Changes in serum dioxin and PCB levels in residents around a municipal waste incinerator in Bilbao, Spain

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ABSTRACT

There is a great concern in the Basque Country regarding emissions from waste incineration, in particular that of organochlorines (OCs), including dioxins, furans and polychlorinated biphenyls (PCBs), and their potential effect on human health. In 2005, a municipal solid waste plant (MSWP) started to operate in Bilbao, representing an opportunity to assess the exposure to the aforementioned pollutants among people living at various distances from the plant. In 2006 and 2008, we carried out two cross-sectional studies to quantify and assess changes in levels of these pollutants. The objective of this study was to describe the levels of OCs in the blood serum in 2013 of 127 adults of this prospective cohort, in four centres of population, near to and further away from the MSWP, and to study trends over time since it started to operate. This study shows the levels of OCs have decreased significantly, by 78.9% or more. Further, levels of OCs in areas near to the MSWP were not found to be higher than those in areas further afield.

ABBREVIATIONS

CI: confidence interval
dl-PCB: dioxin-like PCB
GM: geometric mean
MSWP: municipal solid waste plant
OC: organochlorine
OCDD: octachlorodibenzodioxin
PCB: polychlorinated biphenyl
PCDD: polychlorinated dibenzodioxin
PCDF: polychlorinated dibenzofuran
TEQ: toxic equivalency factor
DLC: dioxin-like compounds

1. INTRODUCTION

The management of the solid waste we produce is among the great challenges facing society today. One of several methods for reducing the volume of this waste is incineration. However, municipal solid waste incineration is a potential source of pollution, given that during the process there is combustion of residues containing chlorine, as well as of heavy metals (Van Caneghem et al., 2010). During combustion processes, there is a release of various pollutants such as organochlorines, including the polychlorinated-*p*-dibenzodioxins, the polychlorinated dibenzofurans, commonly known as dioxins and furans or simply dioxin like compounds (DLCs), and polychlorinated biphenyls (PCBs). Indeed, combustion is the main source of DLCs released into the environment (Domingo et al., 2000). Specifically, among thermal sources, solid waste

incineration has been identified as the principal source of DLCs during the 1980s and early 1990s (Jones et al., 1993; Eduljee et al., 1996; Quaß et al., 2000; Kim et al., 2001; Quaß et al., 2004; Burns et al., 2010). Since then, municipal solid waste plants (MSWPs) have become a more efficient method for treating solid waste and more effective at controlling DLC emissions (Ranzi et al., 2011).

Nevertheless, there is still concern regarding the environmental and health consequences of waste incineration, and hence, it is important to assess its impact at the environmental level and on people living around MSWPs. A systematic review concluded that the level of evidence for MSWPs having an effect on health was limited for all types of cancers, soft tissue sarcoma, and reproductive and birth defects (orofacial and genitourinary) and inadequate for all the other health problems considered (Mattiello et al., 2013).

The opening of a MSWP in Bilbao in 2005 represented a great opportunity to assess exposure to certain pollutants among people living at various distances from the plant. In 2006 and 2008, two cross-sectional studies were carried out to quantify and assess changes in the levels of OCs in a cohort of the general urban population, those with no work-related exposure and living in areas near to and further away from the MSWP (Zubero et al., 2009; Zubero et al., 2011). Given the high toxicity of dioxins and PCBs, a study was carried out to verify if there was a health risk to those close to the MSWP. The objective of studying this new cross-section of the cohort was to assess the levels of DLCs in people in four centres of population, near to and further away from the MSWP and study trends over time since it started to operate.

2. MATERIAL AND METHODS

We carried out prospective cohort study with three cross-sectional analyses among people living in areas near to and further away from the MSWP. The sampling was performed in 2006 (Zubero et al., 2009), 2008 (Zubero et al., 2011) and in the third quarter of 2013. The first stage of the study was conducted when the MSWP started to operate, and the data collected then is considered the baseline for subsequent stages of the study.

2.1. STUDY POPULATION

Regarding distance to the MSWP, we selected four areas: two areas nearby, Alonsotegi and Rekalde, and two areas further away, Santutxu and Balmaseda, (Zubero et al., 2011). The areas considered close to the MSWP were the town of Alonsotegi and the borough of Rekalde in Bilbao, both less than 2 km away from the plant and in urban environments with high traffic density. The areas further away, used as control areas, were the borough of Santutxu in Bilbao, also an urban environment with high traffic density (5 km away

from the plant) and a small town with low traffic density, Balmaseda (20 km away from the plant); both of these areas have little industrial activity and are out of the path of prevailing winds.

In relation to the origin of the samples, in 2006, 36% of the participants came from the census in the areas close to the plant, and the rest were volunteers from the health centre. In remote areas, 25% were recruited from the census, and the rest were volunteers. In 2008, in nearby areas, 71% were previous participants, whereas in the remote areas this percentage was 68%. In 2013, we carried out the third stage of the study, recruiting people who had participated in the two previous studies, with the aim of recruiting 30 people from each study area, stratified by sex and age group at baseline (20-69 years old). The exclusion criteria at all stages were: having lived in the area for less than the previous 5 years, having worked in a company which carried out production processes involving potential exposure to DLCs in the previous 15 years, and being 70 years old or above.

Having telephoned 223 candidate participants, we finally obtained blood samples from 127 individuals. The main reasons why participants in earlier stages of the study did not participate in this stage were work commitments, ill health/death, age, or declining further participation, as well as not being possible to contact them, or that they had moved away.

All participants signed an informed consent form to take part in the study, which was approved by the Ethics Committee for Research and Teaching of the University of the Basque Country.

2.2. STUDY VARIABLES

We interviewed all participants and collected data on anthropometric and socioeconomic characteristics; reproductive and breastfeeding history, intake of local food produce, and smoking and drinking habits. Weight gain or loss of more than 5 kg in the previous 5 years was noted. Regarding social class, participants were classified into one of two categories, manual or non-manual workers, according to their most recent occupation (not as possible source of DLC but as possible confounding factor), or that of the head of the family if they did not work, in line with the Spanish Adaptation of the British OPCS Classification of Occupations (social classes I+II+III and IV+V). Participants were also classified into two groups by level of education, depending on whether or not they had university qualifications. All participants were interviewed by the same researcher to avoid interviewer bias. We collected data on the frequency of intake of local food produce (fruit and vegetables, cow's milk and cheese, poultry and other meat), with four response options: never or less than once every 2 months, occasional (around once a month), around once a week, or daily (more than twice a week). These data were recoded to a dichotomous variable: never or occasionally versus weekly or daily intake.

2.3. BLOOD TEST ANALYSIS

A 70-ml blood sample was collected from all study participants. They were not required to fast, but were recommended to avoid eating fatty foods the night before. Blood samples were collected in blood collection tubes (Vacutainer®) without anticoagulant and transferred immediately to a glass tube for centrifugation. To obtain the serum, the blood was left to clot for approximately 60-75 minutes at room temperature. The sample was then centrifuged at 1500 G for 15 minutes. The serum was separated from the clot with a Pasteur pipette. Unlike in previous stages of the study, in which the analysis was performed using pools of samples grouped by age and sex, the analysis in this study was based on individual samples. Samples were frozen at -20ºC within 90 minutes after collection. Blood extraction, and serum preparation, storage and transport were carried out in accordance with the conditions established by the reference laboratories (Patterson et al., 1991).

Next, workflow analysis is performed as follows: about 40 mL of serum was spiked with a $^{13}C_{12}$ labelled dioxin and PCB standard mixture. Afterwards, analytes were extracted from the matrix by solid-phase extraction (SPE) approach using C_{18} cartridges (SPE Cartridges – C_{18}; ISOLUTE, Charlotte, NC, USA). Elution was performed with n-hexane. Organic components, fat and other interfering substances were removed by treating the hexane extracts with silica gel (Supelco, Bellefonte, PA, USA) modified with sulphuric acid (44%). Further, the extracts were cleaned up by solid-liquid adsorption chromatography, using multilayer silica, basic alumina and activated carbon adsorbents. Purified extracts were rotary concentrated, transferred into vials and concentrated to dryness by a gentle stream of nitrogen prior to the mass spectrometry analysis. To evaluate the recovery rates, final extracts were reconstructed in a known amount of a mixture of labelled $^{13}C_{12}$-PCDD/Fs (EPA-1613ISS, Wellington Laboratiores Inc., Guelph, Canada), $^{13}C_{12}$-dl-PCBs (WP-ISS, Wellington Laboratories Inc, Guelph, Canada) and $^{13}C_{12}$-marker PCBs (MBP-118, Wellington Laboratories Inc, Guelph, Canada).

Instrumental analysis was based on the use of high resolution gas chromatography coupled to a high resolution mass spectrometry (HRGC-HRMS). Analyses were performed on an Agilent gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an AutoSpec Premier high resolution mass spectrometer (Waters, Manchester, UK) at 10,000 resolving power (10% valley definition). Gas chromatographic separation was performed on a DB-5ms fused silica column (60 m x 0.25 mm i.d. x 0.25-µm film thickness; J&W Scientific, CA, USA) for PCDD/Fs and dl-PCBs, whereas for marker PCBs, the analysis was carried out on a DB-XLB fused silica column (60 m x 0.25 mm i.d. x 0.25 µm-film thickness; J&W Scientific, CA, USA). Quantification was carried out by the isotopic dilution method. Relative response factors were measured for each individual compound by the analysis of six different calibration solutions for PCDDs/Fs, dl-

PCBs and marker PCBs. Finally, the results were expressed as TEQ (toxic equivalent) values using WHO-TEQ (World Health Organization Toxic Equivalent). WHO-TEQ values were calculated in "upperbound", assuming the limit of detection (LOD) for those non-detected or below the LOD congeners. More detailed information on instrumental analysis is reported elsewhere (Abad et al., 2000).

Enzymatic lipid determinations were performed using 100 µL serum sub-sample. Overall, four types of lipids were considered: triglycerides, total cholesterol, non-esterified (free) cholesterol and phospholipids. A well-documented summation method was used to calculate the total lipid concentrations (Patterson et al., 1991). The total lipid content was expressed in g/L. For the conversion between volumetric and gravimetric data, a value of 1.026 g/mL was used for serum specific gravity. The requirements for ensuring high quality data include the application of quality assessment and quality control measures. A series of control blanks were analysed to assess laboratory cross-contamination. Detailed information on the analytical method is included as SI.

2.4. STATISTICAL METHODS

The sample was described using the absolute and relative frequencies of the variables collected during the interview. The levels of congeners were described by mean, median, and range (minimum and maximum values), assessing their contribution to the WHO-TEQs. Pearson's coefficient of correlation between the levels of congeners was calculated. Further, the geometric means (GMs) and 95% confidence intervals (CIs) of the lipid-corrected data were calculated for each group of pollutants and compared as a function of socioeconomic and reproductive variables.

For the temporal analysis of the levels of PCDD/Fs and dl-PCBs, given that the previous samples were analysed in serum pools, the corresponding value of a new pool was calculated using the arithmetic mean of each individual value in 2013, because the measured concentration in a pooled sample is comparable to the arithmetic mean of individual specimens making up the pool (Heffernan et al., 2014). The pooled samples of 2006, 2008 and 2013 were adjusted to the normal distribution. Likewise, the distributions of the different groups of OCs, after logarithmic transformation, analyzed on individual samples were adjusted to the normal (Kolmogorov-Smirnov Z test $p > 0.10$). Changes in the arithmetic mean levels of dioxins, furans, and dl-PCBs in the pools from 2006 to 2008 and from 2006 to 2013 were analysed using a paired t-test. Similarly, changes in the geometric means of marker PCBs (individual samples) over these periods were analysed using a paired t-test.

A multiple linear regression analysis was performed to identify the socioeconomic variables associated with each group of contaminants. All variables were included in any of the models with a $p < 0.10$ according to the

likelihood ratio test. All the models included the geographical location, age and sex variables, since they were related to the selection of the sample in previous stages. Results are expressed as a percentage change [(exp(b)-1) * 100]. The relation between OCs and intake of local food produce was plotted, taking into account the distance to the MSWP. The criterion for statistical significance was $p< 0.05$. The statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 20.0 (IBM SPSS, Armonk, NY, USA).

3. RESULTS

Table 1 shows the characteristics of the sample. Slightly more than half of the 127 participants were women. Categorizing age into tertiles, approximately a third of participants were under 47 years of age, another third were between 47 and 60 and the last third over 60 years of age. The highest participation rate was observed in Balmaseda. A total of 29.1% of participants had normal weight, and a similar percentage was overweight, while 35.4% was obese. Nearly half of the sample reported weight change in the previous 5 years, 35.4% having put on weight and 14.2% having lost weight (≥ 5 kg). Regarding occupation and education, 42.5% of participants were manual workers and 28.3% had university qualifications. Overall, 20% were smokers at the time of the interview, 34.6% were ex-smokers and 43.3% were never-smokers. Among the women, more than half were in menopause, while 76.1% had had children and 77.8% had breastfed. Compared with previous years, in 2013, in addition to the corresponding increase in age, there was an increase in BMI and, also an increase in the number of women who had children. Social class showed non-manual workers to a greater extent in the 2013 sample than in the previous two. There were no significant differences in the characteristics of the rest of the variables.

Table 2 shows the geometric mean, median, minimum and maximum levels of each of the congeners studied, as well as the WHO-TEQ values and the weight of each in the total level of substances with dioxin activity (DLC). Although a higher concentration of mono-ortho-PCBs was observed, especially PCB 118, they because of their lower toxicity, contributed a small part of the total WHO TEQ. Marker PCBs showed a clear decrease in levels, lower than DLCs. Three compounds, PCB 126, 12378-PeCDD (1,2,3,7,8-pentachlorodibenzo-p-dioxin) and 23478-PeCDF (2,3,4,7,8-pentachlorodibenzofuran), accounted for 62.81% of the total WHO-TEQs. In contrast, other compounds with a high concentration in blood serum such as mono-ortho PCBs and to a lesser extent octachlorodibenzodioxin (OCDD) and PCB77 contributed very little to the WHO-TEQs, due to their low toxic equivalency factor. Supplementary Table 1 shows the correlations observed between the congeners; the coefficients being the highest among the PCBs and low between these and both dioxins and furans, while there was an intermediate correlation between dioxins and furans.

Table 3 shows a dramatic decrease in the mean values of OCs by 2013 compared to 2008 and 2006, in the total and in the four studied areas. There was not a significant decrease in mean values between 2006 and 2008. Indeed, in this period, we found a significant increase in the concentration of dioxin-like PCBs.

Table 4 lists the GMs and 95% CIs for each pollutant with respect to the socioeconomic, reproductive and breastfeeding variables. Men had significantly higher levels of dl-PCBs than women. Among the four study areas, we found the highest mean OC levels in Santutxu, the difference being significant in all cases except for PCDD/Fs. We observed that individuals with university qualifications had significantly lower mean levels of dioxins, but higher levels of dl-PCBs. We did not observe significant differences between OCs and social class, body mass index (BMI) or weight change (data not shown). Levels of dl-PCBs were higher in women who had had children than those who had not, the difference being marginally significant; while those who had breastfed had lower levels of marker PCBs, the difference again being marginally significant. Post-menopausal women had significantly higher levels of dl-PCBs than other women.

Multiple linear regression analysis (Table 5) showed that compared to the coefficients for Balmaseda, which is the least urban and industrialised of the areas studied and the furthest from the ~~incinerator~~ incinerator, the highest coefficients for all OCs were found for Santutxu, the differences being statistically significant for dl-PCBs, DLC and marker PCBs, but not for PCDD/Fs. In the other two zones, those closest to the MSWP, the coefficients did not show differences from those for Balmaseda, though Alonsotegi showed the lowest coefficients for all the OCs. Comparing the sexes, men did not show differences in marker PCB levels with women. Levels of both types of PCBs were positively associated with age; PCDD/Fs levels, however, were not significantly associated with age.

Individuals who had only completed non-university education had significantly higher levels of PCDD/Fs than participants with university qualifications. The levels of PCDD/Fs and DLC were inversely related to weight gain in the previous 5 years, but the differences were not significant. Women who had breastfed had significantly lower levels of PCBs than the rest of all the participants.

Figure 1 shows the relationship of OC levels with intake of local food produce and distance from the MSWP studied (Rekalde and Alonsotegi being nearby, while Balmaseda and Santutxu are further away). We did not observe any significant increases in the mean levels of OCs with intake of local food produce in areas near the MSWP.

4. DISCUSSION

In our study, individuals living near to a solid waste MSWP did not have higher blood levels of OCs than those living further afield. On the contrary, the highest levels of these pollutants were found in one the more distant areas, even after adjusting for confounding factors. Further, the intake of local food produce near the MSWP was not associated with an increase in the blood concentration of the pollutants analysed. These data are in agreement with those reported by Ranzi et al. (2011), who found that the ratio of emissions in 2008 to those in 1994-1996 for two Italian incinerators was about 0.0001 (10,000 times less) for dioxins and furans. Other studies analysing trends over time in areas close to MSWPs made similar observations, with no increase in OC levels in areas close to MSWPs (Deml et al., 1996; Evans et al., 2000; González et al., 2000; Chen et al., 2004; Huang et al., 2007; Reis et al., 2007; De Felip et al., 2008; Parera et al., 2013).

In addition, we observed significant decreases in the levels of OCs, both in the areas near to and further away from the plant between 2006 or 2008 and 2013. Wittsiepe et al. (2000) also observed a decrease from 43.77 to 20.7 pg TEQ/g lipid between 1989 and 1998, and Humblet et al. (2011) a decrease from 36 to 25 pg TEQ/g lipid in women between 2000 and 2009. Similarly, Porta et al. (2012) reported OC levels to have decreased by 34 to 56% between 2002 and 2006, and Llobet et al. (2003) also found a decrease in OC levels over time. A general tendency for PCB levels to decrease was also observed by Linderholm et al. (2010) between 1990 and 2007 and Humblet et al. (2011), who found a decrease from 291 to 211 ng/g lipid in women. A systematic review of studies published up to 2010 on the blood levels of dioxins, furans and dl-PCBs reported a mean annual decrease of 7% for dioxins and 6% for both furans and dl-PCBs (Consonni et al., 2012). A study based on the incinerator in Mataró, Spain (Parera et al., 2013) found a decrease in the levels of PCDD/Fs and marker PCBs, as in our study. A recent study showed also an important decrease of serum PCBs in a population living near a contaminated area after public health intervention (Magoni et al., 2016).

Unlike various other studies (Deml et al., 1996; Evans et al., 2000; González et al., 2000; Reis et al., 2007), we did not find significant differences between the levels of PCDD/Fs by age. On the other hand, dl-PCB and marker PCB levels were positively associated with age. The aforementioned systematic review of blood OC levels, showed a 2% increase in levels for the three groups of OCs (PCDDs, PCDFs and dl-PCBs) with each year of age (Consonni et al., 2012). A previous study of samples collected from our study population between 2006 and 2008 (Zubero et al., 2015) showed an increase in the levels of OC pesticides and marker PCBs with age, consistent with a cohort effect, and a decrease between samples taken in the two periods (a decreasing period effect). This suggests that the higher levels found in older individuals are due to the fact that OC exposure has decreased over the years, and hence has less impact on younger cohorts, rather than a process of bioaccumulation over the years.

The mean levels of PCDD/Fs were higher in women than in men, after adjusting for other variables, although the differences were not significant. Similar results have been observed by other authors (Deml et al., 1996; Reis et al., 2007). Such a trend could be explained by a higher intake of these compounds as part of their diet (Agramunt et al., 2005). Women have significantly higher levels of dl-PCBs than men. On the other hand, men had higher levels of marker PCBs, although the difference was not significant.

We observed that the levels of dioxin-like and marker PCBs were inversely and significantly correlated with BMI, the relationship being close to significance for PCDD/Fs. Agudo et al. (2009) found a similar pattern. Further, PCDD/F levels and total levels of dioxins and DLCs were lower, though not significantly, in individuals who had gained weight in the previous 5 years. Humblet et al. (2010 and 2011) observed, in two different samples, decreases in OC levels in mothers in relation to recent increases in BMI.

Women who had breastfed had lower levels of marker PCBs. Similarly, Humblet et al. (2010) found lower levels of PCBs and dioxins in mothers with long breastfeeding periods. In our study, we did not find OC levels to be significantly related to menopause or parity. Fernández-Rodríguez et al. (2015) also reported no relationship between OCs and parity, although unlike in our study, they did not find an association with breastfeeding.

The 127 individuals of this study are part of a cohort and all of them participated in the two previous cross-sections. The strengths of this study include the fact that the study sample was initially taken from the general population, selecting from the census, though we also recruited individuals attending their health centre. Moreover, we have analysed data on OCs levels on an individual basis, rather than pooling data for all participants, as in the previous stages. OCs levels were quantified in the same laboratory as in the previous two stages, except in the case of marker PCBs, which for the previous stages were measured in a different laboratory. Hence, interlaboratory variability would only affect this latter compound. This type of variability would be expected to be considerably high, given that we analysed OCs in blood samples with small amounts of fat, and in this study, there were very low concentrations of PCDDs and PCDFs. The weaknesses of the study include the sample size, although few dioxin-based studies have used larger sample sizes than that reported here, it being plausible that studies are constrained by the high costs involved in the measurement of the compounds analysed.

While in the emissions from MWSP the congener profile showed a higher concentration of OCDD, followed by 1,2,3,4,6,7,8-HpCDD, OCDF and 1,2,3,4,6,7,8-HpCDF (Abad et al., 2006), in the serum of this sample the highest concentration observed is mono-ortho PCBs, followed by PCB 77 (non-ortho) and OCDD. On the other hand, the observed decrease between 2006/08 and 2013 occurs in a similar measure in PCDD / Fs and dl-PCBs. This indicates a different source of exposure. Given that in the vicinity there are no paper

manufacturers, cement companies or other companies associated with significant emission of DLCs. The Basque Country presents a varied Mediterranean diet with a high consumption of lean fish (Welch et al., 2002). However, this consumption is not associated with high levels such as the oil-fish, the fish-liver or the seagull eggs (Kvalem et al., 2009), food not included in our diet.

To summarise, this study found very low OC levels and a significant decrease in these levels in 2013 compared to 2006 and 2008. These results may be due to the application of the wide range of legal measures and international agreements aimed at limiting or prohibiting the use, commercialisation and production, and minimising the generation of these compounds. Further, understanding that the diet is the main source of OCs (Kogevinas et al., 2000; Malisch and Kotz, 2014), these decreases would be also attributable to the recommendations of various organisations made over the last decade to set thresholds with the goal of reducing the presence of dioxins, furans and PCBs in animal feed and foodstuffs (as reflected in European Directive 2006/88/EC). This study also shows that monitoring of the OCs is an effective tool for assessing their health effects, without waiting for consequences to appear after long latency periods (Ibarluzea et al., 2016).

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Figure 1: Levels of PCDD/Fs, dl-PCBs, DLC (pg WHO-TEQ/g fat) and marker PCBs (ng/g fat) associated with the intake of local food produce (near to/further away from the incinerator). Box plot.

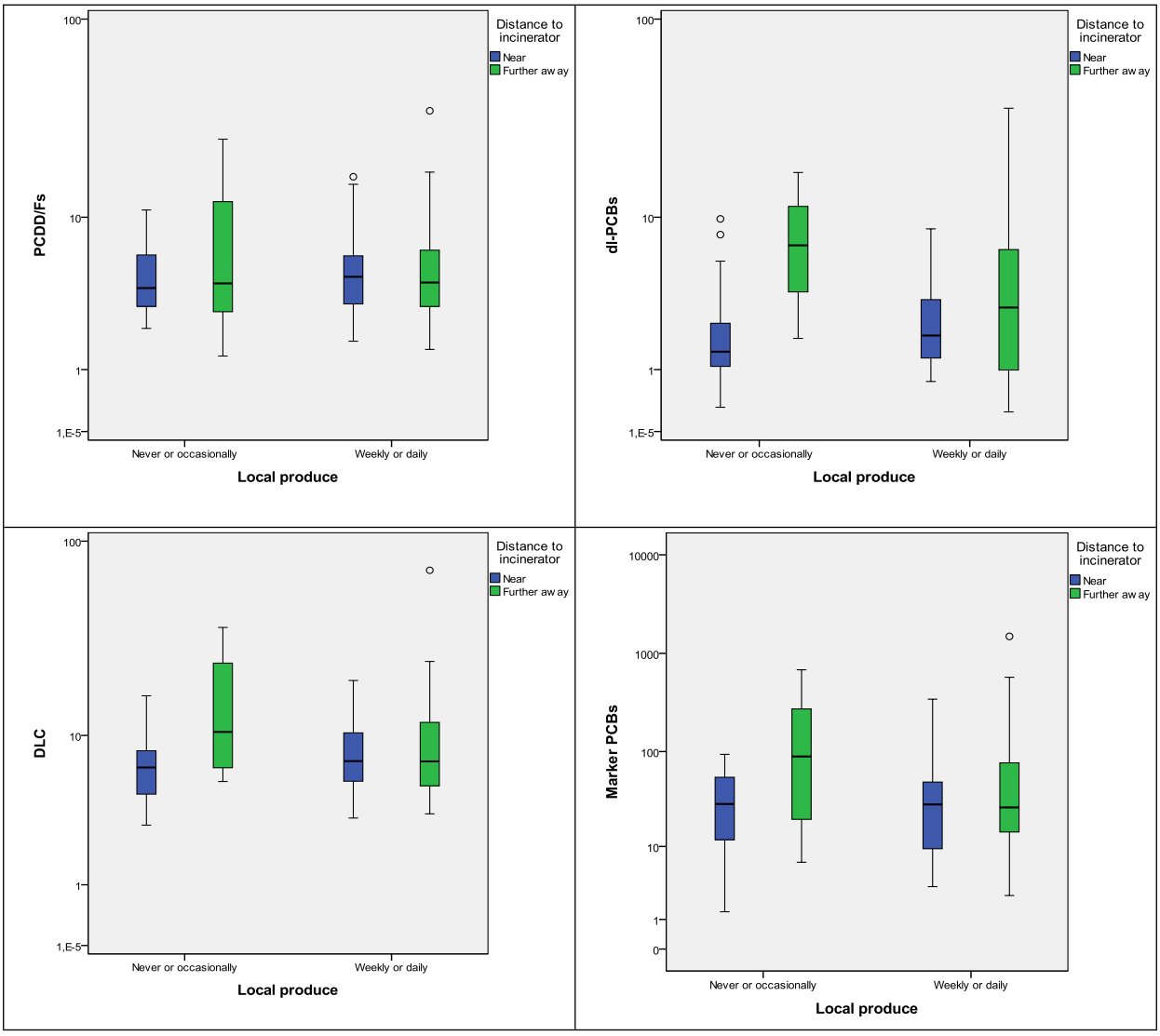

Table 1: Description of the sample. Socioeconomic variables. Years 2006, 2008 and 2013.

Variable		Year 2006			Year 2008			Year 2013	
	Category	**N**	**%**	**p[a]**	**N**	**%**	**p[b]**	**N**	**%**
Total		322	100	-	326	100	-	127	100
Sex	Male	159	49.4	0.313	162	49.7	0.284	56	44.1
	Female	163	50.6		164	50.3		71	55.9
Age, years	< 47	171	53.1	<0.001	176	54	<0.001	42	33.1
	47-60	91	28.3		100	30.7		43	33.9
	> 60	60	18.6		50	15.3		42	33.1
Municipality	Alonsotegi	82	25.5	0.852	82	25.2	0.816	30	23.6
	Rekalde	80	24.8		81	24.8		31	24.4
	Santutxu	79	24.5		82	25.2		29	22.8
	Balmaseda	81	25.2		81	24.8		37	29.1
Body mass index, kg/m^2	< 25	138	42.9	0.012	144	44.2	<0.001	37	29.1
	25-29	106	33		129	39.6		45	35.4
	≥ 30	78	24.2		53	16.2		45	35.4
Weight gain ≥ 5 kg in previous 5 years[c]	No	-	-			-		82	64.6
	Yes	-	-			-		45	35.4
Weight loss ≥ 5 kg in previous 5 years	No	265	82.3	0.367	244	74.8	0.013	109	85.8
	Yes	57	17.7		81	24.8		18	14.2
Level of education	Primary or secondary	238	73.9	0.626	236	72.4	0.652	91	71.7
	University	84	26.1		84	25.8		36	28.3
Social class (occupation)	Manual	194	60.2	0.001	195	59.8	0.001	54	42.5
	Non manual	128	39.8		131	40.2		73	57.5
Smoking status	Never-smoker	237	73.6	0.339	232	71.2	0.144	55	43.3
	Ex-smoker [c]	-	-		-	-		44	34.6
	Smoker	85	26.4		94	28.8		28	22.0
Intake of local produce [c]	Never or occasionally	-	-		-	-		37	29.1
	Weekly or daily	-	-		-	-		90	70.9
Menopause (only in women) [c]	No	-	-		-	-		29	40.8
	Yes	-	-		-	-		42	59.2
Had had children (only in women)	No	56	34.6	0.090	60	18.4	0.050	17	23.9
	Yes	103	65.4		102	31.3		54	76.1
Had breastfed (only in women with children)	No	25	23.4	0.871	23	22	0.988	12	22.2
	Yes	82	76.6		81	77.9		42	77.8

a: 2013 vs. 2006
b: 2013 vs. 2008
c: variable or category not collected in previous years

Table 2: Levels of PCDD/Fs and dl- PCBs (pg/g fat) in serum, toxic equivalency factor (WHO TEF), WHO-TEQ (pg/g fat) values and contribution to the DLC of the total PCDD/Fs (%) and marker PCB congeners (ng/g fat).

	> Limit of Detection (%)	GM	Median	Min	Max	WHO TEF (2005)	WHO TEQ	Contribution to total (%)
Dioxins								
2,3,7,8-TCDD	27.6	0.59	0.56	0.10	2.30	1	0.59	5.90
1,2,3,7,8-PeCDD	37.8	2.38	1.76	0.40	14.49	1	2.38	23.86
1,2,3,4,7,8-HxCDD	43.3	1.40	1.06	0.17	6.41	0.1	0.14	1.40
1,2,3,6,7,8-HxCDD	70.9	4.50	2.27	0.30	56.71	0.1	0.45	4.51
1,2,3,7,8,9-HxCDD	49.6	1.77	1.16	0.26	10.41	0.1	0.18	1.77
1,2,3,4,6,7,8-HpCDD	83.5	5.32	2.97	0.28	86.18	0.1	0.53	5.32
OCDD	89.8	34.86	18.39	0.93	442.28	0.0003	0.01	0.10
Total dioxins							4.28	42.87
Furans								
2,3,7,8-TCDF	59.1	0.82	0.49	0.12	10.92	0.1	0.08	0.82
1,2,3,7,8-PeCDF	66.1	2.01	1.54	0.23	10.02	0.03	0.06	0.60
2,3,4,7,8-PeCDF	79.5	3.12	2.14	0.36	29.73	0.3	0.94	9.39
1,2,3,4,7,8-HxCDF	82.7	1.91	1.35	0.14	12.06	0.1	0.19	1.91
1,2,3,6,7,8-HxCDF	64.6	1.76	1.10	0.18	22.26	0.1	0.18	1.77
2,3,4,6,7,8-HxCDF	65.4	1.43	1.10	0.16	9.08	0.1	0.14	1.43
1,2,3,7,8,9-HxCDF	56.7	1.72	1.31	0.24	9.37	0.1	0.17	1.72
1,2,3,4,6,7,8-HpCDF	56.7	2.24	1.45	0.27	11.17	0.01	0.02	0.22
1,2,3,4,7,8,9-HpCDF	78.7	1.50	0.99	0.13	12.93	0.01	0.02	0.15
OCDF	57.5	3.94	2.85	0.29	21.77	0.0003	0.00	0.01
Total furans	-	-	-	-	-	-	1.80	18.03
Non-ortho PCBs								
PCB-77	70.1	52.19	15.06	2.40	1032.77	0.0003	0.02	0.16
PCB-81	21.3	6.92	4.26	0.57	53.70	0.0003	0.00	0.02
PCB-126	23.6	29.52	17.79	0.93	255.11	0.1	2.95	29.56
PCB-169	39.4	23.85	14.79	1.13	204.39	0.03	0.72	7.17
Total non-ortho PCBs	-	-	-	-	-	-	3.69	36.91
Mono-ortho PCBs								
PCB-105	98.4	644.99	211.64	24.67	16316.96	0.00003	0.02	0.19
PCB-114	81.1	167.85	68.25	4.69	2788.42	0.00003	0.01	0.05
PCB-118	100	2930.80	1052.55	101.57	74839.11	0.00003	0.09	0.88
PCB-123	59.8	66.19	28.76	4.22	1080.77	0.00003	0.00	0.02
PCB-156	100	1943.97	834.44	29.90	29341.22	0.00003	0.06	0.58
PCB-157	96.1	389.36	173.54	8.72	6257.31	0.00003	0.01	0.12
PCB-167	98.4	729.62	282.77	12.92	17285.08	0.00003	0.02	0.22
PCB-189	96.1	426.37	174.20	8.62	5236.37	0.00003	0.01	0.13
Total mono-ortho PCBs	-	-	-	-	-	-	0.22	2.19
DLC	-	-	-	-	-	-	9.98	100
Marker PCBs								
PCB28	100	3.60	0.54	0.05	79.00	-	-	-
PCB52	100	2.39	0.34	0.02	62.05	-	-	-
PCB101	100	1.79	0.29	0.02	36.46	-	-	-
PCB138	100	11.73	4.07	0.13	325.70	-	-	-
PCB153	100	29.92	10.74	0.37	640.06	-	-	-
PCB180	100	34.33	12.99	0.45	490.07	-	-	-

Table 3: Changes in mean levels of PCDD/Fs, dl-PCBs, DLC (pg WHO-TEQ/g fat) (arithmetic means and 95% CI) and total marker PCBs (ng/g fat) (geometric means and 95% CI) in 2006, 2008 and 2013 in the 127 individuals with successful laboratory measurements of repeat blood samples. T-test to compare means of repeated measures. Percentage change in OC level between sampling periods.

		2006	2008	2013	% change 2006-08	p	% change 2006-13	p
Total sample	PCDD/Fs[a,b] (n= 16 pools)	23.45 (19.28-27.62)	23.60 (17.29-29.90)	4.67 (3.95-5.38)	0.6%	0.967	-80.1%	<0.001
	dl-PCBs[a,b] (n= 16 pools)	15.56 (11.66-19.47)	23.61 (15.73-31.49)	3.13 (2.25-4.01)	51.7%	0.042	-79.9%	<0.001
	DLC[a,b] (n= 16 pools)	39.01 (31.67-46.35)	47.20 (31.15-61.26)	8.23 (7.30-9.16)	21.2%	0.260	-78.9%	<0.001
	Marker PCBs[b,c] (n= 127)	254.06 (232.68-277.40)	229.18 (204.98-256.24)	32.36 (25.75-40.66)	-1.86	0.008	-37.21	<0.001
Alonsotegi	PCDD/Fs[a,b] (n= 4 pools)	24.46 (15.85-33.08)	23.98 (13.01-34.95)	4.61 (3.41-5.80)	-2.0%	0.915	-81.2%	0.024
	dl-PCBs[a,b] (n= 4 pools)	16.08 (4.49-27.68)	23.74 (8.43-39.05)	1.45 (1.27-1.64)	47.6%	0.142	-91.0%	0.090
	DLC[a,b] (n= 4 pools)	40.55 (20.54-60.56)	47.72 (21.61-73.83)	6.31 (5.16-7.46)	17.7%	0.434	-84.4%	0.047
	Marker PCBs[b,c] (n= 30)	235.19 (189.38-292.07)	202.41 (161.30-254.00)	19.27 (12.96-28.64)	-2.7%	<0.001	-45.8%	<0.001
Rekalde	PCDD/Fs[a,b] (n= 4 pools)	29.29 (18.09-40.49)	17.67 (8.63-26.72)	4.50 (4.16-4.85)	-39.7%	0.114	-84.6%	0.024
	dl-PCBs[a,b] (n= 4 pools)	18.93 (8.81-29.06)	17.00 (5.36-28.65)	2.81 (2.00-3.64)	-10.2%	0.431	-85.1%	0.047
	DLC[a,b] (n= 4 pools)	48.23 (29.28-67.17)	34.68 (14.17-55.19)	8.01 (7.12-8.89)	-28.1%	0.159	-83.4%	0.024
	Marker PCBs[b,c] (n= 31)	212.38 (176.62-255.39)	195.76 (157.95-242.62)	27.58 (18.57-40.96)	-1.5%	0.374	-38.1%	<0.001
Santutxu	PCDD/Fs[a,b] (n= 4 pools)	21.36 (13.37-29.34)	24.50 (13.37-29.34)	3.52 (2.58-4.47)	14.7%	0.263	-83.5%	0.025
	dl-PCBs[a,b] (n= 4 pools)	11.61 (6.74-16.46)	23.17 (12.62-33.73)	5.19 (2.91-7.47)	99.6%	0.043	-55.3%	0.031
	DLC[a,b] (n= 4 pools)	32.97 (21.57-44.36)	47.67 (21.57-44.36)	8.93 (7.41-10.46)	44.6%	0.073	-72.9%	0.020
	Marker PCBs[b,c] (n= 29)	276.73 (230.63-332.05)	218.49 (164.04-291.01)	93.76 (54.65-160.84)	-4.2%	0.052	-19.2%	0.001
Balmaseda	PCDD/Fs[a,b] (n= 4 pools)	18.68 (16.61-20.75)	28.23 (6.54-49.92)	6.03 (4.12-7.95)	51.1%	0.437	-67.7%	0.001
	dl-PCBs[a,b] (n= 4 pools)	15.63 (12.55-18.71)	30.52 (12.62-33.73)	3.05 (2.49-3.62)	95.3%	0.347	-80.5%	0.005
	DLC[a,b] (n= 4 pools)	34.31 (33.08-35.54)	58.75 (11.80-105.70)	9.67 (7.44-11.90)	71.2%	0.383	-71.8%	0.001
	Marker PCBs[b,c] (n= 37)	293.90 (300.29-348.48)	300.29 (258.76-348.48)	24.46 (17.59-34.02)	0.4%	0.628	-43.6%	<0.001

a: % change 2006-08= 100 * (Mean OC_{2008} - Mean OC_{2006}) / Mean OC_{2006}; % change 2006-13= 100 * (Mean OC_{2013} - Mean OC_{2006}) / Mean OC_{2006}:

b: Student's t-test for paired samples, 2006 and 2008 or 2006 and 2013

c: % change 2006-08= 100 * ((GM OC_{2008} / GM OC_{2006}) -1) ; % change 2006-13= 100 * ((GM OC_{2013} / GM OC_{2008}) -1)

Supplementary Table 1: Pearson's correlation coefficient between different congeners (dioxins, furans, dl-PCBs and marker PCBs).

	2378TCDD	12378PeCDD	123478HxCDD	123678HxCDD	123789HxCDD	1234678HpCDD	OCDD	2378TCDF	12378PeCDF	23478PeCDF	123478HxCDF	123678HxCDF	234678HxCDF	123789HxCDF	1234678HpCDF	1234789HpCDF	OCDF	PCB77	PCB81	PCB126	PCB169	PCB105	PCB114	PCB118	PCB123	PCB156	PCB157	PCB167	PCB189	PCB28	PCB52	PCB101	PCB138	PCB153	PCB180
2,3,7,8-TCDD	**1**																																		
1,2,3,7,8-PeCDD	**.33**	**1**																																	
1,2,3,4,7,8-HxCDD	**.49**	**.50**	**1**																																
1,2,3,6,7,8-HxCDD	**.26**	**.38**	**.31**	**1**																															
1,2,3,7,8,9-HxCDD	**.39**	**.55**	**.61**	**.51**	**1**																														
1,2,3,4,6,7,8-HpCDD	**.25**	**.45**	**.43**	**.71**	**.53**	**1**																													
OCDD	**.18**	**.33**	**.29**	**.65**	**.44**	**.66**	**1**																												
2,3,7,8-TCDF	**.41**	**.32**	**.27**	.13	.16	**.18**	.12	**1**																											
1,2,3,7,8-PeCDF	**.34**	**.40**	**.35**	**.21**	**.29**	**.25**	**.18**	**.34**	**1**																										
2,3,4,7,8-PeCDF	**.23**	**.50**	**.28**	**.69**	**.42**	**.59**	**.58**	**.18**	**.39**	**1**																									
1,2,3,4,7,8-HxCDF	**.33**	**.51**	**.50**	**.44**	**.52**	**.54**	**.39**	**.33**	**.41**	**.40**	**1**																								
1,2,3,6,7,8-HxCDF	**.32**	**.42**	**.42**	**.34**	**.41**	**.44**	**.31**	**.25**	**.46**	**.37**	**.67**	**1**																							
2,3,4,6,7,8-HxCDF	**.34**	**.40**	**.44**	**.33**	**.45**	**.33**	**.28**	**.32**	**.53**	**.38**	**.56**	**.49**	**1**																						
1,2,3,7,8,9-HxCDF	**.32**	**.40**	**.49**	**.19**	**.44**	**.20**	.13	**.16**	**.46**	**.24**	**.50**	**.42**	**.44**	**1**																					
1,2,3,4,6,7,8-HpCDF	**.25**	**.47**	**.42**	**.55**	**.44**	**.62**	**.53**	**.31**	**.35**	**.60**	**.53**	**.45**	**.44**	**.39**	**1**																				
1,2,3,4,7,8,9-HpCDF	**.40**	**.41**	**.46**	**.22**	**.44**	**.23**	.15	**.36**	**.50**	**.21**	**.44**	**.45**	**.61**	**.55**	**.36**	**1**																			
OCDF	**.28**	**.45**	**.37**	**.19**	**.46**	**.20**	.12	**.28**	**.44**	**.19**	**.38**	**.29**	**.42**	**.49**	**.29**	**.61**	1																		
PCB-77	**.23**	.13	**.19**	**.26**	.12	**.27**	**.30**	.13	.16	**.24**	.14	**.25**	.17	.07	**.23**	.13	-.08	**1**																	
PCB-81	**.18**	.10	**.18**	.15	.10	.07	.076	.04	.01	.13	.01	.05	.03	.11	.12	.11	-.03	**.68**	**1**																
PCB-126	.14	.09	**.21**	**.23**	.06	.17	.17	-.01	-.11	**.20**	.01	.07	-.02	-.03	.13	-.05	-.10	**.49**	**.67**	**1**															
PCB-169	**.23**	.16	**.24**	**.45**	**.21**	**.27**	**.30**	-.02	-.05	**.39**	.11	.11	.09	.13	**.28**	.07	-.08	**.51**	**.79**	**.73**	**1**														
PCB-105	**.19**	**.24**	.12	**.56**	**.23**	**.55**	**.56**	.15	.10	**.47**	**.27**	**.32**	.08	-.04	**.33**	-.01	-.16	**.66**	**.36**	**.44**	**.42**	**1**												
PCB-114	**.25**	**.25**	.15	**.69**	**.29**	**.61**	**.64**	.10	.10	**.60**	**.28**	**.30**	.12	.04	**.42**	.02	-.09	**.58**	**.38**	**.49**	**.55**	**.91**	**1**											
PCB-118	**.19**	**.23**	.11	**.60**	**.25**	**.57**	**.59**	.13	.09	**.50**	**.27**	**.32**	.06	-.04	**.36**	-.02	-.16	**.61**	**.33**	**.44**	**.44**	**.99**	**.93**	**1**										
PCB-123	**.22**	**.25**	**.30**	**.46**	**.34**	**.47**	**.46**	.03	.10	**.40**	**.30**	**.34**	.05	.14	**.29**	.05	-.03	**.65**	**.64**	**.62**	**.64**	**.72**	**.74**	**.73**	**1**									
PCB-156	**.20**	**.24**	.11	**.71**	**.30**	**.58**	**.67**	.11	.07	**.62**	**.27**	**.26**	.07	.03	**.45**	-.01	-.04	**.40**	**.22**	**.39**	**.48**	**.82**	**.92**	**.88**	**.60**	**1**								
PCB-157	**.21**	**.25**	.13	**.71**	**.32**	**.60**	**.67**	.10	.06	**.62**	**.29**	**.26**	.07	.06	**.46**	.00	-.04	**.40**	**.25**	**.41**	**.51**	**.83**	**.94**	**.88**	**.63**	**.99**	**1**							
PCB-167	**.18**	**.25**	.11	**.68**	**.28**	**.59**	**.65**	.10	.03	**.59**	**.27**	**.28**	.05	-.02	**.41**	-.04	-.11	**.44**	**.23**	**.41**	**.49**	**.86**	**.91**	**.91**	**.64**	**.96**	**.95**	**1**						
PCB-189	**.25**	**.24**	.17	**.65**	**.29**	**.54**	**.64**	.12	.06	**.58**	**.27**	**.24**	.05	.09	**.44**	.04	-.03	**.41**	**.27**	**.44**	**.54**	**.75**	**.85**	**.79**	**.60**	**.92**	**.91**	**.87**	**1**					
PCB-28	**.30**	**.20**	.17	**.47**	**.22**	**.48**	**.52**	**.21**	**.20**	**.48**	**.23**	**.31**	**.23**	.03	**.44**	.09	-.10	**.80**	**.39**	**.38**	**.42**	**.77**	**.70**	**.74**	**.59**	**.61**	**.60**	**.62**	**.59**	**1**				
PCB-52	**.31**	.16	**.22**	**.39**	.17	**.38**	**.42**	.17	**.19**	**.39**	.16	**.23**	**.19**	.03	**.35**	.07	-.12	**.86**	**.51**	**.44**	**.46**	**.72**	**.65**	**.68**	**.61**	**.52**	**.52**	**.54**	**.53**	**.94**	**1**			
PCB-101	**.25**	.10	.17	**.30**	.13	**.32**	**.37**	.14	.16	**.30**	.12	**.20**	.14	-.03	**.24**	.04	-.15	**.87**	**.52**	**.44**	**.41**	**.74**	**.63**	**.69**	**.62**	**.48**	**.48**	**.51**	**.48**	**.90**	**.97**	**1**		
PCB-138	**.24**	**.23**	.17	**.64**	**.30**	**.54**	**.68**	.10	.08	**.57**	**.25**	**.27**	.09	-.02	**.38**	.01	-.10	**.50**	**.26**	**.40**	**.49**	**.86**	**.88**	**.89**	**.63**	**.90**	**.90**	**.90**	**.84**	**.73**	**.65**	**.64**	**1**	
PCB-153	**.24**	**.22**	.17	**.65**	**.30**	**.54**	**.68**	.09	.09	**.59**	**.25**	**.27**	.10	-.01	**.40**	.00	-.10	**.48**	**.24**	**.40**	**.49**	**.84**	**.88**	**.88**	**.62**	**.92**	**.91**	**.91**	**.86**	**.73**	**.65**	**.63**	**.99**	**1**
PCB-180	**.24**	**.19**	.17	**.64**	**.30**	**.51**	**.68**	.09	.09	**.59**	**.21**	**.23**	.08	.01	**.40**	-.01	-.07	**.45**	**.25**	**.42**	**.51**	**.78**	**.85**	**.83**	**.60**	**.92**	**.91**	**.89**	**.88**	**.70**	**.63**	**.60**	**.96**	**.98**	**1**

Bold: p< 0.05

Table 4: Mean and 95% confidence intervals for levels of PCDD/Fs, dl-PCBs, DLC (pg WHO-TEQ/g fat) and total marker PCBs (ng/g fat) expressed as pg WHO-TEQ/g fat and marker PCBs expressed as ng/g fat, as a function of socioeconomic characteristics. 2013

			PCDD/Fs	dl-PCBs	DLC	Marker PCBs
		N	GM (CI)	GM (CI)	GM (CI)	GM (CI)
TOTAL		127	4.66 (4.22-5.14)	2.53 (2.15-2.98)	7.93 (7.21-8.73)	32.36 (25.75-40.66)
Sex	Male	56	4.75 (4.13-5.46)	3.17 (2.54-3.96)	8.66 (7.58-9.89)	35.00 (24.59-49.82)
	Female	71	4.58 (3.98-5.27)	2.12 (1.69-2.66)	7.40 (6.47-8.47)	30.41 (22.52-41.07)
	P		.731	**.016**	.112	.551
Age, years	< 47	42	4.69 (4.05-5.43)	2.29 (1.71-3.06)	7.69 (6.62-8.92)	26.43 (17.35-40.25)
	47-60	43	4.65 (3.86-5.60)	2.54 (1.87-3.44)	7.82 (6.44-9.50)	35.23 (23.23-53.42)
	> 60	42	4.63 (3.85-5.56)	2.79 (2.17-3.59)	8.30 (7.14-9.66)	36.31 (25.68-51.35)
	p value		.916	.341	.524	.271
Municipality	Alonsotegi	30	4.48 (3.74-5.36)	1.44 (1.15-1.80)	6.23 (5.38-7.22)	19.27 (12.96-28.64)
	Bilbao - Rekalde	30	4.55 (3.83-5.40)	2.82 (2.12-3.76)	8.21 (7.07-9.54)	26.48 (17.74-39.53)
	Bilbao - Santutxu	30	4.96 (3.76-6.54)	6.18 (4.86-7.85)	11.55 (9.13-14.60)	93.75 (55.66-157.92)
	Balmaseda	37	4.65 (3.94-5.48)	1.77 (1.32-2.38)	6.91 (5.88-8.13)	24.46 (17.59-34.02)
	p value		.910	**.000**	**.000**	**.000**
Body mass index, kg/m²	< 25	37	4.94 (4.06-6.02)	2.81 (2.05-3.86)	8.52 (7.01-10.35)	33.90 (21.69-52.98)
	25-29	45	4.76 (4.06-5.59)	2.72 (2.07-3.56)	8.13 (6.87-9.62)	34.51 (22.88-52.03)
	> 30	45	4.33 (3.67-5.11)	2.16 (1.66-2.83)	7.30 (6.35-8.39)	29.20 (20.73-41.13)
	p value		.291	.199	.203	.595
Weight loss ≥ 5 kg	Yes	63	4.68 (4.19-5.24)	2.43 (2.03-2.92)	7.84 (7.02-8.75)	33.04 (25.50-42.80)
(previous 5 years)	No	64	4.48 (3.71-5.41)	3.21 (2.33-4.43)	8.52 (7.47-9.72)	28.52 (19.52-41.67)
	p value		.764	.248	.555	.662
Weight gain ≥ 5 kg	Yes	45	4.89 (4.34-5.50)	2.61 (2.15-3.16)	8.22 (7.35-9.21)	31.45 (23.87-41.43)
(previous 5 years)	No	82	4.26 (3.56-5.09)	2.40 (1.78-3.24)	7.42 (6.23-8.85)	34.07 (22.66-51.24)
	p value		.195	.643	.318	.744
Level of education	Pre-university	91	4.96 (4.43-5.56)	2.27 (1.87-2.76)	7.96 (7.14-8.89)	29.74 (22.71-38.94)
	University	36	3.96 (3.27-4.80)	3.32 (2.48-4.43)	7.85 (6.44-9.56)	40.03 (26.09-61.44)
	p value		**.044**	**.041**	.891	.252
Social class	Manual	54	4.87 (4.22-5.62)	2.54 (1.97-3.27)	8.26 (7.24-9.41)	29.68 (21.15-41.65)
	Non manual	73	4.50 (3.92-5.16)	2.52 (2.03-3.13)	7.70 (6.72-8.82)	34.49 (25.31-47.00)
	p value		.439	.967	.483	.526
Intake of local produces	Never, occasionally	37	4.64 (2.48-6.80)	2.57 (0.25-4.89)	8.02 (5.86-10.19)	33.09 (30.61-35.58)
	Weekly, daily	90	4.66 (2.58-6.74)	2.52 (0.36-4.67)	7.89 (5.82-9.97)	31.81 (29.57-34.05)
	p value		.965	.907	.880	.878
Had had children	No	17	4.72 (3.68-6.06)	2.09 (1.31-3.33)	7.34 (5.59-9.64)	31.83 (17.88-56.68)
	Yes	54	4.54 (3.84-5.37)	2.13 (1.64-2.76)	7.42 (6.35-8.67)	29.97 (21.05-42.69)
	p value		.915	**.055**	.283	.827
Had breastfed	No	12	4.22 (2.96-6.04)	2.79 (1.71-4.54)	7.43 (5.20-10.62)	55.30 (28.54-107.15)
	Yes	42	4.64 (3.82-5.62)	1.97 (1.45-2.67)	7.42 (6.23-8.83)	25.16 (16.84-37.60)
	p value		.657	.283	.994	**.069**
Menopause	No	29	4.31 (3.58-5.20)	1.71 (1.19-2.47)	6.64 (5.50-8.01)	24.87 (15.32-40.38)
	Yes	42	4.78 (3.91-5.84)	2.46 (1.85-3.26)	7.98 (6.63-9.60)	34.94 (23.86-51.15)
	p value		.719	**.014**	.108	.474

Table 5: Multiple linear regression including variables associated with the blood levels of PCDD/Fs, dl-PCBs, DLC (pg WHO-TEQ/g fat) and marker PCBs (ng/g fat). Percentage change and 95% confidence intervals.

		PCDD/Fs	dl-PCBs	DLC	Marker PCB
Geographical areas	Alonsotegi	-7.6* (-30.6 to 22.9)	-22.0 (-46.3 to 13.3)	-12.8 (-32.0 to 11.9)	-16.5 (-53.5 to 49.7)
	Bilbao - Rekalde	-7.4 (-30.0 to 22.5)	48.1 (2.8 to 113.3)	12.0 (-12.2 to 42.9)	16.6 (-34.2 to 106.5)
	Bilbao - Santutxu	25.9 (-6.6 to 69.8)	263.0 (145.7 to 436.2)	90.3 (46.6 to 146.9)	357.3 (148.3 to 742.2)
	Balmaseda	Reference	Reference	Reference	Reference
Sex	Female	Reference	Reference	Reference	Reference
	Male	1.3 (-21.8 to 31.0)	-7.7 (-34.1 to 29.2)	-3.9 (-23.3 to 20.3)	49.3 (-11.9 to 153.0)
Age	(years)	-0.1 (-1.1 to 0.8)	1.7 (0.5 to 2.9)	0.5 (-0.3 to 1.3)	2.6 (0.6 to 4.5)
Level of education	Non-university	Reference	Reference	Reference	Reference
	University	-32.7 (-47.8 to -13.2)	0.9 (-27.6 to 40.7)	-22.1 (-37.6 to -2.8)	-16.7 (-50.5 to 40.0)
Body mass index	(kg/m^2)	-1.6 (-3.4 to 0.2)	-2.3 (-4.6 to -0.01)	-1.4 (-3.0 to 0.2)	-4.6 (-8.2 to -1.0)
Weight gain in previous 5 years	No	Reference	Reference	Reference	Reference
	Yes	-17.1 (-33.3 to 3.0)	-19.4 (-39.3 to 7.1)	-17.3 (-31.5 to 0.0)	-11.0 (-42.9 to 38.8)
Women who had breastfed	No	Reference	Reference	Reference	Reference
(compared to the all rest of subjects)	Yes	-2.6 (-26.0 to 28.2)	-27.8 (-49.6 to 3.3)	-7.4 (-27.1 to 17.6)	-50.5 (-71.8 to -13.3)

* % change= (exp(b)-1) * 100; percentage change in OC levels associated with a change in the reference of the current category or an increase of one unit in the continuous variables.

Hihglights:

- This study shows a dramatic decrease in the mean values of OCs from 2006 and 2008 to 2013
- We found differences in mean OC levels in relation to de area of sample
- Men had significantly higher levels of dl-PCBs than women
- Levels of both dl-PCBs and marker PCBs were positively associated with age
- Individuals with lower level of education had higher levels of PCDD/Fs than participants with university qualifications
- Women who had breastfed had significantly lower levels of PCBs than the rest of all the participants