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Decreasing temporal trends of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in adipose tissue from residents near a hazardous waste incinerator

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ABSTRACT

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are very toxic chemicals which are emitted in waste incineration and whose exposure has important adverse effects for the human health. In 2019, adipose tissue samples were collected from 15 individuals with a median age of 61 years, who had been living near a hazardous waste incinerator (HWI) in Constantí (Catalonia, Spain). The content of PCDD/Fs in each sample was analyzed. The results were compared with data from previous studies, conducted before (1998) and after (2002, 2007 and 2013) the facility started to operate, and based on populations of similar age. In 2019, the mean concentration of PCDD/Fs in adipose tissue was 6.63 pg WHO-TEQ/g fat, ranging from 0.95 to 12.95 pg WHO-TEQ/g fat. A significant reduction was observed with respect to the baseline study (1998), when a mean PCDD/Fs concentration of 40.1 pg WHO-TEQ/g fat was found. Moreover, the current level was much lower than those observed in the 3 previous studies (9.89, 14.6 and 11.5 pg WHO-TEQ/g fat in 2002, 2007 and 2013, respectively). The body burdens of PCDD/Fs were strongly correlated with age. The significant reduction of PCDD/Fs levels in adipose tissue fully agreed with the decreasing trend of the dietary intake of PCDD/Fs by the population of the zone (from 210.1 pg I-TEQ/day in 2018 to 8.54 pg WHO-TEQ/day in 2018). Furthermore, a similar decrease has been also observed in other biological, such as breast milk and plasma. The current data in adipose tissue, as well as those in other biological monitors, indicate that the population living near the HWI is not particularly exposed to high levels of PCDD/Fs. However, biomonitoring studies cannot differentiate the impact of the HWI emissions from food consumption patterns. This question can be only solved by conducting complementary investigations and contrasting the results of monitoring and epidemiological studies.

Keywords: PCDD/Fs, hazardous waste incinerator, adipose tissue, temporal trend, biological monitoring.

1. Introduction

Incineration, especially without energy recovery, is not one of the most desirable methods in the EU priority hierarchy of solid waste treatment. In fact, it is located at the bottom of the current disposal alternatives, together with landfilling. In turn, prevention, reuse, recycling and recovery are considered as better options, if feasible, in the "waste hierarchy" (EC, 2010). According to data from the Eurostat (2016), 100.7 million of tons of waste were classified as hazardous waste, meaning 4% of the total waste. Hazardous waste is defined as "a waste with properties that make it dangerous or capable of having a harmful effect on human health or the environment" (US EPA, 2020). Hazardous waste is generated in a number of industrial activities, including production/management of raw chemical materials and chemical products, mining and processing of nonferrous metals ores, smelting, and petroleum processing and coking, among others (Duan et al., 2008). In 2016, in the EU-28, 51% of the hazardous waste was still landfilled, while 14% of the amount was incinerated (6% without energy recovery, and 8% with energy recovery). Socially, incineration is not well accepted socially, being an issue of great strong public opposition (Yuan et al., 2019).

In 1996-1998, a new HWI was built in Constantí (Tarragona County, Catalonia, Spain). At that time, it was the first HWI across Spain, and it is still being the only HWI in the country. Because of the important concern of the residents living nearby, a wide monitoring program was initiated before the plant started to operate in 1999 (Domingo et al., 1999; Schuhmacher et al., 1999a,b). So far, this surveillance program has been ongoing for 20 years, with periodical samplings of biological, food and environmental samples for the analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and metals (Batista et al., 1996; Nadal et al., 2008, 2013; Schuhmacher et al., 2004b, 2009, 2013; Marquès et al., 2018). Since then, biological samples have been collected every 5 years to determine any temporal trends in the exposure levels of the local population. Between 2017 and 2019, a new set of biological samples, including breast milk and plasma were collected for determining the concentrations of PCDD/Fs (Nadal et al., 2019; Schuhmacher et al., 2019), whereas samples of autopsy tissues, blood and human hair were collected for metal analysis (Esplugas et al., 2019, 2020; García et al., 2020).

In the context of this biological monitoring survey, this study was aimed at determining the temporal trends of PCDD/Fs in samples of adipose tissue collected from residents who had been living near the HWI of Constantí. Data were compared with the trends of PCDD/Fs burdens in other biological monitors (e.g., breast milk and plasma), as well as the dietary intake of these chemicals.

2. Materials and methods

2.1. Sample collection

In 2019, 15 autopsy samples of adipose tissue were collected from individuals who, at the time of death, had been living near the HWI of Constantí for, at least, the last 10 years. As in previous campaigns, the cause of death varied among the 15 study subjects, but it was unrelated to dioxin exposure, according to the current epidemiologic evidence.

Exceptionally, and given its high scientific value, a sample of a child who died at the age of 5 years was collected in order to confirm the correlation between PCDD/Fs and age. All samples were stored in polyethylene containers and immediately kept at -20ºC until the time of PCDD/Fs analyses.

Samples were collected from 14 men and 1 woman, whose age ranged from 5 to 86 years (mean: 56 years; median: 61 years). The samples were collected in collaboration with forensic doctors from the Tarragona Division of the Institute of Forensic Medicine and Forensic Sciences of Catalonia (Spain). None of the subjects had been occupationally exposed to PCDD/Fs. The study was approved by two ethical committees. On one hand, the protocol of the biological surveillance program, number 07/2017, was reviewed and approved by the Ethical Committee for Clinical Research (CEIm) of the Pere Virgili Health Research Institute (IISPV), Reus/Tarragona, Spain. On the other hand, the specific protocol for the biomonitoring of autopsy tissues, number PR164/19, was complementarily evaluated and approved by the Clinical Research Ethics Committee (CEIC) of the Bellvitge University Hospital, Barcelona, Spain.

2.2. Analytical procedure

The concentrations of PCDD/Fs were analyzed following the US EPA method 8290. Further details on the analytical treatment have been given elsewhere (Nadal et al., 2009; Schuhmacher et al., 2014). Briefly, samples were firstly dissolved in hexane (Merck, Darmstadt, Germany), and a mixture of $^{13}C_{12}$-PCDD/Fs standards was added to control for potential losses during the extraction and cleaning processes. The lipids were destroyed by the addition of acid silica. To eliminate possible interfering components, a multi-stage clean-up process was carried out. The first step consisted of cleaning with a multilayer silica column. The extract was then eluted through a basic alumina column by passing through several solvents, in order to separate the PCDD/Fs from other compounds. The PCDD/Fs fraction was collected and concentrated to dryness by a stream of nitrogen. Finally, 25 µL of C_{13}-labeled injection standards were added.

The analytical determination of PCDD/Fs was performed by high-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC-HRMS). The extract was injected into an Agilent 6890 gas chromatograph equipped with a ZB5-MS capillary column and coupled to a Waters Autospec mass spectrometer. The chromatographic process separated the 17 2,3,7,8-chlorinated PCDD/Fs congeners from the remaining compounds. Mass spectrometric parameters allowed the separation of PCDDs and PCDFs, between different degrees of chlorination, and between C_{13}-labeled and native (C_{12})-labeled congeners. The mass spectrometer measured, at a resolution of 10,000, two ions selected per congener group.

The calculation of the concentrations was performed using the corresponding level of congeners marked as internal standard, applying an automatic correction based on the potential losses occurred during the analytical process.

2.3. Data processing

Total PCDD/Fs concentrations were calculated using the most recent toxic equivalence factors (WHO-TEF) (van den Berg et al., 2006). Statistical processing of the results was performed with the SPSS 25.0 software package. First, the Levene test was applied to verify the homogeneity of the variances. Statistical significance was determined by applying the ANOVA or Kruskal-Wallis statistical test based on whether the data followed a normal probability or not, respectively. A probability less than 0.05 ($p <0.05$)

was considered as significant. For the WHO-TEQ calculations, when a congener was below its detection limit (LOD), its concentration was assumed to be equal to half of its respective detection limit (ND = 1/2 LOD). LOD values were 0.025 pg/g fat for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 2,3,4,7,8-PeCDF; 0.05 pg/g fat for 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, and 2,3,4,6,7,8-HxCDF; 0.1 pg/g fat for 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDF, and 1,2,3,4,7,8,9-HpCDF; and 0.5 pg/g fat for OCDD and OCDF. Recovery percentages ranged from 66% to 90%.

A multivariate analysis was also conducted to analyze in more detail any temporal trends on the levels of PCDD/Fs in adipose tissue, as well as potential changes of source apportionments. More specifically, a Principal Component Analysis (PCA) was executed using data collected from 1998 to 2019. This is a vastly extended method (Mari et al., 2017; Škrbić and Marinković, 2019; Lin et al., 2020), which allows to handle a great amount of data reducing them into a few variables in order to extract as much information as possible (Nadal et al., 2004). Prior to the execution of the PCA, all data were properly standardized.

3. Results and discussion

The levels of PCDD/Fs (in pg WHO-TEQ/g fat) in adipose tissue samples, obtained in autopsies of 15 individuals, are summarized in Table 1. Concentrations ranged between 0.95 and 12.95 pg WHO-TEQ/g fat, with a mean of 6.63 pg WHO-TEQ/g fat and a median of 5.30 pg WHO-TEQ/g fat. Table 1 also provides additional information on the age and sex of each subject. Samples were collected from 14 men and 1 woman, aged between 5 and 86 years at the time of death (mean: 56 years; median: 61 years). The median age was similar to that of the participants in the baseline survey (1998) and the first study after the HWI started to operate (2003), when the subjects showed a median age of 66 and 62 years, respectively (Schuhmacher et al., 1999c, 2004a) . In turn, it was slightly higher to those corresponding to the individuals of the campaigns performed in 2008 and 2013, when the subjects were 53 and 52 years old, of median, at the time of death (Nadal et al., 2009; Domingo et al., 2017).

The temporal evolution of PCDD/Fs concentrations in adipose tissue in the baseline study (1998), as well as in the sampling campaigns performed in 2002, 2007 and 2013 (Schuhmacher et al. 1999c, 2004a, 2014; Nadal et al., 2009; Domingo et al., 2017), are shown in Table 2. In the baseline survey (1998), the mean concentration of PCDD/Fs in adipose tissue was 40.1 pg WHO-TEQ/g fat (range: 24.2-72.1 pg WHO-TEQ/g fat). In 2002, the average level of PCDD/Fs was 9.89 pg WHO-TEQ/g fat (range: 1.4-36.1 pg WHO-TEQ/g fat), while in 2007, the mean concentration was 14.6 pg WHO-TEQ/g fat (range: 3.3-55.4 pg WHO-TEQ/g fat). In the previous survey, conducted in 2013, the mean concentration of PCDD/Fs in adipose tissue was 11.5 pg WHO-TEQ/g fat (range: 2.8-46.3 pg WHO-TEQ/g fat). The current (2019) values mean a non-significant decrease of 42% ($p>0.05$) compared to the immediately previous study (2013), and a statistically significant reduction of 83% ($p <0.001$) for the global period 1998-2019. The decreasing trend of PCDD/Fs concentrations in adipose tissue samples is graphically depicted in Figure 1. The concentration-time curve followed a hyperbolic trend, with data from the second (2002) to the current (2019) surveys being more than 2-fold lower than the mean level of PCDD/Fs in the baseline (1998) study. The concentration range varied from 6.63 to 14.6 pg WHO-TEQ/g fat since 2002, while the mean level in 1998 was 40.1 pg WHO-TEQ/g fat. It could indicate that PCDD/Fs concentrations in these biomonitors might have reached some kind of stasis, as already found elsewhere (Bichteler et al., 2017).

The PCDD/Fs congener profile in adipose tissue, obtained in each sampling campaign (1998, 2002, 2007, 2013 and 2019), is shown in Figure 2. Despite there were differences in the respective levels, the profiles were very similar in all the studies. In 2019, OCDD was the predominant congener, followed by 1,2,3,6,7,8-HxCDD and 1,2,3,4,6,7,8-HpCDD. Regarding PCDD/Fs homologues, PCDDs showed much higher concentrations than PCDD/Fs.

Since 14 out of the 15 samples corresponded to men, a statistical analysis of differences according to the sex could not be carried out. In contrast to previous studies (Schuhmacher et al., 2004a; Nadal et al., 2009), PCDD/Fs concentration in adipose tissue of the woman of the present study was lower than the mean levels found in 14 men (1.89 vs 7.44 pg WHO-TEQ/g fat) (Table 3). It is well established that the accumulation of PCDD/Fs in the human body increases with age (Kiviranta et al., 2005; Uemura et al., 2008; Alawi et al., 2018). In the current survey, a significant Pearson correlation ($p < 0.001$) was observed between the age and the concentrations of PCDD/Fs in adipose tissue.

In order to establish potential differences between samples, a PCA was applied to the results obtained in all the sampling campaigns (1998, 2002, 2007, 2013 and 2019). A three-dimensional model was obtained (Figure 3). The first principal component (PC1), which accounted for 53% of the variance, was related to PCDDs and hexa-PCDFs, while the second principal component (PC2), with 17% of the variance, was correlated with the lighter furans (2,3,7,8-Tetra-CDF and 1,2,3,7,8-Penta-CDF). Finally, the third principal component (PC3), which accounted for 11% of the variance, was related to the heaviest furans (1,2,3,4,7,8,9-Hepta-CDF and OCDF). Most samples from the baseline (1998) survey showed a high value of CP1 due to a higher concentration of PCDD/Fs. In contrast, all samples corresponding to the current (2019) study were grouped in a single "cluster".

Together with other biological monitors such as blood/plasma and breast milk, adipose tissue is one of the main matrices used to determine the accumulation of polychlorinated compounds in humans (Domingo et al., 2017; Jackson et al., 2017). In the last decade, only a limited number of articles focused on analyzing the burdens of PCDD/Fs in adipose tissue has been published. Moon et al. (2011) determined the concentrations and accumulation features of PCDD/Fs and dioxin-like polychlorinated biphenyls (DL-PCBs) in samples of adipose tissue collected in 2007-2008 from 53 Korean women aged 40-68. The total mean concentrations of PCDDs and PCDFs were 3.6 and 3.4 pg WHO-TEQ/g fat, being the PCDD/Fs level very similar to that reported here found for the population living near the HWI of Constantí (7.0 vs. 6.63 pg WHO-TEQ/g fat).

Most scientific investigations have been targeted on the potential association of PCDD/Fs and other dioxin-like chemicals in adipose tissue with several adverse health outcomes, including cancer, endometriosis or cardiovascular risks (Cano-Sancho et al., 2019; Ferro et al., 2019; Koual et al., 2019; Ploteau et al., 2016; Qiu et al., 2020). In France, Koual et al. (2019) recently measured the occurrence of 49 persistent organic pollutants (POPs) in adipose tissue from breast cancer patients, with or without lymph node metastasis. The median concentration of PCDD/Fs in adipose tissue from 38 women with metastatic breast cancer was 11.8 pg WHO-TEQ/g fat. However, this value was not significantly different from that observed in 53 women with non-metastatic breast cancer (11.6 pg WHO-TEQ/g fat). Notwithstanding, when analyzing individually the chemicals, it was found that 2,3,7,8-TCDD exhibited substantially higher adjusted odd ratios than the rest of PCDDs or their sum (Koual et al., 2019). The same French research group conducted a similar study focused on the associations between internal exposure levels of POPs in adipose tissue and deep infiltrating endometriosis (DIE) with or without concurrent ovarian endometrioma (Ploteau et al., 2017). Adipose tissue and serum samples were collected from surgically confirmed cases (n=55) and controls (n=44) enrolled during 2013 and 2015 in Pays de la Loire, France. The levels of POPs were determined. The median concentration of PCDD/Fs in control samples was 5.53 pg WHO-TEQ/g fat, while that in samples from women with DIE was 5.80 pg WHO-TEQ/g fat. Significantly higher concentrations were found in women with DIE and ovarian endometrioma (8.75 pg WHO-TEQ/g fat; $p<0.05$). Regarding PCDD/F congeners, significant associations between DIE and adipose tissue levels of 1,2,3,7,8-PeCDD and OCDF were noticed. This conclusion agrees with the results published by Martínez-Zamora et al. (2015), who compared the concentrations of several dioxin-like substances in adipose tissue of 30 patients with DIE and those from a control group without endometriosis (n=30). Significantly ($p<0.05$) higher levels of PCDD/Fs were observed in the DIE group (6.90 pg WHO-TEQ/g fat) as compared with the control group (6.10 pg WHO-TEQ/g fat). This difference was basically due to the significant ($p<0.01$) differences in the levels of 3 congeners: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF.

In Jordan, Alawi et al. (2018) carried out a comparative study of several POPs concentrations in cancer-affected human organs with those of healthy organs. Thirteen adipose tissue samples of healthy people were collected from Jordan University Hospital, while 33 adipose tissue samples of cancer-affected patients were collected from King Hussein Cancer Center. PCDD/Fs concentrations in adipose tissue samples for cancer-affected patients were 3 times higher than those in healthy persons, with the congener 2,3,4,7,8-PeCDFs showing the highest value. In turn, Koskenniemi et al. (2015) conducted a case–control study consisting of 44 cryptorchid cases, and 38 controls operated for inguinal hernia, umbilical hernia, or hydrocele at the Turku University

Hospital (Finland) or Rigshospitalet, Copenhagen (Denmark) in 2002–2006. During the operation, a subcutaneous adipose tissue biopsy was taken, and the content of PCDD/Fs was analyzed. In Finland, the median concentration of PCDD/Fs in adipose tissue from new-born boys affected by cryptorchidism was 7.44 pg TEQ/g fat, while that in healthy (control) boys was 5.43 pg TEQ/g fat. Substantially higher levels were found for Denmark, as median levels in cases and controls were 18.5 and 13.0 pg TEQ/g fat, respectively. In the US, Li et al. (2013) analyzed the burdens of PCDD/Fs in prostate cancer patients who underwent radical prostatectomy. This was performed in a large study focused on establishing whether the Agent Orange exposure in Vietnam veterans was associated to the biochemical recurrence. The median dioxin level in 37 Agent Orange-exposed patients was significantly higher than that in 56 unexposed patients (22.3 vs 15.0 pg TEQ/g fat; $p<0.001$). In Japan, Watanabe et al. (2013) analyzed the levels of PCDD/Fs in the liver and adipose tissue of Japanese cadavers, and their toxicokinetic in association with hepatic cytochrome P450 (CYP) 1A protein expression levels was examined. Concentrations of PCDDs and PCDFs in adipose tissue of men from Ehime Prefecture were found to be 27 and 11 pg TEQ/g fat, respectively. In women, levels were slightly lower: 21 and 7.7 pg TEQ/g fat for PCDDs and PCDFs, respectively. Summing up, PCDD/Fs concentrations in Japanese were found to be 38 and 28.7 pg TEQ/g fat in men and women, respectively. Kim et al. (2011) characterized the POPs total body burden and their redistribution in obese individuals before and after drastic weight loss. The results were compared with a variety of molecular, biological, and clinical parameters. For that purpose, adipose tissue samples were obtained from 71 obese subjects who underwent bariatric surgery, as well as from 18 lean women. Total POPs body burden was 2-3 times higher in obese individuals. However, significantly higher concentrations of PCDD/Fs were surprisingly found in adipose tissue from lean subjects compared to obese individuals (Kim et al., 2011).

In comparison to data from the scientific literature, the current mean value of 6.63 pg WHO-TEQ/g fat, found for the population living near the HWI of Constantí, is in the lower part of the range observed in most industrialized countries. It must be remarked that values of PCDD/Fs have also decreased considerably compared to those found in studies conducted in the 90s (Takenaka et al., 2002; Kiviranta et al., 2005), a trend also recorded in other biomonitors, such as blood (Consonni et al., 2012). Also in Spain, Zubero et al. (2017) found a dramatic decrease in the mean values of PCDD/Fs and other organochlorinated compounds in blood serum of people living near a municipal solid waste plant in Bilbao (Basque Country) between 2006 and 2013, with a 80% drop of the body burdens of PCDD/Fs. Campo et al. (2019) recently published a systematic review of peer-reviewed literature on human biological monitoring to exposure by individuals living near or working at solid waste incinerators. According to this extensive review, some earlier studies showed an increase of PCDD/Fs and other chemicals in individuals (mainly workers) exposed to emissions from old incinerators, while studies from the year 2000 showed no increase of biomarkers. In addition, decreasing trends were observed in prospective studies. According to Weldon and LaKind (2016), most of the available data indicate that PCDD/F levels in humans have been declining over the years in the general population as well as in populations with excessive exposure. It confirms that the decreasing trends detected in biomonitors collected from the population living near the HWI of Constantí has been observed elsewhere.

As it has been widely reported in the scientific literature, diet is the main source of human exposure to PCDD/Fs (Charnley and Doull, 2005; Llobet et al., 2008; Linares et al., 2010), with percentages of up to more than 95% of total exposure. In fact, the significant reduction in PCDD/Fs levels in adipose tissue observed in the period 1998-2019 fully agrees with the decreasing trend in the dietary intake of PCDD/Fs. In the baseline survey (1998), the daily intake of PCDD/Fs by the adult population living near the HWI of Constantí was estimated in 210.1 pg I-TEQ/day (Domingo et al., 1999). A gradual reduction was observed in subsequent surveys, being 63.8, 27.8 and 33.1 pg WHO-TEQ/day in 2002, 2006 i 2012, respectively (Bocio and Domingo, 2005; Martí-Cid et al., 2008; Domingo et al., 2012). In 2018, the intake of PCDD/Fs through the diet had decreased considerably, until reaching a value of 8.54 pg WHO-TEQ/day (González et al., 2018).

The significant decrease in the levels of PCDD/Fs in adipose tissue of residents in the area under evaluation also agrees with the significant reduction in the concentrations of the same compounds in other biological monitors evaluated in the same surveillance program: breast milk and plasma. In 2017, the mean concentration of PCDD/Fs in 20 breast milk samples was 2.26 pg I-TEQ/g fat, 81% lower than the value observed in the baseline study (11.8 pg I-TEQ/g lipid) (Schuhmacher et al., 2019). In plasma, the percentage of decrease in dioxin levels in the period 1998-2018 was 75%, being reduced from 27.0 to 6.79 pg I-TEQ/g lipid (Nadal et al., 2019). Likewise, the levels of PCDD/Fs in breast milk and adipose tissue compared to the 2013 study also decreased: 48% and 58%, respectively. In turn, the concentration of PCDD/Fs in plasma had increased by a 10% in the last period evaluated (2013-2018). The graphical comparison of the temporal evolution of PCDD/Fs in these three biological monitors, together with that on their dietary intake, is depicted in Figure 4.

4. Conclusions

After 20 years of regular operations, the concentrations of PCDD/Fs in samples of adipose tissue of people who -at the time of death- had spent (at least) the last 10 years living near the HWI, significantly decreased. This reduction follows the same trend as that previously observed in other biomonitors (e.g., breast milk and plasma) collected in 2017 and 2018 in non-occupationally exposed individuals living in the same zone. The information from this large dataset, retrieved through 20 years, is an indicator that the 3 compartments (adipose tissue, breast milk or plasma) may be good biological monitors for the follow-up of human exposure to PCDD/Fs. Therefore, and if needed for economic reasons, any of them could be even excluded from the surveillance program, and the loss of information would be minimal. The decrease in the body burdens of PCDD/Fs is also parallel to their dietary intake, which significantly reduced between 1998 and 2018 (from 210.1 pg I-TEQ/day to 8.54 pg WHO-TEQ/day). A global analysis of the data indicates that the HWI of Constantí should not have an important contribution to exposure to PCDD/Fs for the population living nearby. However, the single execution of biomonitoring studies does not allow to determine the contribution of the HWI emission on the body burdens of PCDD/Fs, as their human exposure occurs though different pathways, mostly food consumption. Therefore, it cannot be stated that the reduction in the accumulation of PCDD/Fs would have been even more pronounced if the HWI had not been built. The only way to solve this question is by conducting an epidemiological study in the area under potential influence of this HWI, as it has been strongly demanded to the local and regional authorities (Domingo et al., 2020). Furthermore, since the HWI emissions may influence locally grown products, future studies should be conducted to assess the contribution of the emissions on the inhalation and the dietary intake of local products by the local population. Finally, the next monitoring campaigns of the surveillance program should try to overcome the limitations of their study, including the use of a larger sample size, being not limited to 15. By doing this, and increasing the number of samples from women, a stratified analysis of the data will be then possible.

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Table 1. Concentrations of PCDD/Fs in samples of adipose tissue of 15 subjects who had been living near the HWI of Constantí.

No. of sample	Sex	Age (years)	pg WHO-TEQ/g fat
1	Man	86	9.69
2	Man	65	10.51
3	Man	5	0.95
4	Man	45	3.79
5	Man	42	4.44
6	Man	72	11.32
7	Woman	40	1.89
8	Man	83	9.25
9	Man	59	12.95
10	Man	85	9.96
11	Man	34	3.08
12	Man	72	8.83
13	Man	62	5.30
14	Man	61	4.74
15	Man	35	2.82
Mean		56	6.63
St. Dev.		23	3.87
Median		61	5.30
Min		5	0.95
Max		86	12.95

Table 2. Mean levels of 17 PCDD/Fs congeners (in pg/g fat) in samples of adipose tissue from 15 subjects who had been living near the HWI of Constantí.

Congener	1998	2002	2007	2013	2019
2,3,7,8- Tetra-CDD	4.13 ± 3.03[a]	1.39 ± 1.53[b]	1.68 ± 1.86[b]	1.24 ± 1.14[b]	0.71 ± 0.31[b]
1,2,3,7,8 Penta-CDD	11.37 ± 4.74[a]	3.73 ±3.51[b,c]	5.28 ± 4.80[b]	4.11 ± 3.48[b,c]	2.41 ± 1.35[c]
1,2,3,4,7,8 Hexa-CDD	5.61 ± 2.86[a]	2.78 ± 1.73[a]	3.30 ± 3.61[a,b]	2.26 ± 2.26[b,c]	1.14 ± 0.74[c]
1,2,3,6,7,8 Hexa-CDD	59.4 ± 30.2[a]	19.2 ± 18.9[b]	28.1 ± 29.3[b]	25.3 ± 26.6[b]	15.3 ± 16.5[b]
1,2,3,7,8,9 Hexa-CDD	8.12 ± 6.45[a]	2.08 ± 2.03[b]	3.55 ± 4.47[b]	2.63 ± 4.37[b]	1.22 ± 1.06[b]
1,2,3,4,6,7,8-Hepta-CDD	84.9 ±60.9[a]	10.2 ± 8.0[b]	20.0 ± 28.9[b,c]	20.3 ± 52.9[b,c]	4.83 ± 4.55[c]
OCDD	477 ± 320[a]	53.6 ± 51[b,c]	152 ± 188[b]	113 ± 196[b,c]	42.9 ± 41.4[c]
2,3,7,8-Tetra-CDF	0.94 ± 0.58[a]	0.34 ± 0.40[b,c]	0.40 ± 0.40[b]	0.35 ± 0.27[b,c]	0.18 ± 0.09[c]
1,2,3,7,8-Penta-CDF	0.92 ± 0.47[a]	0.50 ± 0.45[a]	1.40 ± 1.33[a,b]	0.31 ± 0.26[b]	0.12 ± 0.07[c]
2,3,4,7,8-Penta-CDF	21.1 ± 11.5[a]	5.71 ± 5.95[b,c]	9.94 ± 9.43[b]	7.43 ± 6.93[b,c]	4.43 ± 2.24[c]
1,2,3,4,7,8-Hexa-CDF	7.0 2± 3.33[a]	2.32 ± 1.75[b,c]	3.29 ± 3.25[b]	2.83 ± 2.97[b,c]	1.31 ± 0.67[c]
1,2,3,6,7,8-Hexa-CDF	8.22 ± 3.99[a]	2.03 ± 1.86[b]	3.31 ± 3.47[b]	2.69 ± 2.86[b]	1.46 ± 0.95[b]
1,2,3,7,8,9-Hexa-CDF	0.62 ± 0.35[a]	0.39 ± 0.41[b,c]	0.06 ± 0.05[b]	0.07 ± 0.05[b]	0.45 ± 0.27[c]
2,3,4,6,7,8-Hexa-CDF	2.2 ± 1.28[a]	0.38 ± 0.44[a]	0.88 ± 0.73[a]	0.69 ± 1.15[a]	0.06 ± 0.07[b]
1,2,3,4,6,7,8-Hepta-CDF	4.81 ± 2.17[a]	2.06 ± 0.65[a]	2.99 ± 2.54[a,b]	2.34 ± 3.13[b,c]	1.07 ± 0.69[c]
1,2,3,4,7,8,9-Hepta-CDF	0.39 ± 0.1[a]	0.31 ± 0.52[a,b]	0.10 ± 0.06[b]	0.11 ± 0.05[b]	0.13 ± 0.13[b]
OCDF	0.72 ± 0.27[a]	2.59 ± 1.27[b]	0.49 ± 0.30[a,c]	0.31 ± 0.15[a,c]	0.39 ± 0.36[c]
WHO-TEQ	40.1 ± 12.7[a]	9.89 ± 9.27[b]	14.6 ± 14.2[b]	11.5 ± 11.1[b]	6.63 ± 3.86[b]

Data given as mean ± standard deviation.

[a,b]*Different superscripts indicate that the difference between years (columns) is statistically significant (p <0.05).*

Table 3. Concentration of PCDD/Fs (in pg WHO-TEQ/g fat) in 15 adipose tissue samples according to the sex of subjects.

	Number of samples					Mean ± Standard deviation				
	1998	2002	2007	2013	2013	1998	2002	2007	2013	2019
Total	15	15	15	15	15	40.1 ± 12.7	9.9 ± 9.3	14.6 ± 14.2	11.5 ± 11.1	6.63 ± 3.86
Sex										
Men	10	11	11	9	14	35.8 ± 8.0	7.2 ± 3.5	11.2 ± 8.1	9.3 ± 17.9	7.44 ± 3.49
Women	5	4	4	6	1	48.7 ± 16.8	17.4 ± 16.1	23.8 ± 23.9	18.5 ± 5.9	1.89

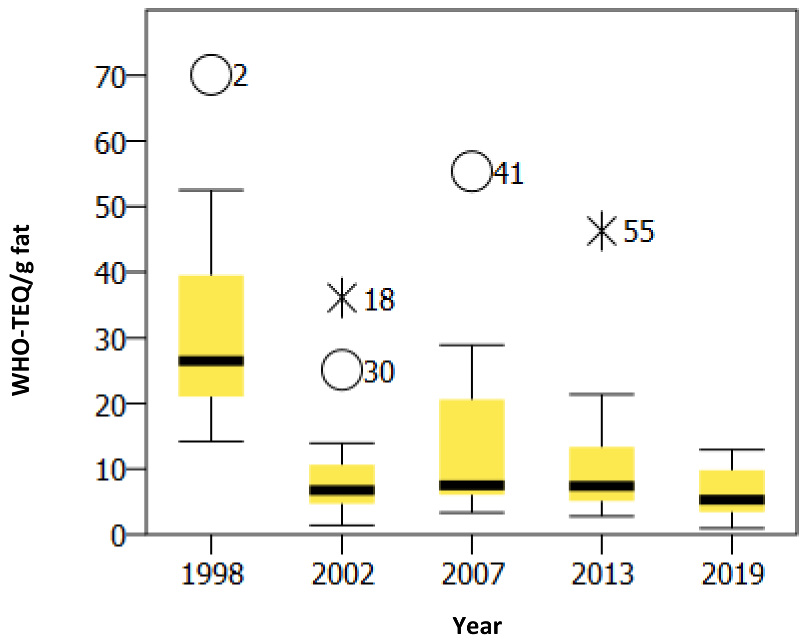

Figure 1. Boxplot of PCDD/Fs concentrations in adipose tissue samples obtained from autopsies of individuals who had been living near the HWI of Constantí.

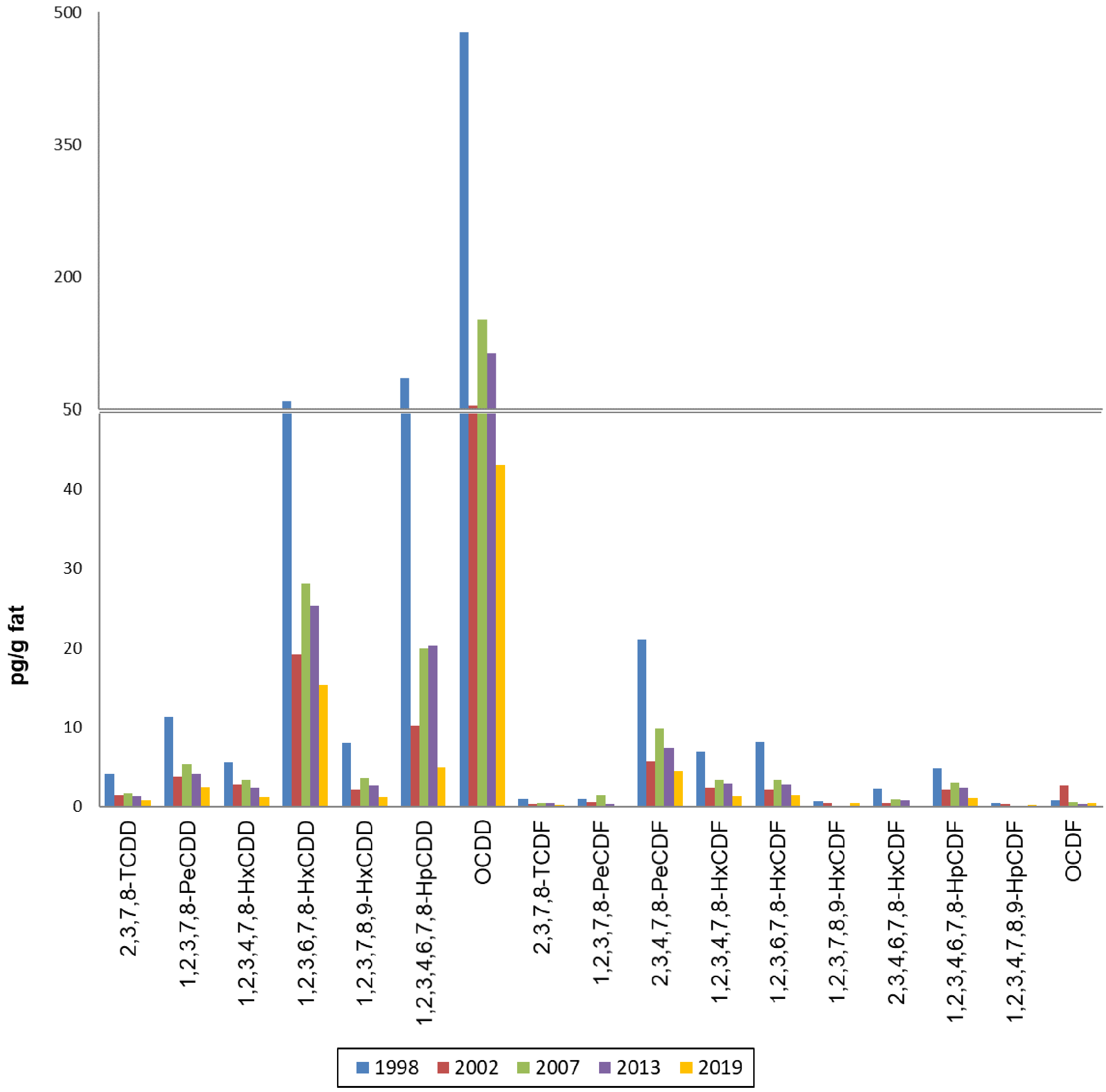

Figure 2. PCDD/Fs congener profiles in samples of adipose tissue from subjects who had been living near the HWI of Constantí.

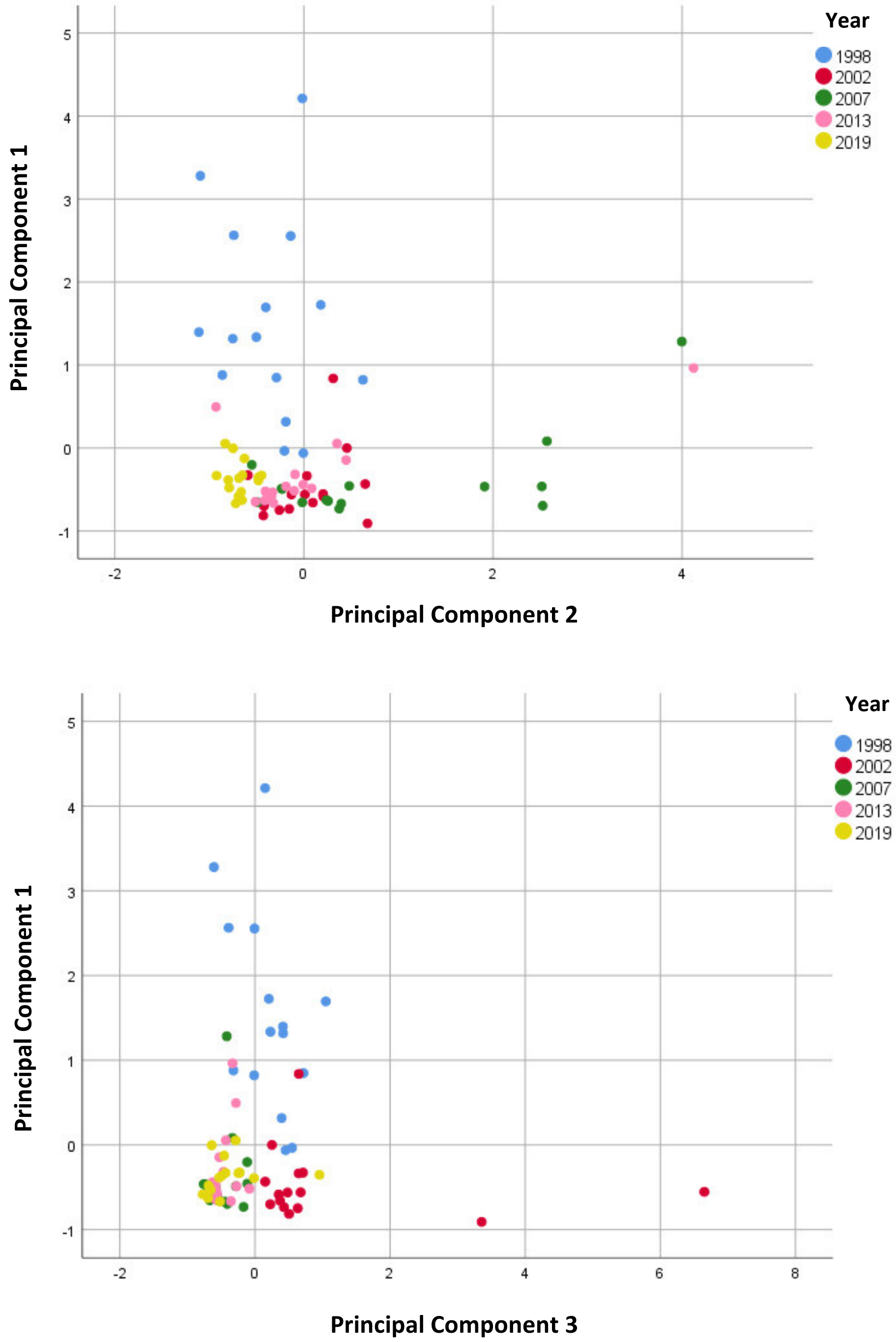

Figure 3. Principal Component Analysis applied to PCDD/Fs concentrations in adipose tissue.

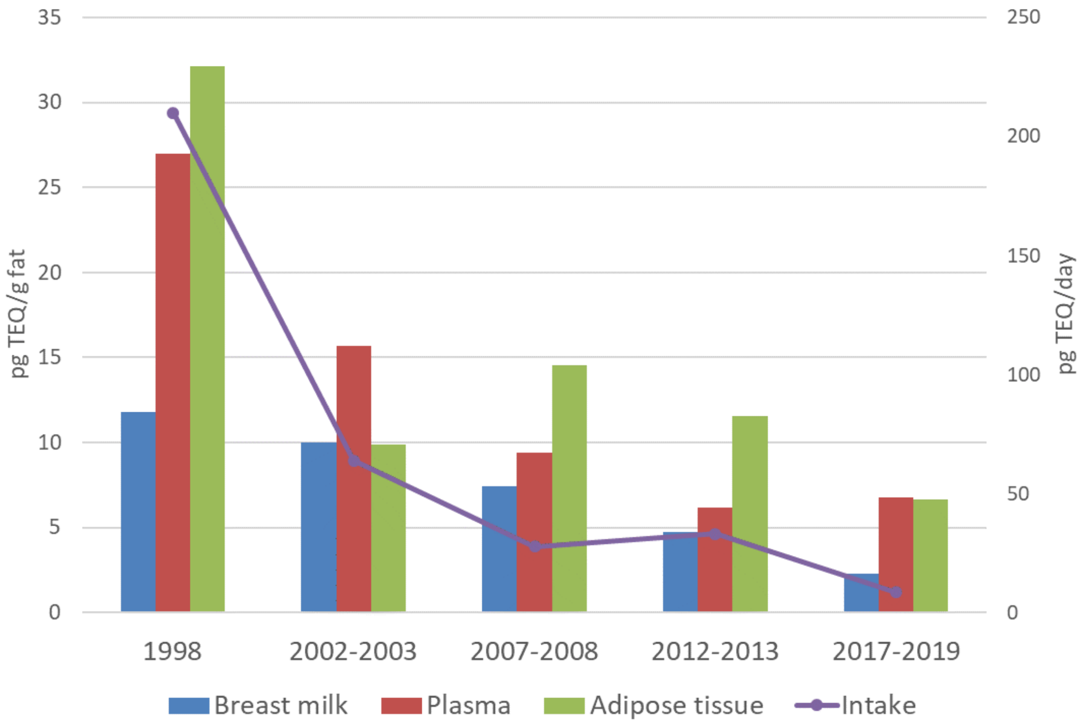

Figure 4. Temporal trend of PCDD/Fs concentrations in 3 biological monitors (in pg TEQ/g fat) and comparison with the dietary intake (in pg TEQ/day) by the adult population living near the HWI of Constantí. Sources: breast milk, Schuhmacher et al. (2019); plasma, Nadal et al. (2019); dietary intake, González et al. (2018).