Time trends in serum organochlorine pesticides and PCBs in the general population of Biscay, Spain

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ABSTRACT

Despite the measures adopted to avoid the production and reduce environmental levels of organochlorine compounds (OCs), pesticides and polychlorinated biphenyls (PCBs), they continue to be detected in the human body. This study aimed to explore time-dependent factors, age and, especially, changes in the concentrations of PCBs and pesticides, OCs quantified for reasons of opportunity, in a general population cohort composed of the individuals from whom a second blood sample was obtained. Two cross-sectional samples were taken from 162 adults between 20 and 75 years of age, with a gap of two years, from four areas in Biscay selected for evaluating exposure to dioxins and metals from an urban waste treatment plant. The levels of eight pesticides and seven PCBs were measured for reasons of opportunity. More than 75% of the individuals had quantifiable levels of hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH), dichlorodiphenyldichloroethylene (p,p'-DDE) and PCBs 138, 153 and 180. Over this time, significant changes were observed; PCB 180 and HCB levels increased and PCB 138 and β-HCH levels decreased. This study shows a clear decrease of levels in relation to age, suggesting a cohort effect. The period (with two years increase) was not related to the decrease of levels in all age intervals, but a statistically increase of PCBs in older people was found. High body mass index was associated with lower PCB 180 levels and higher HCH levels. On the other hand, higher levels of HCB and β-HCH were seen in those who had lost weight prior to the study. Levels of HCB and β-HCH were also higher in women who had had children, though they were lower in those who breastfed. Levels of these same OCs (HCB and β-HCH) were higher in fish consumers while those of PCBs 138 and 153 were higher in those who consumed local produce, all these diet related trends being close to significance. The evolution levels in OCs were very different by area of residence. Efforts should continue to reduce exposure to these pollutants and assess whether levels in the general population fall with time.

Keywords: Serum, Pesticides, Polychlorinated Biphenyls, Organochlorine Compounds, Public Health Surveillance, Biomonitoring

Summary of acronyms:

BMI: Body mass index

EPIC: European Prospective Investigation into Cancer and Nutrition

HCB: hexachlorobenzene

HCH: hexachlorocyclohexane

IARC: International Agency for Research on Cancer

LOQ: limit of quantification

OCs: organochlorine compounds

PCBs: polychlorinated biphenyls

p-p’-DDE: dichlorodiphenyldichloroethylene

p-p’-DDT: p,p'-dichlorodiphenyltrichloroethane

REML : Restricted maximum likelihood

α-HCH: alfa-hexachlorocyclohexane

β-HCH: beta-hexachlorocyclohexane

γ-HCH: gamma-hexachlorocyclohexane

1. INTRODUCTION

Organochlorine pesticides are a group of chemical compounds that are very effective against a wide variety of insects and other pests. The drawback of using them is their toxicity and high persistence. Among them, p,p'-dichlorodiphenyltrichloroethane (p,p'-DDT), and its main metabolite dichlorodiphenyldichloroethylene (p,p'-DDE), beta-hexachlorocyclohexane (β-HCH) and hexachlorobenzene (HCB) are the most commonly found in the environment (WHO, 2003). In the past, p-p'-DDT was widely used as an insecticide in agriculture, as well as in public health programmes against the malaria vector. Once absorbed, p-p'-DDT is metabolized to p-p'-DDE, a more stable and persistent form in the body. As a consequence, this compound is considered a good marker of chronic exposure (Jaga and Dharmani, 2003). Though prohibited in Spain in the seventies, p-p'-DDT is still being used in some countries (Rogan and Chen, 2005). As for HCH, while it has several isomers, only lindane (gamma-hexachlorocyclohexane) has insecticidal properties. In the production process of lindane, various HCH isomers are produced and β-HCH is the most persistent. Hexachlorobenzene (HCB) was used as a fungicide, but today is an industrial by-product (WHO, 2003). Lastly, Heptachlor epoxide and Endosulfan were used as an insecticides and acaricides. Endosulfan became a highly controversial agrichemical due to its acute toxicity and role as an endocrine disruptor (WHO, 2003).

Another group of organochlorine compounds (OCs) is polychlorinated biphenyls (PCBs). They are not naturally produced and they have been used in many industrial applications, including as dielectric fluids in capacitors and transformers and additives in pesticides, paints and plastifying agents, as well as pesticides. These compounds are also lipophilic and tend to bioaccumulate. There are more than 200 types of PCBs, those most commonly found being 118 and, above all, 138, 153 and 180. Notably, IARC recently upgraded PCBs to Group 1, as human carcinogens (IARC, 2013). It is believed that the exposure of the general population to these compounds is decreasing (Hagmar et al., 2006; Porta et al., 2012; Petrik et al., 2006; Sjödin et al., 2004).

Diet, through the fat of fish and meat as well as other fats, is the most common source of exposure of OCs pesticides or PCBs, in the general population (WHO, 2003; Domingo and Bocio, 2007). The load of these compounds in the body remains a concern across the world, due to their potential adverse effects on health and the environment. In the Basque Country, the intake of pesticides through the diet was found to be very low, with values of less than 7% of the tolerable daily intake (Jalón et al., 1997).

In Spain, despite most having been banned in the seventies and eighties, relatively recent studies have detected residues of OCs in foodstuffs, as well as in the general population (Agudo et al., 2009; Carreño et al., 2007; Jakszyn et al., 2009; Luzardo et al., 2006; WHO, 2003). Among publications available, two studies are of particular interest: one analyzing the concentrations of organochlorine pesticides in serum in a representative sample of the general population in the Canary Islands (Zumbado et al., 2005) and the other assessing organochlorine pesticides and PCBs in serum in a representative sample of the population in Catalonia (Porta et al., 2010). In both studies, DDE was present in more than 80% of the samples. In addition, it has been found that there is considerable prenatal exposure to these substances (Vizcaino et al., 2011).

Given their high persistence and liposolubility, it is possible to study the presence of these compounds in adipose tissue, blood serum and breast milk (LaKind et al., 2001). The advantage of serum is that it can be collected from any individual, although the amount of fat is lower than in other biological matrices.

In relation to time trends, Petrik et al., (2006) observed a 30% decrease in the levels of PCBs and organochlorine pesticides comparing two groups whose samples were taken in 1998 and 2002 in Slovakia, and Porta et al., (2012) found 34-56% decreases in OCs between 2002 and 2006 in Barcelona (Spain). Although these studies were carried out in general population samples, they were cross-sectional study designs with great inter-individual variability, in which various confounding factors could influence the results. The cohort studies, however, have another limitation for investigating trends in OCs levels in that, over the study period, the age of the participants also increases. With adjustments, considering these two time-dependent factors, age can be an indicator of the birth cohort and of the timescale for the changes in OCs. Older cohorts have had more exposure and the period is related to the balance between current exposure and metabolic clearance of the compound. As well as these two time-dependent variables, exposure is related to geographical area and sex. These four variables are clearly associated with the observed variability (Porta et al., 2008).

In February 2006 and February 2008, the levels of dioxins and furans and heavy metals were quantified in blood samples of the general population in Biscay (Basque Country) in order to evaluate their exposition from an urban waste treatment plant (Zubero et al., 2010, 2011). The aim of this study was to explore time-dependent factors, age and, especially, changes in the concentrations of PCBs and pesticides, OCs quantified for reasons of opportunity, in a cohort composed of the individuals from whom a second blood sample was obtained.

2. METHODS

2.1. Population and sample:

Systematic random sampling was used, stratifying by age and sex, from the municipal census provided by the local councils of four areas in Biscay, two towns and two districts in the city of Bilbao, within a 60 km radius. Two of the four areas selected were located close to a waste incinerator (the town of Alonsotegi and the district of Rekalde in Bilbao); while the other two were further away (the district of Santutxu and the town of Balmaseda). Initially, 120 individuals aged between 20 and 75 years old were selected for each area, 80 candidate participants plus potential substitutes. These 480 individuals received a letter explaining the objectives of the study and a telephone call to invite them to participate. People known to be exposed to OC pesticides, PCBs, dioxins or furans at work were excluded. To compensate for people declining to participate and thereby complete the target sample size for the study, neighbourhood volunteers from social associations and, finally, people attending the local health centres were included. In 2006, 30.7% of participants were recruited through the census, 43.5% were neighbourhood volunteers from social associations and 25.8% were recruited in the health centres. In 2008, a total of 69.6% participants had taken part in the previous survey, while 11.3% were neighbourhood volunteers and 19% were recruited from the health centres.

This analysis was based on the 162 individuals who completed both surveys. All participants signed an informed consent form prior participation in the study. The study was approved by the Clinical Research Ethics Committee of the local referral hospital.

2.2. Sampling and Laboratory Analysis

A blood sample was taken from each participant. They were not required to fast prior to the blood collection, but were recommended to avoid eating fats the night before. Samples were taken, treated, stored and transported in accordance with the conditions established by the reference laboratories (Patterson et al., 1991). Analyses were carried out in the Public Health Laboratory of Gipuzkoa, following a previously described method (Goñi et al., 2007).

The levels of the following eight organochlorine pesticides were analyzed: HCB, α-HCH, β-HCH, γ-HCH, heptachlor epoxide, endosulfan beta, p,p'-DDE and p,p'-DDT. The levels of the most common PCBs (PCB 28, 52, 101, 118, 138, 153 and 180) were also measured. The limits of quantification (LOQ) at both surveys were 0.10 ng/ml for PCBs and HCH isomers, and 0.20 ng/ml for the other pesticides.

The final results were corrected for enzyme-based measurements of lipid concentration and were expressed in nanograms of compound per gram of lipid (ng/g lipid). To express the concentration of the chemical residue as a function of the lipid content of the samples, we applied the algorithm of Philips et al. (1989). The adjustment is recommended because lipid changes in serum can be detected over time and gives a more stable estimate (Koppen et al., 2002), especially considering the need to allow for the contribution of the weight of the lipids in the calculation (Bernert et al., 2007).

2.3. Study variables

As time dependent variables, we considered age and study period itself. We recorded sex and geographical area; BMI, categorizing participants into three groups; any weight gain or loss of five or more kilograms in the last five years before the survey; number of children, and in the case of women, whether they were breastfeeding or not. In addition, socioeconomic status was defined using the Spanish adaptation of the British Registrar General classification system (Domingo-Salvany et al., 2000), grouping the social classes into three categories: high (classes I and II), medium (class III), and low (classes IV and V). Education level was also grouped into three categories: primary or less, secondary and university. Information was also collected on whether individuals often consumed locally grown produce, (yes or no), and finally, the consumption of saltwater fish, with three categories: 0-1, 2-4 and more than 4 times per week.

2.4. Statistical analysis

Before correcting for lipids, samples that were lower the LOQs were replaced with the LOQ divided by the square root of 2 (CDC, 2005). Statistical analysis included OCs with quantification frequency >75%. Logarithmic transformation of the lipid-corrected data brought it closer to a normal distribution and reduced the variance. For hypothesis testing, the McNemar test was used for qualitative variables. Geometric means and 95% confidence intervals were calculated and the Student's t-test for paired samples was used for comparing the means of the biological indicators between 2006 and 2008 in the each year's analysis. The Student's t-test for independent samples and analysis of variance were employed for comparing the means of variables with two or more categories, respectively, using the trend test for ordinal variables in the each year's analysis.

Determinants of the OCs were explored taking into account the intra-individual correlation between surveys using mixed-effects linear models (Pinheiro and Bates, 2000). The concentration of each compound expressed in ng/ml was logarithmically transformed and adjusted using a specific model. Starting from a full model, variables were excluded using a backward selection process, using a p-value above 0.10 in the likelihood ratio (LR) test as the exclusion criterion. The lipid variable was considered as an independent term of the models (Schisterman et al., 2005). In all the models, regardless of their statistical significance, we adjusted for the following variables: lipids (logarithmically transformed), time point/survey, age, geographical area and sex. Final models were estimated by restricted maximum likelihood (REML). The percentage of the unexplained variance that was attributable to the individual (intraclass correlation coefficient) was estimated as the quotient between the estimate of the variance of the intercept random effects and the sum of the variance of the intercept random effects plus the variance of the within-group errors, $\rho = \sigma^2_{between} / (\sigma^2_{between}+\sigma^2_{within})$ (Pinheiro and Bates, 2000).

The presence of interactions with the survey/time point variable was explored to assess whether any study variables interfered with the changes in OCs levels over time. The approximate normality of residuals was confirmed graphically. It was also explored whether there was a linear relationship between the variable age and the concentration of the compounds analyzed. To avoid individual observations having an excessive influence on the results, the cases with intra-individual Pearson residuals greater than 4 in absolute value were removed from the final models. The level of significance was set at $\alpha=0.05$. Data were analyzed using the SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL) statistical package and R version 2.15.3 (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org).

3. RESULTS

Table 1 describes the characteristics of the 162 participants of the 2006 and 2008 surveys. Table 2 shows the number of samples with values above the LOQs. We did not detect PCBs 52 or 101, endosulfan beta, heptachlor epoxide or α-HCH in any of the surveys. Nevertheless, all individuals had a quantifiable amount of at least one OC. In the second survey, we found a higher proportion of cases above LOQ of PCBs 28 ($p= 0.006$) and 118 ($p< 0.001$) and a lower proportion of detectable cases of p-p'-DDT (0.007) and ß-HCH ($p= 0.015$). Results for PCBs 28, 52, 101 and 118, and heptachlor epoxide, γ-HCH and p-p'-DDT were quantifiable in less than 75% of the samples in both years and, hence, were excluded from the subsequent statistical analysis.

Analyzing the mean levels of the three PCBs and three OC pesticides most commonly found (> 75% above LOQ), and changes therein between the measurements at the two time points, we observed that p-p'-DDE had the highest mean concentrations, followed by HCB and the PCBs 153 and 180 in both years (Table 3). Over the two-year period, we observed a significant increase in the mean levels of PCB 180 and HCB, and a significant mean decrease of PCB 138 and β-HCH.

The supplementary table shows the relationship between the socioeconomic variables and the mean values of OCs for the two survey years. The age was significantly positively associated with the concentration of all the OCs (p< 0.001, for all the OCs). We found statistically significant differences between the different areas, with a non-homogenous geographical pattern. Men had significantly higher levels of PCB 180, while women had significant higher levels of HCB and ß-HCH. Women who had had any children showed significantly higher mean levels of OCs than women who had not, and there was no statistically significant difference between those who had and had not breastfed their children. With respect to BMI, there was a significant gradient, with higher mean levels of HCB, b-HCH and p-p'DDE in obese and overweight people. Body weight change before the first survey was related to a significant trend, with higher levels of the three PCBs (138-153-180) in those who had lost weight. Individuals from the lowest social class and with the lowest level of education had significantly higher levels of all the OCs, except for p-p'-DDE, where no differences were observed. The consumption of local produce was not significantly associated with the concentration of OCs but the frequency of fish consumption was positively associated with the concentration of all OCs except for PCB 180.

Figure 1 show the distribution of OCs in 2006 and 2008 (period, with an increase in age of two years) by age (year of birth or cohort). All OCs showed a clear decrease of levels over the date of birth. Only ß-HCH showed decrease of levels across the sampling period in all cohorts. Among older people levels of PCBs and HCH increased.

After adjustment (Table 4), between 2006 and 2008 (sampling time), there was a significant decrease in the levels of PCBs 138 and 153, ß-HCH and p-p'-DDE, but not of PCB 180 or HCB. The levels of all the OCs significantly increased with age (p<0.01). By area of residence, we observed a high geographical variability, with significant differences for PCB 180, HCB and p-p'-DDE, the pattern being consistent for PCB 180 and HCB, with higher levels in the most rural area, Balmaseda. As showed before adjustment, there were significant higher levels of PCB 180 in men than in women, but HCB and ß-HCH levels were significantly higher in women. Higher BMI levels were significantly related to low levels of PCB 180 and to high levels of HCB. A significant increase in the levels of HCB and ß-HCH was associated with having lost weight before the first survey. Similarly, the levels of these two pesticides were significantly higher in women who had children, but significantly lower in those who had breastfed, in relation to those women who had not children and did not breast-feed them, respectively. In addition, there were significant increases in the levels of these two pesticides among those who consumed saltwater fish. We did not observe any significant associations between the levels of OCs and consumption of local produce, social class or level of education. The intra-class correlation coefficients ranged between 0.73 to 0.83 for PCBs, HCB and ß-HCH, and 0.95 for p-p'-DDE, indicating the elevated persistence of these compounds in the organism.

Table 5 shows the interactions observed between the sampling time when the blood samples were taken for the analysis and the variables entered in the regression models of Table 4. It indicates whether changes in various subgroups were significantly different. No significant interactions were observed in the case of ß-HCH. We found a significant interaction between sampling time and age, were older people increased the levels of PCBs, but decreased this of p-p'-DDE. In addition, we observed that the OCs level changes varied among the geographical areas, with a trend towards the decreases in levels being larger in Alonsotegi and Rekalde and smaller in Santutxu and Balmaseda, where PCB 180 actually increased significantly. Other variables, such as parity, breastfeeding and BMI were not associated with the changes in OCs levels. On the other hand, there were trends towards the levels of HCB decreasing in those who had lost weight and increasing significantly in those who gained weight.

4. DISCUSSION

All individuals had detectable levels of any of the analyzed OCs in their blood. Moreover, PCBs 138, 153 and 180 and pesticides HCB, ß-HCH and p-p'-DDE were detectable in more than 75% of individuals.

The presence of samples above the LOQ in the blood and the mean levels of PCB 180 and HCB increased over the study period. Only the levels of ß-HCH and PCB 138 decreased significantly. For all the other OCs, either there were no significant changes. The fact that the use of these substances is prohibited in our setting is not sufficient for them to totally disappear from our bodies or even that the balance between intake and clearance to favour to their elimination from the body. Porta et al., (2012), Petrik et al., (2006), Sjödin et al., (2004) and Hagmar et al., (2006) observed more marked decreases in OCs than we found in this study. Nevertheless, this study only covered a period of two years. It included repeated measurements in the same individuals, and hence the results show the balance between OCs intake and metabolism. Hagmar et al., (2006), also observed decreases in the levels of PCB 153 by 34%, p-p'-DDE by 55% and HCB by 53% in a sample of 39 individuals over 10 years. On the other hand, Tee et al., (2003) did not observe a reduction of the total PCBs among 78 not freshwater fish consumers from 1980 to 1994, but only freshwater fish consumers, with a reduction of fish consumption. Hovinga et al., (1992) did not observe decrease of PCB levels among 95 not freshwater fish consumers from 1982 to 1989, with a little decrease among consumers.

In our study there was a marked association between age and all the OCs, suggesting that these compounds are increasing in the body (age effect). However, the design of this study with repeated measures on the same individuals can show that this increase does not coincide with the actual time, and this suggests more a cohort effect, as Porta et al., (2008) referred. Nøst et al., (2013) observed throughout five surveys of a cohort of 53 men, a decrease in the levels of PCB 153 in relation to age, controlling for birth date, using an age-cohort-period graphic analysis.

The association between age and OCs has been widely reported in the literature (Porta et al., 2008; Jakzsin et al., 2009). Further, in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort in Spain, the levels found in samples collected between 1992 and 1996 for PCBs (Agudo et al., 2009) and pesticides (Jakszyn et al., 2009) were higher, especially in the Gipuzkoa subset, than in this study for all the pesticides and PCBs, except for PCB 180. Gipuzkoa region is close to Biscay (both are in the Basque Country, Spain) with similar social and industrial structure as well as diet. On the other hand, the EPIC study used blood samples of people aged between 35 and 65 years old (birth year from 1927 to 1961), increasing the comparability between both studies. This suggests that the association observed in these studies is not only due to an accumulative effect with age but are also due to a cohort effect.

This study provides analysis not only of changes in OCs levels, but also of interactions with the sampling time point. Older individuals increased the levels of PCBs, but decreased this of p-p'-DDE. An explanation of this observation would be that the current exposure of PCBs were greater in older. On the other hand, the interaction was negative in the case of p-p'-DDE, suggesting a faster decrease among elders, individuals with higher levels of these compounds.

In our study, the levels of PCBs were lower and of pesticides were higher among women than among men, as reported by other authors (Agudo et al., 2009; Cerná et al., 2008; Charlier and Plomteux, 2002; Costopoulou et al., 2006; Jakszyn et al., 2009; Jönsson et al., 2005; Park et al., 2007; Petrik et al., 2006; Porta et al., 2010; Tee et al., 2003), although this is not a constant finding (Bates et al., 2004; Cruz et al., 2003; Lino and da Silveira, 2006; Porta et al., 2012). On the other hand, in this study, we did not find differences in the evolution of OCs as a function of sex.

Another aspect to take into account to understand the distribution of OCs is geographical variability (Bachelet et al., 2011; Porta et al., 2012). The levels of OC pesticides in our study were lower than those found in other areas in Spain, namely, Catalonia (Porta et al., 2010), Andalusia (Carreño et al., 2007; Cerrillo et al., 2006) and the Canary Islands (Zumbado et al., 2005; Luzardo et al., 2006). On the other hand, the levels of PCBs were slightly higher than in Catalonia (Porta et al., 2010) and much higher than in the Canary Islands (Henríquez-Hernández et al., 2011). Both of these observations may be explained by the fact that the Basque Country has a low level of agricultural activity but a considerable industrial sector. In our study, after adjustments, we observed significant differences between urban districts and towns less than 60 km apart, with different patterns of changes in levels over time. That is, the balance between intake and metabolic clearance of OCs did not seem to be homogenous across the groups, even in closely located areas, suggesting a different pattern of exposure as a function of the area, given that the elimination should be similar, after adjustment for confounding variables. PCBs and OC pesticides are not related to emissions from waste incinerators. In this study most of the pollutants showed higher means in the areas far away from the plant.

The consumption of saltwater fish was associated with higher levels of all the OCs studied, except for PCB 180. In the Basque Country, there is a high consumption of fish (81.21 g/day; Government of the Basque Country, 2007). Indeed, in the EPIC study, comparing 19 zones in 10 European countries, it was concluded that the consumption of fish and fish products was highest in the Basque Country, it being mainly white fish (Welch et al., 2002). Kvalem et al., (2009) reported that the greatest source of PCBs (and dioxins) was oily and semi-oily fish. In a review of Spanish studies, Gasull et al., (2011) concluded that the consumption of animal products (fish, milk and dairy products and meat) was associated with higher levels of OCs in the body. Greater consumption of fish (saltwater fish) has also been associated with higher levels of PCBs and p-p'-DDE (Bachelet et al., 2011), as well as of PCBs (Bräuner et al., 2011; Llop et al., 2010) and organochlorine pesticides (Bräuner et al., 2012). Petrik et al., (2006) found an association between the decrease in levels of PCBs and organochlorine pesticides and the decrease in the intake of fish in their second study survey. Similarly, Hagmar et al., (2006) related decreases in the levels of PCB 153, HCB and p-p'-DDE to changes in BMI and the consumption of Baltic Sea fish. Another Norwegian study reported an association between the consumption of fresh fish liver oil and seagull eggs in a sample of people with a diet based on seafood with higher levels of PCBs, but not of p-p'-DDE (Rylander et al., 2009). Similarly, the EPIC study (which is designed to assess the effect of diet on the health) found a significant association between the concentration of PCBs and the consumption of fish (Agudo et al., 2009). In contrast, other research has not found an association between the levels of pesticides and diet (Jakszyn et al., 2009; Ibarluzea et al., 2011). In short, it seems that the consumption of fish is associated with an increase in the levels of OCs in the body, in particular PCBs. On the other hand, our study did not show that eating fish changed the trend in OCSs levels over time. Fish consumption may be related to the body burden rather than changes therein.

No association was found among consumers of locally grown products and PCBs or pesticides. Bräuner et al., (2012) found an association between the levels of organochlorine pesticides and the consumption of fruits and vegetables. In any case, the consumption of local products was not associated with an increase in OCs levels over the study period, suggesting that it is not currently a significant source of these compounds.

Another controversial issue in the literature regarding OCs has been the influence of body weight. This study found, after adjusting for other variables, higher levels of HCB in high BMI levels, while the opposite pattern was observed for PCB 180. Porta et al., (2012) did not find a clear pattern with regards to the relationship between BMI and OCs, while Bräuner et al., (2012) observed higher levels of pesticides with higher BMI, as we found in this study. On the other hand, Bachelet et al., (2011) found a negative association between PCB concentrations and BMI in women, as we found for PCB 180 in our study. Tee et al., (2003) observed also lower levels of total PCBs in high BMI levels.

In any case, there was a consistent pattern with loss of body weight prior to the first survey, with an increase of levels for HCB and ß-HCH. In contrast, we found a significant increase in the levels of HCB from 2006 to 2008 in those who had previously gained weight. Porta et al., (2012) also noted lower levels of OCs in individuals who had gained weight in the previous 6 months, although these results were not adjusted. Bachelet et al., (2011) found that in women, after adjustments, the higher the BMI the higher the levels of p-p'-DDE and the lower the levels of PCBs, as well as observing a decrease in these compounds in people who had gained weight in the previous 10 years. Therefore, the role of the BMI in determining the levels of OCs is not clear, not even the direction of the association (Moysich et al., 2002). There may be differences in the metabolism of OCs as a function of BMI. Wolff et al., (2005) suggested that greater quantity of fat in obese people may imply that metabolic clearance of OCs from the exposure earlier in life would be slower. Certainly, BMI and weight change do seem to modify the concentration of pollutants in blood (Carreño et al., 2007).

Parity and breastfeeding were found to be associated only with the levels of ß-HCH and HCB after adjustments, with an increment and a decrement, respectively. Porta et al., (2012) did not observe significant differences in the concentrations of OCs either of these variables. Similarly, the EPIC study did not find a significant trend between PCBs and maternal breastfeeding, and even found lower levels in those who did not breastfeed (Agudo et al., 2009). Ibarluzea et al., (2011) and Llop et al., (2010) showed decrease of levels of OCs in women breastfeeding during a long period of time. Social position, measured by social class or by the level if education has been associated with the levels of OCs found in biological samples (Ibarluzea et al., 2011; Porta et al., 2012), nevertheless we did not observed such an association between social position and the levels of OCs in our study, after adjusting for age, time point, sex and geographical area.

The cohort nature of this study restricts the inter-individual variability, characteristically high in cross-sectional studies. On the other hand, some aspects could reduce reliability to the study; the small sample size, the fact that they were volunteers and the short time period between the two surveys (2 years). Nevertheless, due to difficulties in the recruitment and, especially, in the maintenance of the willingness among the participants with repeated measurements, it would have been even harder to conduct this study with a longer period between surveys.

To conclude, these results indicated that the general adult population of Biscay has been exposed to a greater or less extent to OCs and continues to be exposed to many of these compounds. In this population, the levels of these persistent pollutants are within the range observed by other authors and we suspect that there is low level diffuse exposure, probably through diet. We observed a downward trend in the levels of OCs in the general, the exception being PCBs and HCB in older people. The measures taken after the banning of these compounds or following adoption of the Stockholm Convention seem to have had a beneficial effect, but there is ongoing exposure to OCs, the effects of which are still unknown. Further research may by necessary to elucidate how dietary habits or other factors could explain the differences in evolution of OC levels as well as the effects of this exposure on health.

Contributors: All authors contributed to various aspects of this paper. JJA and JMI designed the study. JJA, MM and MBZ analysed the data. FG and CJ analysed OCs in serum samples. MBZ, MM, FG, CJ and FB revised the design of the study and the results. MBZ and JJA redacted the manuscript and the other authors participated in the review of the different drafts and approved the final version.

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Table 1 Description of the study sample

Variable	*N*	(%)
All participants	162	100
Origin of the sample		
2006		
Census volunteers	50	30.9
Neighbourhood volunteers	70	43.2
Health centres	42	25.9
2008		
Previous volunteers	113	69.8
Neighbourhood volunteers	18	11.1
Health centres	31	19.1
Zone		
Alonsotegi	44	27.2
Rekalde	30	18.5
Santutxu	37	22.8
Balmaseda	51	31.5
Sex		
Woman	102	63.0
Man	60	37.0
Age (year) (mean SD)[a]	47.0	12.6
Age range in years (mean SD)[a]		
20–44	73	45.1
45–69	89	54.9
BMI (mean SD)	26.4	4.9
BMI group		
\25	71	43.8
25–29	63	38.9
C30	28	17.3
Weight change[b]		
Weight gain	27	16.7
No change	126	87.7
Weight loss	9	5.6
Level of education		
University or other tertiary	44	27.2
Secondary	54	33.3
Primary or less	64	39.5
Social class		
I–II (highest)	37	12.8
III	32	19.8
IV–V (lowest)	93	57.4
No. of children		
0	92	56.8
1–2	54	33.3
C3	16	9.9
Had children and breastfed		
No	15	21.4
Yes	55	78.6
Consumption of fish (portions/week)		
0–1	27	16.7
2–4	121	74.7
C5	14	8.6
Consumption of locally grown produce		
No	71	43.8
Yes	91	56.2

[a] Mean (SD) age at the first survey

[b] Body weight change in the 5 years (C5 kg) before the first survey

Table 2 Blood serum samples with values greater than the LOQ

Compounds	LOQ (ng/g lipid)	2006		2008		p^a
		n	(%)	*n*	(%)	
PCB 28	0.10	1	0.6	10	6.2	0.004
PCB 52	0.10	0	0	0	0	–
PCB 101	0.10	0	0	0	0	–
PCB 118	0.10	49	30.2	81	50.0	<0.001
PCB 138	0.10	161	99.4	160	98.8	1
PCB 153	0.10	162	100	160	98.8	–
PCB 180	0.10	161	99.4	162	100	–
Hepta chlorepoxide	0.20	0	0	0	0	–
HCB	0.20	135	83.3	145	89.5	0.013
a-HCH	0.10	0	0	0	0	1
b-HCH	0.10	142	87.7	129	79.6	0.015
c-HCH	0.10	2	1.2	6	3.7	0.219
p-p^0-DDT	0.20	28	17.3	15	9.3	0.007
p-p^0-DDE	0.20	160	98.8	158	97.5	0.500

[a] *p* value from exact McNemar test comparing the detection of compounds in 2006 and 2008; *p* values were two-sided

Table 3 Geometric means and 95 % CIs for OC concentrations measured in 2006 and 2008

Compounds	2006		2008		% ch[a]	p^b
	GM	95 % CI	GM	95 % CI		
PCB 138	68.0	62.2–74.3	64.8	58.7–71.6	–4.6	0.030
PCB 153	93.8	85.2–103.3	92.4	83.2–102.7	–1.5	0.500
PCB 180	85.6	78.3–93.6	92.4	84.0–101.7	7.9	0.004
HCB	99.3	85.7–115.1	107.2	92.7–124.1	8.0	0.028
b-HCH	46.2	40.6–52.5	36.2	31.6–41.4	–21.7	<0.001
p-p^0-DDE	191.8	165.8–221.9	187.4	164.2–213.7	–2.3	0.508

Compounds were quantified in ≥75 % of individuals in both surveys. Percentage change and comparison of means were applied to the logarithm of the concentrations. Concentrations are expressed as nanograms per gram of lipid

[a] $\%ch = 100 * ((GM\ OC_{2008}/GM\ OC_{2006})-1)$: percentage change of OCs are associated with the period of sampling

[b] Student *t* test for comparing the means of paired samples

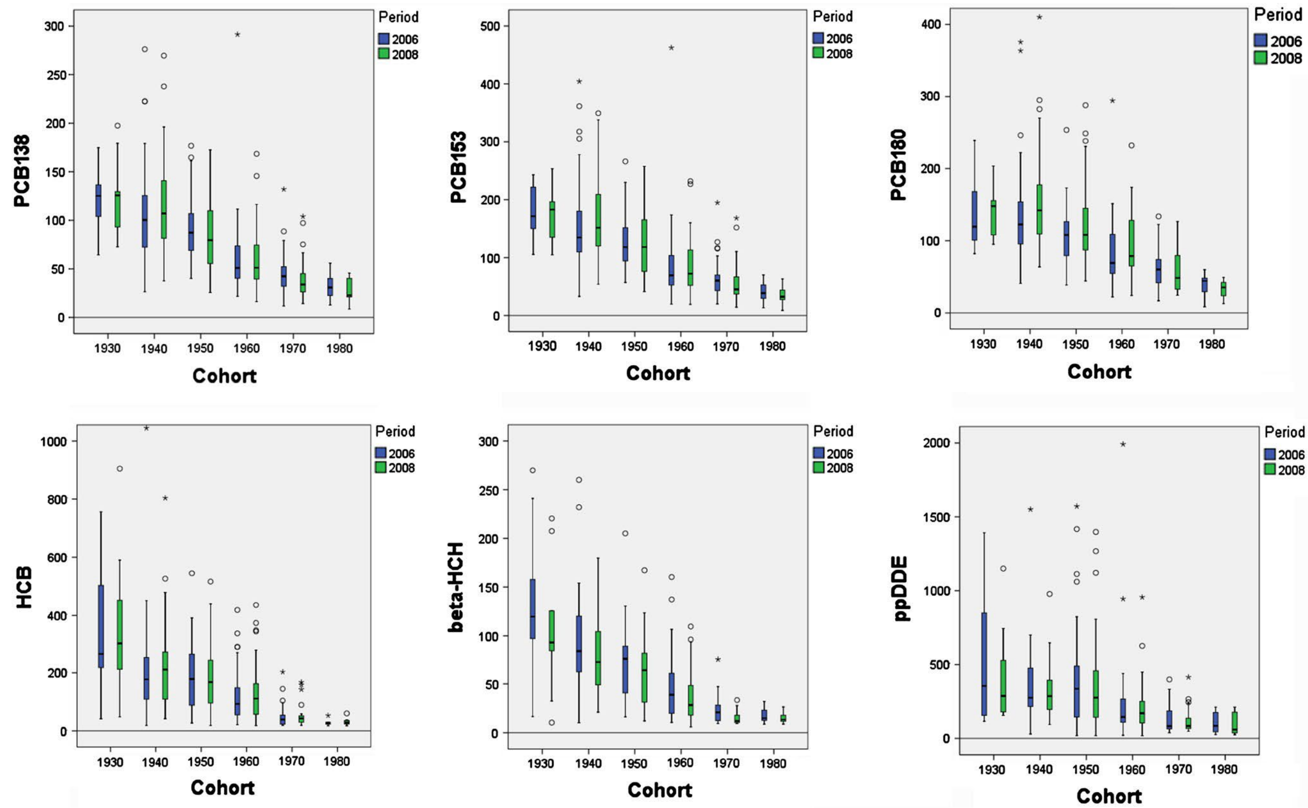

Fig. 1 OC distribution (ng/g lipid) by cohort (birth year [age]) and period (sampling year [age]) in 162 individuals (paired samples)

Table 4 Variables associated with PCBs and pesticides

Variables	PCB 138			PCB 153			PCB 180			HCB			ß-HCH			*p*-*p*^{0}-DDE		
	%ch[a]	95 % CI	*p*[b]	%ch	95 % CI	*p*	%ch	95 % CI	*p*	%ch	95 % CI	*p*	%ch	95 % CI	*p*	%ch	95 % CI	*p*
Lipids (mg/dl)[c]	80.3	50.2–116.5	<0.001	73.6	43.5–109.9	<0.001	77.3	44.6–117.5	<0.001	34.9	4.4–74.4	0.021	113.3	64.0–177.3	<0.001	31.1	8.8–58.1	0.005
Time point			<0.001			0.003			0.271			0.736			<0.001			0.006
2006	–			–			–			–			–			–		
2008	−9.4	−13.3 to −5.4		−6.6	−10.7 to −2.3		3.0	−2.2 to 8.4		1.1	−5.1 to 7.7		−29.0	−33.5 to −24.1		−6.0	−10.0 to −1.8	
Area			0.144			0.060			0.019			<0.001			0.203			0.026
Alonsotegi	–			–			–			–			–			–		
Rekalde	5.1	−14.3 to 29.0		0.0	−19.5 to 24.2		−4.9	−21.3 to 15.0		−7.1	−28.0 to 19.9		−0.3	−21.8 to 27.0		17.6	−17.4 to 67.4	
Santutxu	22.4	0.3–49.4		24.5	0.8–53.9		8.4	−9.3 to 29.4		19.2	−6.4 to 51.7		9.0	−13.4 to 37.2		63.6	18.5–125.8	
Balmaseda	16.9	−2.1 to 39.6		19.9	−0.7 to 44.7		24.0	4.9–46.6		41.5	12.7–77.8		−13.6	−30.5 to 7.3		19.2	−12.1 to 61.7	
Sex			0.220			0.091			<0.001			<0.001			0.011			0.669
Female	–			–			–			–			–			–		
Male	8.8	−5.1 to 24.7		13.2	−2.1 to 30.8		26.0	10.1–44.3		−43.4	−57.1 to −25.4		−27.6	−43.7 to −6.9		−5.0	−25.2 to 20.7	
Age (year)	3.6	3.1–4.2	<0.001	3.9	3.3–4.5	<0.001	3.5	2.9–4.0	<0.001	4.6	3.7–5.5	<0.001	4.9	4.0–5.7	<0.001	4.2	3.2–5.2	<0.001
BMI (kg/m^2)									0.003			0.031						
<25							–			–								
25–29							−18.2	−29.5 to −5.0		6.9	−13.1 to 31.5							
C30							−24.8	−37.4 to −9.8		39.5	7.9–80.5							
Weight change												<0.001			0.012			
None										–			–					
Gain										−2.1	−23.5 to 25.2		8.8	−13.2 to 36.3				
Loss										125.0	54.9–226.9		70.0	19.0–142.9				
Had children												0.001			0.009			
No										–			–					
Yes										88.7	31.0–171.7		57.8	11.4–123.5				
Breastfed												0.017			0.014			
No										–			–					
Yes										−32.5	−51.5 to −6.1		−32.0	−50.2 to −7.0				
Fish consumption (portions/week)												0.093			0.054			
0–1										–			–					
2–4										19.5	−5.2 to 50.7		11.5	−10.5 to 38.9				
C5										47.3	2.1–112.6		51.8	7.3–114.8				

Table 4 continued

Variables	PCB 138			PCB 153			PCB 180			HCB			ß-HCH			p-p^0-DDE		
	%ch[a]	95 % CI	p[b]	%ch	95 % CI	p	%ch	95 % CI	p	%ch	95 % CI	p	%ch	95 % CI	p	%ch	95 % CI	p
Consumes local produce			0.092			0.076												
No	–			–														
Yes	14.3	−2.3 to 33.7		16.1	−1.7 to 37.1													
ICC[d]	0.82			0.83			0.73			0.77			0.73			0.95		

Mixed-effects linear regression model in paired samples

[a] %ch = (exp(b)−1) × 100; percentage change in OCs were associated with a change from the reference to the current category or with an increment of one unit in continuous variables

[b] p value from the likelihood ratio test

[c] Natural logarithm of lipid concentration

[d] ICC = intraclass correlation coefficient: $\rho = \sigma^2_{between}/(\sigma^2_{between} + \sigma^2_{within})$

Table 5 Variables associated with the change in OC concentrations between 2006 and 2008: Evaluation of the interaction with time point in 162 individuals (paired samples)

Variables	PCB 138				PCB 153				PCB 180				HCB				p-p^0-DDE			
	%ch[a]	95 % CI		p[b]	%ch	95 % CI		p	%ch	95 % CI		p	%ch	95 % CI		p	%ch	95 % CI		p
Time point × age (year)	0.5	0.2	0.9	<0.001	0.5	0.2	0.8	0.003	0.5	0.1	0.9	0.015					−0.4	−0.7	−0.1	0.005
Time point × area[c]				0.071				0.026				0.038								<0.001
Alonsotegi	−16.6	−22.7	−9.9		−13.5	−20.0	−6.4		−1.6	−10.3	7.9						−7.7	−14.0	−0.8	
Rekalde	−7.4	−15.6	1.5		−11.2	−19.2	−2.3		−8.0	−17.7	2.8						−18.2	−25.2	−10.5	
Santutxu	−3.8	−11.5	4.5		1.2	−7.0	10.3		8.9	−1.5	20.3						−5.2	−12.2	2.3	
Balmaseda	−7.7	−14.1	−0.7		−2.7	−9.7	4.9		10.3	1.0	20.3						2.3	−4.4	9.6	
Time point × weight change																0.043				
None													–	–	–					

The models included the same variables listed in Table 4, and the interactions with time point were additionally included if they had a p value from the likelihood ratio test ≤ 0.10

[a] % ch = (exp(b)−1) $\times$ 100; percentage change in OCs levels were associated with a change from the reference to current category or with an increment of one unit in continuous variables

[b] p value from the likelihood ratio test

[c] The coefficients represent the joint effect of time point and area to improve interpretability. The p value is that for interaction