indirectly proportional to the content of body fat, as individuals with less fat may eliminate 2,3,7,8-TCDD more easily (Emond et al., 2005; Collins et al., 2007). Since BMI tends to increase with age, it is difficult to study the effects of BMI independently of age. Collins et al. (2007) reported that age and BMI are both important factors for assessing background levels of 2,3,7,8-TCDD. Several studies indicate that body fat turnover is regulated at least, in part, by fat patterning, and that upper body fat turns over at a higher rate than peripheral fat located below the hips (Rodin, 1992). Therefore, measures other than

Table 3
Concentrations of PCDD/Fs (in pg WHO-TEQ/g fat) in adipose tissue of individuals who had lived in Tarragona County (Catalonia, Spain), according to gender.

	1998	2002	2007	2013
Men	25.3 ± 9.4 (n = 10)	7.2 ± 3.5 (n = 11)	11.2 ± 8.1 (n = 11)	8.3 ± 5.3 (n = 9)
Women	45.7 ± 16.7 (n = 5)	17.4 ± 16.1 (n = 4)	23.8 ± 23.9 (n = 4)	16.4 ± 15.9 (n = 6)

Data given as mean ± standard deviation; n: number of samples.

BMI may provide further insight into the relationship between the amount and distribution of body fat and the capacity to store and eliminate PCDD/Fs. Although the amount of body fat has been also correlated with PCDD/F levels, it is rarely adjusted when comparing potentially exposed populations to a surveyed background (Landi et al., 1998). Pharmacokinetic properties of POPs can explain the observed relationships. POPs are stored in lipid reservoirs, while their concentrations change predictably with changes in adipose tissue volume. Levels in blood are proportional to blood lipid content, and weight gain increases the POP half-life (Thomaseth and Salvan, 1998). Lim et al. (2011) observed that weight gain over 10 years resulted in lower levels of polychlorinated biphenyls (PCBs) compared with weight stable or weight-loss conditions. However, the pharmacokinetics is complex, and relationships between POPs and a number of factors, such as BMI or weight change, are difficult to evaluate. Temporal changes depend on the magnitude and time of exposure, ongoing exposure, body fat mass, and changes in fat mass during the time between exposure and the time of blood sampling, among others.

The pharmacokinetic characteristics of each PCDD/F congener determine the transport of chemicals, which pass from blood to the fat tissue. These characteristics include, for example, lipophilicity of the substance, molecular diameter, molecular weight, molar volume, and octanol–water partition coefficient (K_{ow}). Some constitutional descriptors such as the number and position of attached halogens and the number of hydrogen-bond acceptors, have also some contribution. The position of the halogen substitutes will determine the rigidity of the molecular structure and, consequently, its ability to pass from blood to other tissues (Mannetje et al., 2012). Recent studies suggest that different PCDD/F congeners have different partition coefficients that are dependent on the properties listed above (Needham et al., 2011). In addition, this ratio can be modified by the half-life of each PCDD/F congener. The ratio between the concentration of each PCDD/F congener in fat tissue and human plasma, expressed on a lipid basis, for the non-occupationally exposed subjects of Tarragona County (Spain) is shown in Table 4. All PCDD congeners showed fat:blood ratios >1, being 2,3,7,8-TCDD and OCDD those presenting the highest and lowest relationships (3.7 and 1.5, respectively). In turn, PCDFs showed a completely different pattern of the fat:blood ratio, with values under the unity for most congeners. The higher ratio was noted for 2,3,4,6,7,8-HxCDF (3.3), while 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, and 1,2,3,6,7,8-HxCDF showed values >1. The reasons behind these patterns need further investigations. These results have certain limitations because the samples of plasma and adipose tissue are not from the same individuals. Although studies did not differ in the timing of the sampling of both plasma and adipose tissue, being the mean age of subjects quite similar (52 and 50 years for donors of fat tissue and blood, respectively), different dietary habits could have altered these ratios.

3.2. Physiologically-based pharmacokinetic (PBPK) model

Physiologically based pharmacokinetic (PBPK) models are mathematical representations of the human body where the tissues are considered well-stirred compartments linked by the blood flow (Nestorov, 2007). The final result is a set of ordinary differential equations that can be mathematically solved to predict the concentration of chemicals in human tissues. In recent years, PBPK models have been used in human health risk assessment to estimate the burdens of chemicals in human tissues, thus avoiding the analysis of this kind of samples (Chiu et al., 2007; Clewell and Clewell III, 2008). In the present study, a previous PBPK model (Maruyama et al., 2003; Fàbrega et al., 2014) was adapted to assess the concentration of PCDD/Fs in adipose tissue in four temporal scenarios: 1998, 2002, 2007 and 2013. The simulations were run for adult individuals living in the same area of study. Oral intake was assumed to be the only exposure pathway. Data about dietary intake of PCDD/Fs were obtained from previous studies conducted in the same area (Domingo et al., 1999; Bocio and Domingo, 2005; Martí-Cid et al., 2008; Domingo et al., 2012) (Table 5). The characteristics and parameters of the model were previously described (Nadal et al., 2013). Briefly, 7 body compartments were considered: blood, muscle, skin, richly perfused, adipose, kidney, and liver. The intake of PCDD/Fs, as well as other PBPK model parameters (e.g., volume, absorption, elimination and cardiac output of each tissue), were considered constant along time. The physical basis of the adipose tissue diffusion is related to the octanol:water partition coefficient (*K*ow). At steady state, the log *K*ow predicts the capacity of the chemical to diffuse into adipose tissue, being therefore accumulated in fat.

PCDD/F concentrations in the flow limited compartments (muscle, skin, richly perfused, fat, kidney and liver) were estimated by applying the following equation:

$$\frac{dCi}{dt} = \frac{Qi \times \left(Ca - \frac{Ci}{Ki:p}\right)}{Vi} \quad (1)$$

where *Ci* is the concentration of PCDD/Fs in the tissue *i* (pg/L), *Qi* is the blood flow in the tissue *i* (L/h), *Ca* is the arterial concentration (pg/L), *Ki:p* is the partition coefficient of tissue *i*, and *Vi* is the volume of the tissue *i* (L).

Table 4
Fat:blood ratio of PCDD/Fs in subjects living in Tarragona County (pg/g lipid).

Congener	PCDD/Fs in fat tissue (pg/g lipid)[a]	PCDD/Fs in plasma (pg/g lipid)	Fat:blood (unitless)
2,3,7,8-TCDD	1.2	0.3	3.7
1,2,3,7,8-PeCDD	4.1	2.1	1.9
1,2,3,4,7,8-HxCDD	2.3	1.1	2.1
1,2,3,6,7,8-HxCDD	25.3	10.2	2.5
1,2,3,7,8,9-HxCDD	2.6	1.4	1.8
1,2,3,4,6,7,8-HpCDD	20.3	9.0	2.3
OCDD	113	75.8	1.5
2,3,7,8-TCDF	0.3	4.4	0.07
1,2,3,7,8-PeCDF	0.3	2.5	0.12
2,3,4,7,8-PeCDF	7.4	4.4	1.7
1,2,3,4,7,8-HxCDF	2.8	2.4	1.2
1,2,3,6,7,8-HxCDF	2.7	1.9	1.4
1,2,3,7,8,9-HxCDF	0.1	0.6	0.17
2,3,4,6,7,8-HxCDF	0.7	0.2	3.3
1,2,3,4,6,7,8-HpCDF	2.3	5.4	0.43
1,2,3,4,7,8,9-HpCDF	0.1	0.5	0.20
OCDF	0.3	6.5	0.05

[a] From Nadal et al. (2013).

Table 5
Absorption and dietary intake of 17 PCDD/F congeners used in the PBPK model.

	Absorption (%)	Intake (pg/day)			
		1998	2002	2007	2012
2,3,7,8-TCDD	97	28.6	6.1	3.9	5.6
1,2,3,7,8-PeCDD	99	14.9	9.6	8.5	7.6
1,2,3,4,7,8-HxCDD	98	43.4	11.6	5.3	3.6
1,2,3,6,7,8-HxCDD	97	114	21.5	7.1	10.9
1,2,3,7,8,9-HxCDD	96	41.6	11.5	4.8	6.9
1,2,3,4,6,7,8-HpCDD	86	1292	92.4	28.3	69.6
OCDD	76	9623	525	141	297
2,3,7,8-TCDF	97	192	48.9	25.2	40.6
1,2,3,7,8-PeCDF	99	125	28.2	11.4	9.2
2,3,4,7,8-PeCDF	98	109	46.8	12.4	25.7
1,2,3,4,7,8-HxCDF	97	231	53.3	20.9	33.4
1,2,3,6,7,8-HxCDF	97	107	27.6	12.3	13.9
1,2,3,7,8,9-HxCDF	95	10.2	10.4	3.1	6.6
2,3,4,6,7,8-HxCDF	96	42	32.7	5.8	9.3
1,2,3,4,6,7,8-HpCDF	87	708	69.8	72.2	127
1,2,3,4,7,8,9-HpCDF	99	89.7	15.8	13	23.9
OCDF	95	4420	93.4	476	201

The PCDD/F content in the blood compartment was assessed by using the following equation:

$$\frac{dCblood}{dt} = \frac{\sum_i \left(\frac{Qi \times Ci}{Ki:p}\right) - \left(Cblood \times \sum_i Qi\right) + D \times Abs}{Vblood} \quad (2)$$

where *Cblood* is the concentration of PCDD/Fs in blood (pg/L), *Qi* is the blood flow in the tissue *i* (L/h), *Ci* is the concentration in the tissue *i* (pg/L), *Ki*:*p* is the partition coefficient of tissue *i*, *D* is the oral dose (pg/day), *Abs* is the gastrointestinal absorption (%), and *Vblood* is the volume of the tissue *i* (L).

Since liver was considered as an elimination organ, the predicted concentration of PCDD/Fs in this tissue was estimated by the following equation:

$$\frac{dCliver}{dt} = \frac{Qliver \times \left(Ca - \frac{Cliver}{Kl:p}\right) - Cliver \times Vliver \times K_1}{Vliver} \quad (3)$$

where *Cliver* is the concentration of PCDD/Fs in blood (pg/L), *Qliver* is the blood flow in the liver, *Ca* is the arterial concentration, *Kl*:*p* is the partition coefficient in the liver, K_1 is the elimination constant, and *Vliver* is the volume in liver (L). Values of tissue volumes, cardiac output, absorption, partition coefficient (*Kp*), and elimination constants were the same used in our previous models (Nadal et al., 2013) (Tables 6 and 7). A density of 0.92 g/mL in adipose tissue was used to recalculate the final concentration in pg/g tissue (Brown et al., 1997). The final system was a set ordinary differential equations which was handled by using Berkeley Madonna v.8.3.18. Stiff was considered as the method to solve the differential equations, while the step size (SD) in the simulations was set at 0.0001.

The measured and simulated concentrations of PCDD/Fs in fat tissue for the 1998 (baseline), 2002, 2007 and 2013 (current) surveys are summarized in Table 8. When comparing the modeled and experimental concentrations of PCDD/Fs in fat tissue, expressed as total WHO-TEQ, it can be observed that similar values were obtained in the four scenarios. However, experimental results are slightly higher than those simulated for all the surveys. Analyzing PCDD/F congeners individually, the simulated concentrations of all PCDDs, excepting 1,2,3,7,8,9-HxCDD, tended to suffer an underestimation with respect to those observed levels. In turn, the simulated levels of PCDFs, excepting 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, were slightly higher than those experimentally obtained. OCDF showed the most important difference between simulated and measured concentrations of PCDD/Fs, being 2–3 orders of magnitude higher than the modeled results (21.78 vs. 0.31 pg/g fat). Notable dissimilarities between estimated and observed concentrations of OCDF in adipose tissue were also reported in previous campaigns (1998, 2002, and 2007). The transfer of POPs from the vascular environment to other biological tissues is highly influence by pharmacokinetic factors, such as tissue volume, anatomical localization, and blood flow rate. The default approach assumes that the tissue is flow limited. It means that the distribution of chemicals contained in blood across the well-stirred tissue compartment is fast and homogenous. Although this assumption is valid for the distribution of many xenobiotic chemicals into many tissues, because of their diffusion-limited characteristics, it appears to be incorrect for the movement of several highly lipophilic POPs across the adipose tissue (Levitt, 2010; La Merrill et al., 2013). In this case, the distribution of the chemicals is slower and may be incomplete. A comparison between measured and simulated concentrations of PCDD/Fs in samples of human adipose tissue collected in 1998, 2002, 2007 and 2013, is depicted in Fig. 2. Because food intake was assumed to be the only single exposure pathway, a significant reduction of fat concentrations of PCDD/Fs was noted. This is in agreement with the significant decrease found in the dietary intake of PCDD/Fs (Domingo et al., 2012). An important uncertainty, represented by the high standard deviations, was observed in the experimental concentrations of PCDD/Fs. As abovementioned, age and BMI of individuals can affect PCDD/F accumulation in fat tissue. In addition, diverse dietary patterns may be also the reason of the differences found between individuals. Anyhow, simulated concentrations of PCDD/Fs in fat were within the range of experimental values, irrespectively of the sampling year. According to Berezhkovskiy (2011), the classical pharmacokinetic

Table 6
Physiological parameters used in the PBPK model.

	Tissue volume (L)	Cardiac output (L/h)
Blood	4.5	–
Liver	1.5	182.2
Fat	10.3	11.1
Kidney	0.3	3.5
Muscle	24	43.2
Richly perfused	1.8	51.1
Skin	2.2	3.2

Table 7
Partition coefficients (Pk) used in the PBPK model (unitless).

	Liver	Kidney	Fat	Muscle	Richly perfused	Skin
2,3,7,8-TCDD	9.8	3.1	247	17	4.1	2.5
1,2,3,7,8-PeCDD	17	2.9	432	19	4.5	2.0
1,2,3,4,7,8-HxCDD	30	1.2	117	5.1	2.4	2.6
1,2,3,6,7,8-HxCDD	55	3.0	219	13	2.7	1.7
1,2,3,7,8,9-HxCDD	297	11	1466	134	30	2.6
1,2,3,4,6,7,8-HpCDD	34	2.3	143	39	14	14
OCDD	56	2.8	55	26	16	14
2,3,7,8-TCDF	18	0.97	55	4.7	2.3	9.4
1,2,3,7,8-PeCDF	19	1.4	130	10	6.3	9.2
2,3,4,7,8-PeCDF	45	2.1	336	38	3.5	2.6
1,2,3,4,7,8-HxCDF	25	1.6	75	11	6	2.1
1,2,3,6,7,8-HxCDF	45	1.7	130	25	2.2	2.4
1,2,3,7,8,9-HxCDF	1.8	0.4	172	2.9	3.7	11
2,3,4,6,7,8-HxCDF	3.8	0.9	48	3.1	3.5	6.2
1,2,3,4,6,7,8-HpCDF	22	0.9	139	7.3	2.3	11
1,2,3,4,7,8,9-HpCDF	8.7	0.3	113	3.1	1.9	11
OCDF	15	1.7	144	5.5	4.5	14

Table 8
Comparison between simulated (Sim) and measured (Exp) concentrations of PCDD/Fs found in adipose tissue of adults living near the HWI of Tarragona County (Spain).

	1998		2002		2007		2013	
	Sim	Exp	Sim	Exp	Sim	Exp	Sim	Exp
2,3,7,8-TCDD	3.40	4.13	0.81	1.39	0.48	1.68	0.88	1.24
1,2,3,7,8-PeCDD	3.91	11.4	2.25	3.73	1.68	5.28	1.62	4.11
1,2,3,4,7,8-HxCDD	0.89	5.61	0.74	2.78	0.35	3.30	0.22	2.26
1,2,3,6,7,8-HxCDD	10.2	59.4	3.29	19.2	2.03	28.1	0.87	25.4
1,2,3,7,8,9-HxCDD	9.07	8.12	4.37	2.08	2.19	3.55	5.32	2.63
1,2,3,4,6,7,8-HpCDD	64.2	84.9	8.04	10.2	3.10	20.0	3.80	20.3
OCDD	115	478	7.85	53.6	2.67	152	3.53	113
2,3,7,8-TCDF	8.21	0.94	2.10	0.34	1.08	0.40	1.86	0.35
1,2,3,7,8-PeCDF	9.23	0.92	2.18	0.50	0.88	1.40	0.91	0.31
2,3,4,7,8-PeCDF	11.5	21.10	4.76	5.71	1.81	9.94	2.62	7.43
1,2,3,4,7,8-HxCDF	9.70	7.02	2.37	2.32	0.92	3.29	1.50	2.83
1,2,3,6,7,8-HxCDF	4.53	8.22	1.34	2.03	0.60	3.31	0.62	2.69
1,2,3,7,8,9-HxCDF	1.54	0.62	1.43	0.39	0.49	0.06	1.35	0.07
2,3,4,6,7,8-HxCDF	3.93	2.20	3.14	0.38	0.53	0.88	1.14	0.69
1,2,3,4,6,7,8-HpCDF	45.72	4.81	5.98	2.06	4.99	2.99	9.63	2.34
1,2,3,4,7,8,9-HpCDF	9.11	0.39	1.67	0.31	1.32	0.10	3.12	0.11
OCDF	376	0.72	9.59	2.59	40.67	0.49	21.8	0.31
WHO-TEQ	17.2	32.1	6.59	9.94	3.66	14.6	4.77	11.5

Concentrations are given in pg/g tissue.

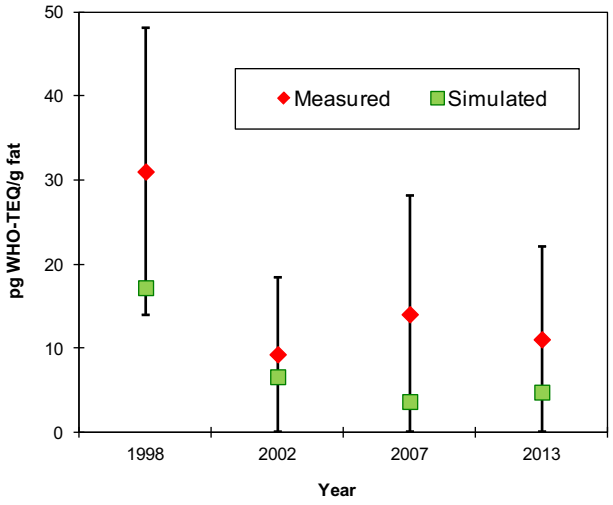

Fig. 2. Comparison between measured and simulated concentrations of PCDD/Fs in samples of adipose tissue collected in 1998, 2002, 2007 and 2013.

analysis of highly lipophilic POPs at low concentrations often leads to a substantial underestimation of the distribution volume during steady state (Vss) in obese people. Therefore, an accurate determination of Vss is required to assess the distribution and kinetics of environmental chemicals. In any case, further research to improve the understanding of the adipose tissue:blood partitioning mechanisms, and the differences according to the PCDD/F congener, is needed. Moreover, the histological and anatomical structure of different types of adipose tissue can influence their contribution to toxicokinetics (Sbarbati et al., 2010). Furthermore, age and BMI may be also important in epidemiology studies, where back-extrapolation from current PCDD/F levels is used. In order to improve the model, different partition ratios for each PCDD/F congener should be taken into account. Another issue for improvement is the inclusion of dynamic parameters of exposure, such as time varying lifetime exposure.

4. Conclusions

The PCDD/F concentrations in adipose tissue of 15 individuals who had been living for at least the last 10 years near a HWI located in Tarragona County (Spain), were here determined. A mean PCDD/F level of 11.5 pg WHO-TEQ/g fat was found, being significantly lower than the concentration observed in 1998 (32.1 pg WHO-TEQ/g fat), when the facility was still inactive (Schuhmacher et al., 1999b). Current values of PCDD/Fs in adipose tissue in Tarragona County are of the same order of magnitude than those recently observed in a number of industrialized countries. The important decrease, which was also noted in other biological monitors such as plasma and breast milk (Nadal et al., 2013; Schuhmacher et al., 2013), agrees well with the notable reduction in the dietary intake of PCDD/Fs recently found for the population living in the same area. Our findings confirm that, after 14 years of regular operations, air emissions of PCDD/Fs from the HWI do not mean a significant additional exposure to these organic pollutants for the population living near the facility.

On the other hand, our adapted PBPK model has demonstrated to be a reliable tool to predict the levels of PCDD/Fs in fat tissue, as it was also in plasma (Nadal et al., 2013). Although the application of the model should be of great interest to estimate the long-term accumulation of PCDD/Fs, not only in fat, but also in other biological tissues, an improvement of the PBPK model is required to obtain more accurate results.

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