Autopsy tissues as biological monitors of human exposure to environmental pollutants. A case study: concentrations of metals and PCDD/Fs in subjects living near a hazardous waste incinerator

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Abstract

Human biomonitoring is of tremendous importance to prevent potential adverse effects derived from human exposure to chemicals. Blood and urine are among the biological monitors more frequently used. However, biological matrices such as breast milk, hair, nails, saliva, feces, teeth, and expired air are also often used. In addition, and focused mainly on long-term exposure, adipose tissue and other human tissues like bone, liver, brain or kidney, are also used as biological monitors of certain substances, especially for long-term biomonitoring. However, for this kind of tissues sampling is always a limiting factor. In this paper, we have examined the role of autopsy tissues as biological monitors of human exposure to environmental pollutants. For it, we have used a case study conducted near a hazardous waste incinerator (HWI) in Catalonia (Spain), in which the concentrations of metals and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), have been periodically determined in autopsy tissues of subjects living in the area under potential influence of the facility. This case study does not show advantages - in comparison to other appropriate biomonitors such as blood- in using autopsy tissues in the monitoring of long-term exposure to metals and PCDD/Fs.

Key words: human biomonitoring; autopsies; adipose tissue; metals; PCDD/Fs.

1. Introduction

Human biomonitoring (HBM) is a method for the protection of human health due to exposure to chemical compounds –including environmental pollutants- by controlling the amounts taken up (Angerer et al., 2007; Morello-Frosch et al., 2015). For HBM, biological materials should be easily accessible in sufficient quantities under routine conditions and without unacceptable discomfort and/or health risks for the involved subjects. Among different biological matrices, urine and blood have been -and remain- the most commonly used (Esteban and Castaño, 2009; Forde et al., 2014; Lopez-Herranz et al., 2016; Wilhelm et al., 2008; Lupsa et al., 2015). Biological matrices such as hair, expired air/breath and breast milk, or even teeth, nails and saliva/sputum, are also used with some frequency (Alves et al., 2014, 2016; Andra et al., 2015; Barton, 2011; Corradi et al., 2015; Esteban et al., 2015; Kucharska et al., 2015; Smolders et al., 2015; Rebelo and Caldas, 2016). In contrast, for ethical and practical reasons, other human tissues like liver, kidney, brain, etc, are much less used since they require invasive sampling. However, for biological monitoring of human exposure to certain pollutants for long periods of time, tissues such as liver, kidney, muscle, bone, etc, may even be more appropriate monitors than blood or urine (i.e., for measuring trace elements), while adipose tissue would be a suitable monitor for organic pollutants.

With respect to this tissue, it has been used in a relatively limited number of studies in order to determine the concentrations of various environmental contaminants in samples obtained of populations from Belgium, Japan, USA, Mexico and Sweden. polybrominated diphenyl ethers (PBDEs) (Covaci et al., 2002, 2008; Johnson-Restrepo et al., 2005; Meyronté Guvenius et al., 2001; Naert et al., 2006), polychlorinated biphenyls (PCBs) (De Saeger et al., 2005; Covaci et al., 2002; Johnson-Restrepo et al., 2005; Naert et al., 2006; Choi et al., 2002; Takenaka et al., 2002), and organochlorine pesticides (OCPs) (Chu et al., 2003; Covaci et al., 2002; Herrero-Mercado et al., 2010; Waliszewski et al. 2011, 2012). The levels of PCBs and OCPs have been also determined in other autopsy tissues such as lungs in subjects from Greece (Rallis et al., 2014), and in muscle, liver, brain and kidney of individuals from Belgium (Chu et al., 2003). Moreover, Meyronté Guvenius et al., (2001), also determined the concentrations of PBDEs in samples of liver obtained from autopsies of Swedish individuals. The levels of a number of perfluoroalkyl substances (PFASs) were also analyzed in various autopsy tissues (brain, liver, lung, bone and kidney) collected from Spanish subjects (Kärrman et al., 2010; Pérez et al., 2013). On the other hand, and regarding metals, Vallascas et al. (2013) analyzed the relationship between lead levels and adipose tissue in 759 children between 11 and 15 years, residents in various municipalities of Sardinia, using hair lead levels as the biomarker of exposure. The results suggested that organic lead tended to accumulate in adipose tissue, and therefore, this tissue could be considered a possible new biological matrix for the evaluation of environmental lead exposure.

This paper is focused on examining the use of autopsy tissues as biological monitors of human exposure to environmental pollutants. It is mainly based on a case study conducted near a hazardous waste incinerator (HWI) in Catalonia (Spain), in which the concentrations of metals, and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in autopsy tissues of subjects living in the area under potential influence of the facility have been periodically determined. The case study covers since 1996-1998, period during which the HWI was being constructed, to 2013 when the last sampling was carried out.

2. Case study

For much of the 20th century, hazardous waste (HW), municipal solid waste (MSW) and other kinds or waste, were dumped indiscriminately, being uncontrollably burned or even being buried irresponsibly. Among various possible options, incineration has demonstrated to be a commercially available technology for HW, MSW and medical waste disposal, being a responsible, environmentally secure manner of treating these kinds of wastes. Thus, regulator organizations have considered incineration as a strategic option for waste reduction and disposal. Nevertheless, not everything has been favorable in relation to waste incineration. In comparison with other treatments for processing HW or MSW, incineration has considerable advantages such as volume reduction, energy recovery, elimination of pathogen agents, etc. However, as for other waste management facilities, incinerators have been traditionally affected by the NIMBY (Not In My Back Yard) syndrome. Consequently, the public opposition to the siting and permitting of MSW and HW incinerators has been important from the outset. With respect to HW incinerators (HWI), the main concern is related with the environmental and health consequences potentially derived from the stack emission of a number of inorganic and organic substances, mainly metals, semivolatile and volatile compounds, being the greatest amount of scientific and public attention given to PCDD/Fs (Sedman and Esparza, 1991; Domingo, 2002; Ferré-Huguet et al., 2006).

During 1996–1998, a new HWI was constructed in Constantí (Tarragona County, Catalonia, Spain), whose regular operations started in 1999. That HWI was the first, and up to now, the only HWI in Spain. Due to the potential exposure to metals and PCDD/Fs for the general population living near the new facility, the concern about its environmental impact and health risks was very important. Consequently, in order to evaluate the human health risks, a biological monitoring program was designed during the stage of construction of the HWI. In a baseline study (1996-1998), samples of human blood, hair, and autopsy tissues (brain, lung, kidney, bone, and liver) were obtained from subjects living in the neighborhood of the facility and the concentrations of arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), tin (Sn), thallium (Tl), and vanadium (V) were analyzed. (Granero et al., 1998; Llobet et al., 1998a,b). Moreover, samples of human blood, breast milk and adipose tissue from the population living in the neighborhood of the HWI were also collected, being analyzed for determining the baseline levels of PCDD/Fs (Schuhmacher et al., 1999a,b,c). While urine and blood are widely used in HBM, due to the complexity of the sampling, the number of studies aimed at measuring the concentrations of metals and PCDD/Fs are much more limited. However, in general terms blood and urine –and also hair or breast milk, for example- reflect better a rather short-exposure to environmental pollutants, while tissues such as kidney, liver, bone or fat may be better indicators of a long-term exposure. We here summarize the results of our surveillance program regarding the levels of metals and PCDD/Fs in autopsy tissues of subjects living in the area under potential influence of the emissions of the HWI. The results have been compared with data taken from the scientific literature.

2.1.Metals

During 1996, samples of brain (frontal lobe), bone (right rib), kidney (cortex), liver and lung were obtained from 20 autopsied subjects who had lived in Tarragona County at least during the 10 years previous to their deaths. The reasons for the autopsies were always medico-legal. Sex, age, occupational and residential information, and drinking and smoking habits were recorded. No occupational exposure to metals was registered for any of the subjects. Samples with gross morphological changes indicating chronic hepatitis, cirrhosis, pyelonephritis, nephritis, renal cysts, tumors or abscesses were excluded, as well as those showing risk of contamination of the respective tissues (e.g., an open cavity or putrefaction initiated). The concentrations of As, Be, Cd, Cr, Mn, Hg, Ni, Pb, Tl, Sn, V and zinc (Zn) were determined in all samples (Llobet et al., 1998b). Beryllium, Tl, and V were under the respective detection limits. Bone showed the highest concentrations of As, Cr, Ni, Pb, Sn, and Zn. In turn, the highest levels of Cd and Hg were found in kidney, while the highest Mn concentrations were observed in liver. No significant differences in metal concentrations (with the exception of Cd, Mn, and Zn in lung) were noted in relation to the age of the subjects. In that baseline survey, the concentrations of the selected metals in tissues of individuals from Tarragona County were in the normal range based on commonly reported levels in previous surveys. In a subsequent study (García et al., 2001), which was conducted just before the HWI started (1999) regular operations, again we collected samples of liver, lung, kidney, brain and bone from a total of 78 non-occupationally exposed subjects, autopsied between 1997 and 1999. Most tissue levels of As, Co, Cr, Hg and V were near to the analytical detection limit, being even lower in some cases. The results of that second survey showed that tissue metal concentrations did not significantly change due environmental and/or a dietary exposure in the population living in the surroundings of the HWI, while it was being constructed.

In 2003, we conducted the first study aimed at establishing if any potential increase in the concentrations of metals in tissues of subjects living in the vicinity of the HWI, could be due to the environmental emissions of these elements by the facility (Bocio et al., 2005a). Based on the same experimental procedure than that followed in the baseline survey (Llobet et al. 1998a), samples of autopsy tissues were obtained from 22 individuals who at the time of death had been living, during at least the last 10 years, in zones of Tarragona County near the HWI. Samples of brain (frontal lobe), bone (right rib), kidney (cortex), liver, and lung were collected. A decrease in the levels of most metals was generally noted, being the differences statistically significant in brain for Cd, Mn, Pb, and Sn. In turn, Cr, Pb, and Sn also showed significant reductions in bone, while in contrast Cd and Ni levels showed a slight increase. Mn and Sn levels were significantly reduced in kidney between 1998 and 2003, while a similar tendency was also noted in liver for Pb and Sn. Finally, Ni levels in lung increased significantly, but a general decrease in lung was observed for the rest of analyzed elements. The results of that survey led to the conclusion that the emissions of metals by the HWI were not meaning an additional exposure to these elements for the population living in the neighborhood.

In 2007, and during 2012-2013, two new studies were conducted. Autopsy tissues (brain, bone, kidney, liver, and lung) were obtained from 20 subjects (in both studies), who at the time of their deaths, had lived in the area around the HWI during at least the last 10 years. The experimental procedures were the same than those in the previous surveys (Llobet et al., 1998a; García et al., 2001; Bocio et al., 2005). The results of both studies were compared with those of the baseline survey (Llobet et al., 1998a). With respect to the results of the 2007 survey (unpublished data), in general terms, metal levels were similar -or lower- than the concentrations found in the baseline survey. However, a number of differences (increases and decreases) were noted in some tissues for the levels of certain metals between the baseline (Llobet et al., 1998a) and the 2014 (Marí et al., 2014) surveys. Interestingly, in 2013, As, Be, Ni, Tl, and V showed levels below their corresponding detection limits. The levels of metals in samples of brain, bone, kidney, liver, and lung of autopsy tissues in the baseline (1998) and 2003, 2007 and 2013 surveys, as well as the temporal trends, expressed as variation percentage between mean values, are summarized in Table 1. Between 1998 and 2013, the highest increase corresponded to Cr in kidney (633%), bone (171%), brain (159%), liver (154%) and lung (94%). In contrast, the most important decrease corresponded to Pb in lung (98%), liver (93%) and bone (65%). These changes could be due to the relatively limited sample size, which would generate some fluctuations. However, as dietary exposure is the main source of human exposure to metals, the changes in the dietary intake of these elements could also contribute significantly to the changes in the metal levels found in the autopsy tissues.

In relation to this, we also determined the dietary exposure to the above-indicated metals by the population living near the HWI, during the period of construction of the facility. The concentrations of the same metals were measured in foodstuffs widely consumed in the area under evaluation. With those results, and based also on the consumption habits, we determined the human dietary intake of the metals, which was considered as “baseline” for our purposes (Llobet et al., 1998c). According to the objectives of the surveillance program on the health risks of the HWI, the dietary intake of metals was again estimated in 2003 (Bocio et al., 2005b), 2006 (Martí-Cid et al., 2009) and 2013 (Perelló et al., 2015). In the 2013 study (Perelló et al., 2015), the intakes of As, Cd, Cr, Hg, Mn, Ni, Pb, Sn, and V for the adult population living in the area under evaluation were 265, 33.9, 695, 8.5, 2120, 180, 41.7, 40.1, and 24 µg/day, respectively. These intakes were similar (even some of them were lower) than those we found in the 2006 survey (Martí-Cid et al., 2009). However, the dietary intakes of Cr and Hg, progressively and significantly increased with respect to those of the baseline study (139 and 4.8, for Cr and Hg, respectively) (Llobet et al., 1998c). These considerable increases in the intakes of Cr (695.5 vs. 139 µg/day) and Hg (8.5 vs. 4.8 µg/day) are in accordance with the important increases also noted in the levels of Cr and Hg in some autopsy tissues. No relevant increases in the human dietary exposure of the rest of the analyzed trace elements were observed. Therefore, the increases in the tissue levels of Cr and Hg should be attributable to the dietary intakes rather than to the metal emissions from the HWI.

2.2.PCDD/Fs

PCDD/Fs are also among the substances emitted by incinerators that have generated most concern between the public opinion, and consequently, a more great interest in the scientific community. In the current case study, the surveillance program included also the analyses of PCDD/Fs in blood, breast milk, and adipose tissue of individuals living in the surroundings of the facility. As for metals, the dietary intake of PCDD/Fs was also estimated through the time. The PCDD/F concentrations in adipose tissue samples collected in the the baseline (1998) and subsequent (2003, 2007 and 2013) surveys, are listed in Table 2, together with values according to age and gender. In the baseline study (1996-1998), samples of adipose tissue were obtained from a total of 15 autopsies of individuals, who at the time of death had lived in the city of Tarragona and surroundings during at least the last 10 years. There was no known occupational exposure to PCDD/Fs for any of the subjects (Schuhmacher et al., 1999c). PCDD/F concentrations ranged between 13.4 and 69.4 pg I-TEQ/g fat, being the mean and median values 31.0 and 26.3 ng I-TEQ/kg fat, respectively (32.1 and 26.7 ng WHO-TEQ/kg fat, respectively). At that time, baseline concentrations were of the same order of magnitude than the levels of PCDD/Fs in adipose tissue reported for various industrialized countries.

The main goal of the second study of this series was to determine the concentrations of PCDD/Fs in adipose tissue of individuals living near the HWI, after 3 years of regular operations in the facility. During 2002, 15 adipose tissue samples were obtained from autopsies of individuals who –as in all the studies belonging to our surveillance program on this HWI- at the time of death had lived in the city of Tarragona and surroundings (zones under potential influence of the HWI) during at least the last 10 years. All samples were collected from the same body compartment (abdominal adipose tissue). The individual levels of PCDD/Fs ranged between 1.5 and 41 pg WHO-TEQ/g fat), being the mean and median values 11.0 and 7.4 pg WHO-TEQ/g, respectively (Schuhmacher et al., 2004). In comparison with the baseline study, the mean concentration of PCDD/Fs decreased by 70%. This notable reduction was in agreement with the important decrease also noted during those years in the dietary intake of PCDD/Fs by the population of the area, which diminished from 210 pg I-TEQ/day in 1998 (Domingo et al., 1999) to 60 pg I-TEQ/day (Bocio and Domingo, 2005). In 2007, after approximately 9 years of regular operations in the HWI, again we measured the concentrations of PCDD/Fs in samples of adipose tissue of 15 autopsied subjects living in the area under potential impact of the facility (Nadal et al., 2009). As for the previous studies, there was no occupational exposure to PCDD/Fs for any of these individuals. The mean PCDD/F concentration was 14.6 pg WHO-TEQ/g fat (with individual values ranging between 3.3 and 55.4 pg WHO-TEQ/g fat), higher than the mean concentration found in the previous (2002) survey, 11.0 pg WHO-TEQ/g fat (Schuhmacher et al., 2004). However, the increase noted during those 5 years was not directly attributed to the exposure to PCDD/Fs emitted by the facility, as other biological monitors such as human plasma (Nadal et al., 2008) and breast milk (Schuhmacher et al., 2009), also included in the surveillance program, decreased during the same period. In addition, the 2007 mean value (Nadal et al., 2009) was still notably lower than that corresponding to (30.98 pg I-TEQ/g fat) the baseline study (Schuhmacher et al., 1999c).

The last study of this series was conducted in order to determine, once again, the concentrations of PCDD/Fs in 15 samples of adipose tissue (collected in 2012-2013) of individuals living in the neighborhood of the HWI, 14 years after the facility started its regular operations. The results were compared with those from our previous surveys carried out just before (baseline study) and after (2002 and 2007) the HWI began to operate. (Schuhmacher et al., 2014). In that last survey, we found mean and median PCDD/F concentrations of 11.5 and 7.4 pg WHO-TEQ/g fat, respectively, with the individual levels ranging between 2.8 and 46.3 pg WHO-TEQ/g fat. It meant an important reduction (64%) with respect to the baseline mean level (30.98 pg I-TEQ/g fat) (Schuhmacher et al., 1999c). However, during the period 2002–2013 no significant differences in the mean PCDD/F levels could be observed. Although an overall assessment of the data shows a similar decreasing trend of PCDD/F burdens in the three biomonitors (plasma, breast milk, and adipose tissue) used in our surveillance program of the facility, the profiles are slightly different, depending on the analyzed tissue. Thus, 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, but higher than that of the last study (2012–2013). In contrast, the most important decrease in the levels of PCDD/Fs in adipose tissue was detected in the period 1998 (baseline)–2002, but no significant changes were noted since then. This would indicate that adipose tissue is acting more as a storage compartment in comparison to plasma and breast milk. Therefore, and based on the set of all these results for this specific surveillance program, it was concluded that the levels of PCDD/Fs in plasma are a better biological indicator of exposure to these organic pollutants than the concentrations in adipose tissue.

3. Discussion and conclusions

With respect to the levels of metals in human tissues, recently we summarized the concentrations in non-occupationally exposed individual from a number of countries in various tissues (bone, brain, kidney, liver and lung) (Mari et al., 2014). Most data corresponded to the toxic elements Cd, Hg and Pb. Differences among the levels of trace elements are considerable, which could be expected given also the important differences in the exposure characteristics of the subjects included in the studies. It cannot be forgotten that human exposure to metals occurs mainly through the diet (Linares et al., 2010). The results of the case study here examined show that, in general terms, the analyzed tissues may be appropriate monitors for HBM. However, there were no significant advantages with respect to other biological tissues also included in the surveillance program of the HWI, such as blood and hair, tissues with a sampling process much easier, and without the ethical problems that entail the collection of autopsy tissues with a certain regularity.

Regarding the concentrations of PCDD/Fs in adipose tissue, the best human tissue to measure the accumulation of liposoluble compounds, data from the scientific literature are much more limited than those corresponding to metals in human tissues. Most of the first surveys were conducted in Japan. Thus, Iida et al. (1999) investigated the concentrations of PCDD/Fs in various human tissues and also in blood obtained from the general population of Japan. In adipose tissue, all PCDD/F congeners had relatively good correlations to those in blood. Therefore, it was concluded that the congener concentrations in human blood might be useful for estimating the congener levels in adipose tissue. In turn, Takenaka et al. (2002) determined the concentrations of PCDD/Fs in samples of liver and adipose tissue of Japanese subjects. A mean total level of 49 pg TEQ/g (lipid basis) was reported for PCDD/Fs in adipose tissue (57 pg/g in liver). Also in Japan, Choi and co-workers (2002) measured the levels of PCDD/Fs in samples of human adipose tissue. Temporal trends were assessed by comparing data obtained in 1970-1971, and 2000. Mean TEQ levels showed a significant decrease from 31.6 pg-TEQ/g fat wt in 1970-1971 to 11.9 pg-TEQ/g fat wt in 2000. In recent years, the concentrations of PCDD/Fs in samples of human adipose tissue have been also determined by Cok et al. (2007, 2008) in Turkey, La Rocca et al. (2008) in Italy, Lopez-Espinosa et al. (2008) in Southern Spain, Shen et al. (2009) in China, and Moon et al. (2011) in Korea. The range of PCDD/F concentrations was very wide depending obviously on the characteristics of each of those studies. Data on PCDD/Fs in samples of adipose tissue from a number of investigations are summarized in Table 3.

Returning to our case study, the mean concentrations of PCDD/Fs in blood and adipose tissue obtained in the baseline study (1998) (Schuhmacher et al., 1999a,c), as well as in the three surveillance studies conducted in 2002, 2007 and 2013 (Nadal et al., 2009; Schuhmacher et al., 2004, 2014) are depicted in Figure 1. As above commented, among 1998 and 2013, a significant decrease in the dietary intake of PCDD/Fs by the population living in the neighborhood of the HWI was noted. As the diet is usually the main route of human exposure to PCDD/Fs (Domingo and Bocio, 2007; Linares et al., 2010), it seems obvious that the blood levels of these pollutants reflected much better the important decrease than the concentrations in adipose tissue. On the other hand, between 1999 and 2013 the emissions of PCDD/Fs from the stack of the facility remained rather similar, and always under the legal limit of 0.1 ng/Nm^3. Therefore, the changes noted in the body burden of PCDD/Fs were not due to air/dermal exposure to the PCDD/Fs emitted by the HWI. Based on these data, and considering also that the collection of human adipose tissue from autopsies (also in general *in vivo*), is much more complicated that the collection of samples of human blood, we conclude that blood is a more appropriate monitor for conducting follow-up studies of facilities emitting persistent organic pollutants such as PCDD/Fs.

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Table 1
Mean concentrations (in µg/g wet wt.) in samples of autopsy tissues from subjects who had been living in the vicinity of the hazardous waste incinerator of Tarragona (Catalonia, Spain) between 1998 and 2013. Temporal trends [Adapted from Mari et al., 2014].

	Brain				% of variation		Bone				% of variation	
	1998	2003	2007	2013	1998-2013	2007-2013	1998	2003	2007	2013	1998-2013	2007-2013
As	<0.05	<0.05	<0.05	<0.05	-	-	0.06	<0.05	0.19	<0.05	-	-
Be	<0.02	<0.05	<0.03	<0.05	-	-	<0.02	<0.05	0.03	<0.05	-	-
Cd	0.03	0.02	0.32	<0.025	-	-	0.04	0.05	0.04	<0.025	-	-
Cr	0.22	<0.25	0.45	0.57	159^{***}	27^{***}	0.51	<0.25	1.39	1.38	171^{***}	-1
Hg	<0.05	<0.05	0.10	<0.05	-	-	<0.05	<0.05	0.05	<0.05	-	-
Mn	0.22	0.03	0.24	0.33	50^{***}	38^{***}	0.06	<0.03	0.25	0.13	117^{***}	-48
Ni	<0.01	<0.10	0.36	<0.025	-	-	0.64	1.16	1.53	<0.025	-	-
Pb	1.41	0.06	0.10	<0.025	-	-	3.99	2.11	2.66	1.39	-65^{***}	-48
Sn	1.32	0.09	0.03	<0.05	-	-	7.40	0.34	0.31	0.17	-98^{***}	-45^{***}
Tl	<0.02	<0.01	<0.01	<0.025	-	-	<0.02	<0.01	<0.01	<0.025	-	-
V	<0.12	<0.25	0.28	<0.25	-	-	<0.12	<0.25	<0.25	<0.25	-	-

	Kidney				% of variation		Liver				% of variation		Lung				% of variation	
	1998	2003	2007	2013	1998-2013	2007-2013	1998	2003	2007	2013	1998-2013	2007-2013	1998	2003	2007	2013	1998-2013	2007-2013
As	<0.05	<0.05	0.06	<0.05	-	-	<0.05	<0.05	0.07	<0.05	-	-	<0.05	<0.05	0.14	<0.05	-	-
Be	<0.02	<0.05	<0.03	<0.05	-	-	<0.02	<0.05	<0.03	<0.05	-	-	<0.02	<0.05	<0.03	<0.05	-	-
Cd	17.52	17.46	14.72	21.15	21	44	0.95	1.36	0.80	1.38	45	73	0.42	0.18	0.27	0.26	-38	-4
Cr	0.09	<0.25	0.42	0.66	633^{***}	57^{***}	0.26	<0.25	0.63	0.66	154^{***}	5^{*}	0.33	0.25	0.58	0.64	94^{***}	10
Hg	0.33	0.23	0.30	0.15	-55^{**}	-50	0.20	0.14	0.14	<0.05	-	-	<0.05	<0.05	<0.05	<0.05	-	-
Mn	1.01	0.74	0.78	1.09	8	40^{**}	1.28	1.07	0.99	1.45	13	46^{*}	0.13	0.04	0.30	0.21	62^{**}	-30
Ni	<0.01	<0.10	<0.05	<0.025	-	-	0.09	<0.1	0.07	<0.025	-	-	0.08	0.12	0.07	<0.025	-	-
Pb	<0.02	0.06	0.77	0.10	-	-87^{***}	2.56	0.30	0.35	0.18	-93^{***}	-49^{**}	2.27	0.13	0.08	0.05	-98^{***}	-38
Sn	1.66	0.17	0.05	<0.05	-	-	5.06	0.19	0.07	<0.05	-	-	2.16	0.20	0.07	<0.05	-	-
Tl	<0.02	<0.01	<0.01	<0.025	-	-	<0.02	<0.01	<0.01	<0.025	-	-	<0.02	<0.01	<0.01	<0.025	-	-
V	<0.12	<0.25	<0.25	<0.25	-	-	<0.12	<0.25	<0.25	<0.25	-	-	<0.12	<0.25	0.58	<0.25	-	-

N= 20. Statistically significant differences at: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 2

Total concentrations of PCDD/s (in pg WHO-TEQ/g fat) in adipose tissue of individuals who had been living in the vicinity of the hazardous waste incinerator of Tarragona (Catalonia, Spain) between 1996-1998 and 2013, according to gender and age [partly adapted from Schuhmacher et al., 2014]

	Number of samples				Mean				Standard deviation			
	1996-1998	2002	2007	2013	1996-1998	2002	2007	2013	1996-1998	2002	2007	2013
Total	15	15	15	15	32.1	9.9	14.6	11.5	15.3	9.3	14.2	11.1
Gender												
Men	10	11	11	9	25.3	7.2	11.2	8.3	9.4	3.5	8.1	5.3
Women	5	4	4	6	45.7	17.4	23.8	16.4	16.7	16.1	23.9	15.9
Age												
<40	4	4	5	3	27.2	5.5	5.3	3.6	16.7	2.2	1.8	1.3
40-60	-	3	4	8	-	7.8	9.5	9.3	-	1.9	5.3	4.9
>60	11	8	6	4	33.9	15.0	25.7	21.9	11.1	13.2	17.0	17.4

Table 3

PCDD/F concentrations in adipose tissue reported in a number of countries.

Country	Year of sampling	No. of samples	Gender	Age range	Mean	St. Dev.	Reference
Turkey	2004	23	23 men	21-45	9.2	4.9	Cok et al. (2007)
Italy	n.a.	9	2 men; 7 women	n.a.	range: 3.4-13.3		La Rocca et al. (2008)
Southern Spain	2003	20	20 women	24-81	19.6	8.43	Lopez-Espinosa et al. (2008)
China	2006	24	20 men; 4 women	26-73	7.73 (range: 1.33-16.4)		Shen et al. (2009)
Korea	2007-2008	53	53 women	40-68	13 (range: 3.4-42)		Moon et al. (2011)
Tarragona County, Spain	1996-1998	15	10 men; 5 women	28-83	32.1	15.3	Schuhmacher et al. (1999c)
	2002	15	11 men; 4 women	19-94	9.9	9.3	Schuhmacher et al. (2004)
	2007	15	11 men; 4 women	28-83	14.6	14.2	Nadal et al. (2009)
	2013	15	9 men; 6 women	30-74	11.5	11.1	Schuhmacher et al. (2014)

n.a.: not available.

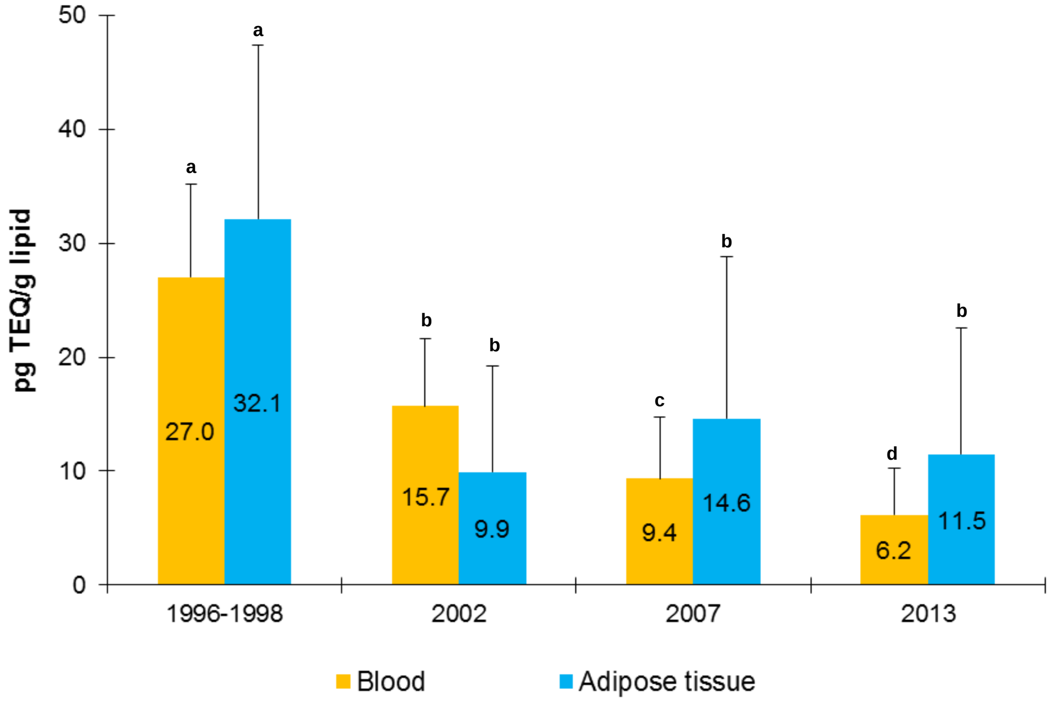

Fig 1. Mean concentrations of PCDD/Fs in blood (in pg I-TEQ/g lipid) and adipose tissue (in pg WHO-TEQ/g fat) from individuals living near the HWI in surveys conducted between 1996-1998 and 2013. Different superscripts (a,b,c,d) indicate significant differences between sampling years, for each biological monitor. Number of samples: Blood: 20 in each study between 1996-1998 and 2007, and 40 in 2013. Adipose tissue: 15 in each survey.