SERUM PCB LEVELS IN A REPRESENTATIVE SAMPLE OF THE SPANISH ADULT POPULATION: THE BIOAMBIENT.ES PROJECT

Huetos, O., Bartolomé, M., Aragonés, N., Cervantes-Amat, M., Esteban, M., Ruiz-Moraga, M., Pérez-Gómez, B., Calvo, E., Vila, M., BIOAMBIENT.ES, & Castaño, A. (2014). Serum PCB levels in a representative sample of the Spanish adult population: the BIOAMBIENT.ES project. *The Science of the total environment*, *493*, 834–844.

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Abstract: This manuscript presents the levels of six indicator polychlorinated biphenyl congeners (PCBs) (IUPAC nos. 28, 52, 101, 138, 153 and 180) in the serum of 1880 individuals from a representative sample of the Spanish working population recruited between March 2009 and July 2010.

Three out of the six PCBs studied (180, 153 and 138) were quantified in more than 99% of participants. PCB 180 was the highest contributor, followed by PCB 153 and 138, with relative abundances of 42.6%, 33.2% and 24.2%,respectively. In contrast, PCBs 28 and 52 were detected in only 1% of samples, whereas PCB 101 was detectable in 6% of samples.

The geometric mean (GM) for the sumatory PCBs138/153/180 was 135.4 ng/g lipid (95% CI: 121.3-151.2 ng/g lipid) and the 95th percentile was 482.2 ng/g lipid.

Men had higher PCB blood concentrations than women (GMs 138.9 and 129.9 ng/g lipid respectively). As expected, serum PCB levels increased with age and frequency of fish consumption, particularly in those participants younger than 30 years of age. The highest levels we found for participants from the Basque Country, whereas the lowest concentrations were found for those from the Canary Islands.

The Spanish population studied herein had similar levels to those found previously in Greece and southern Italy, lower levels than those in France and central Europe, and higher PCB levels than in the USA, Canada and New Zealand.
This paper provides the first baseline information regarding PCB exposure in the Spanish adult population on a national scale. The results will allow us to establish reference levels, follow temporal

trends and identify high-exposure groups, as well as monitor implementation of the Stockholm convention in Spain.

Response to Reviewers: Minor Corrections has been made according to reviewre comments

Majadahonda, June 17th 2014

Dear Adrian,

Many thanks for your comments and suggestions regarding our manuscript "SERUM PCB LEVELS IN A REPRESENTATIVE SAMPLE OF THE SPANISH ADULT POPULATION: THE BIOAMBIENT.ES PROJECT" Ref. No.: STOTEN-D-14-00725.

We have prepared a revised version that takes into account the point from the first reviewer. The suggestion and corresponding modification can be found in a separate file.

We have followed the electronic (online) submission process for the new version of the manuscript, and we have included a clean version and a version that shows all track changes according reviewer comment.

Thank you for your help and we look forward to hearing from you in due course.

Kind regards,

Argelia.

Head
Environmental Toxicology.
CNSA-Instituto de Salud Carlos III
Majadahonda (Madrid) Spain.

Reviewer 1 Comments:

Thank you for the critical reading of the manuscript.

Reviewers/Editor comments:

Reviewer #1:
All the questions I had raised have been answered by the authors and the additional information required provided.

The only issue I still find critical is the comparison with studies carried out in different years and, even more, the comparison with areas known to be among the most polluted in Europe, like Brescia.
About this latter matter, I find the comparison with studies carried out on Brescia useless and misleading, and definitely think it should be eliminated with any loss of the paper significance.

We have eliminated in the the text and in table 5 according to your suggestion.

Highlights

- 6 NDL-PCBs serum levels reported in a representative sample of Spanish adults.
- PCB congener 180 is the largest contributor to PCB load body burden.
- Serum PCBs levels increased with the age and the frequency of fish consumption.
- Basque country had the highest and Canary Islands the lowest PCB levels in the population.
- We confirm the general reduction in human PCB exposure over the last 20 years.

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Abstract

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Three out of the six PCBs studied (180, 153 and 138) were quantified in more than 99% of participants. PCB 180 was the highest contributor, followed by PCB 153 and 138, with relative abundances of 42.6%, 33.2% and 24.2%, respectively. In contrast, PCBs 28 and 52 were detected in only 1% of samples, whereas PCB 101 was detectable in 6% of samples.

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participants from the Basque Country, whereas the lowest concentrations were found for those from the Canary Islands.

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This paper provides the first baseline information regarding PCB exposure in the Spanish adult population on a national scale. The results will allow us to establish reference levels, follow temporal trends and identify high-exposure groups, as well as monitor implementation of the Stockholm convention in Spain.

Keywords: Polychlorinated Biphenyls (PCBs), Human Biomonitoring, Spain, Serum, Working Population.

1. Introduction

The growing interest in protecting human health from the adverse effect of chemicals in the environment, diet and lifestyle is reflected by the development of international policies of different scope (European environment and health strategy, Minamata Convention, UNEP 2013a,b; REACH Regulations, EC 2006; Stockholm Convention, UNEP 2004; etc). Polychlorinated biphenyls (PCBs) are organic chemicals used in a variety of applications, such as in electrical capacitors, fluorescent lights, paints, sealants for construction, etc. These compounds are chemically and thermally stable, resistant to degradation and highly lipophilic. As a result, PCBs bioaccumulate in the environment, living organisms and food chains thereby resulting in significant human exposure and, in some cases, high body burdens (Wang et al., 2007; World Health Organization, 2002, 2000).

PCBs are toxic to animals and humans and their toxicity is directly proportional to the degree of chlorination of the molecule. As such, PCBs are associated with a wide spectrum of adverse health effects and have recently been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) (Lauby-Secretan et al., 2013). PCB exposure is also associated with endocrine disruption, reproductive disorders (Buck Louis et al., Codru et al., 2007; 2010; Diamanti-Kandarakis et al., 2009; Porta, 2006; Rylander et al., 2005; Schell et al., 2013) and neurological, immunological and dermatological effects (Fitzgerald et al., 2008; Grandjean & Landrigan, 2006). PCBs can cross the placenta to the foetus and have also been detected in breast milk (Guo et al., 2004; Konishi et al., 2009; Park et al., 2008; Wang et al., 2007). All these characteristics justify their inclusion amongst the Stockholm convention "dirty dozen" chemicals (UNEP, 2004).

PCBs have been targeted for elimination since the 1970s. In Spain, environmental restrictions initially came into force in 1999 and were strengthened in 2006 (R.D. 1378/1999 and R.D. 228/2006). As a result, background PCB levels have decreased (Agudo et al., 2009; Harmag et al., 2006; Schuhmacher et al., 2013), although they currently appear to have stabilised (Porta et al., 2008).

At a global level, the Stockholm Convention banned PCB production and use in May 2001 (UNEP, 2004), and Regulation (EC) No. 850/2004 is the legal framework which gives effect to the provisions of this Convention in Europe. In Spain, the National Implementation Plan for the Stockholm Convention was approved in 2007 (http://www.magrama.gob.es/es/calidad-y-evaluacion-ambiental/temas/productos-quimicos/pnestocolmofinal_31enero2007_tcm7-3187.pdf) However, as an exception has been made to allow the use of PCB-containing equipment until 2025, important diffuse sources of PCBs continue to exist (Herrick et al., 2004), thus meaning that human exposure continues to occur. Amongst other actions, the treaty encourages the establishment of human biomonitoring (HBM) programs in the general population to check the effectiveness of treaty implementation. As a result, PCBs in humans have been measured in many countries over the last 20 years, thus allowing the elimination trend to be monitored. In Spain, although the PCB level in the general population has been measured in different regions of the country (Agudo 2009), such as Catalonia (Porta et al., 2012, 2010), the Basque Country (Zubero et al., 2009), Madrid (Martínez et al., 2009) and the Canary Islands (Henríquez-Hernandez et al., 2011), to date there has not been a nationwide study at a defined moment that is representative of the general population.

In compliance with the Spanish national implementation plan for POPs, in 2007 the Spanish Ministry of Agriculture, Food and the Environment funded a national HBM program. The purpose of this program was to enhance current understanding of the distribution of priority environmental pollutants, such as metals, pesticides, flame retardants, perfluorinated compounds, PCBs etc., in Spain and to establish reference levels within the Spanish population. The ministerial action was planned in two stages. The first stage (2007-2010) included the BIOAMBIENT.ES project, a nationwide cross-sectional study, with stratified cluster sampling, designed to cover all geographical areas (including the Canary Islands and autonomous city of Ceuta) and occupational sectors, and aimed at obtaining a representative sample of the Spanish workforce (Esteban et al., 2013; Perez-Gomez et al., 2013).

Herein we present the first baseline information regarding PCB exposure in the Spanish adult population on a national scale. The number of PCB congeners selected for analysis (IUPAC numbers 28, 52, 101, 138, 153 and 180) will offer a good picture of general exposure to the most representative non-dioxin-like PCBs (NDL-PCB) (Wingfors et al., 2006).

The results obtained will provide the first nationwide fingerprint of NDL-PCB burden in Spanish adults and will allow us to identify high-exposure regions or population groups as well as enabling comparisons with other countries. They will also allow environmental policymakers to establish the relative European or world-wide position of Spain regarding PCB exposure, to follow temporal trends and to check the degree of implementation of actions taken in compliance with the Spanish national implementation plan for POPs.

2. Materials and Methods

2.1. Study Population

From March 2009 to July 2010, a total of 1936 subjects residing in all 17 Spanish Autonomous Communities and the autonomous city of Ceuta were recruited. Participants were workers aged 18 years or older who had been resident in Spain for five years or more and attended the health facilities of the Societies for Prevention of IBERMUTUAMUR, MUTUALIA, MCPREVENCIÓN, MUGATRA, UNIMAT PREVENCIÓN, and PREVIMAC. The design and field work have already been described in detail elsewhere (Esteban et al., 2013; Perez-Gomez et al., 2013). Briefly, participants agreed to donate biological samples and to complete a short self-administered epidemiological questionnaire on environmental and lifestyle-related exposures. Of the 1936 subjects recruited, the amount of blood was insufficient for the analytical determination in 12 cases and no information was available on the stratification variables in 44 cases. As a result, PCB congeners were determined using the serum from 1880 individuals.

2.2 Ethical approval

This study was approved by both the Ethical Committee and the legal department of IBERMUTUAMUR. Participants were asked to provide written informed consent. Their collaboration was voluntary and altruistic; only a small token pen drive was given to the participants. The study was performed in accordance with legal/ethical principles and regulations concerning research involving individual information and biological samples, including Organic Law 15/1999 on Personal Data protection and its Regulations, Law 41/2002, on the Autonomy of Patients and rights and obligations relating to health information and documentation, as well as General Health Law 14/1986. Since the study involved the collection of blood samples, the principles of the Declaration of Helsinki and those contained in the UNESCO Universal Declaration on the Human Genome and Human Rights were observed.

2.3 Biological Sampling and Storage

Fasting blood specimens were collected in S-Monovette® neutral tubes (Sarstedt Nümbrecht, Germany) by trained healthcare staff. Blood samples were centrifuged within 5 hours of collection and transported at 4ºC within 96 hours post-sampling (Esteban et al., 2013). In the laboratory, samples were left to rest for approximately 60 min at room temperature, then the sample was centrifuged again and the serum separated. As the BIOAMBIENT.ES project included, among others, the study of several POPs in human serum, we tried to reduce volumes as much as possible. Thus, we prepared small serum aliquots (0.5 mL) in glass vials and stored them at -20ºC until analysis.

2.4 Extraction and Analysis

Individual standard solutions (10 ng/μL in isooctane) of the six PCBs (IUPAC numbers 28, 52, 101, 138, 153 and 180) were purchased from Ehrenstorfer (Augsburg, Germany). Individual and working standard solutions as well as $^{13}C_{12}$-PCB 118 internal standard solution (Wellington Laboratories Inc., Guelph, Canada) were prepared in hexane and stored in the dark at 4ºC (range 2-10ºC).

Because of the matrix effect, rabbit serum was used as "blank" in the spiked calibration curves. Rabbit serum was selected because it presents the lowest PCB levels when compared to horse, calf, pig and human serum samples. Rabbit serum blanks and blank reagent samples (demineralized water) were analyzed to identify any contamination during the analytical procedure.

Prior to extraction, all samples were spiked with a known quantity of a solution containing $^{13}C_{12}$-PCB 118 and deproteinized with formic acid (Merck, Darmstadt, Germany). These aliquots (0.5 mL) were extracted by solid-phase extraction (SPE) using an OASIS cartridge (Waters, Milford, USA) and the fat subsequently eliminated on-line by dropping the eluate directly onto a second glass cartridge (J.T. Baker, The Netherlands) containing layers of activated neutral silica gel and sulphuric acid (Merck, Darmstadt, Germany) modified silica gel (Gomara et al., 2002). The eluate obtained was evaporated to dryness under a slow stream of nitrogen in a TurboVap LV evaporator (Zymark, Hopkinton, MA; USA) then dissolved in 50 μL of hexane. All solvents used for extraction and clean-up were Pestipur quality (Lab Scan, Dublin, Ireland).

Finally, the extracts were transferred to a vial with a glass insert (Agilent Technologies Inc, Palo Alto, CA, USA) and a 2 μL aliquot was injected in pulse-splitless mode using an automatic injector, at a temperature of 260ºC, in an Agilent Technologies HP7890A gas chromatograph system with a mass spectrometer detector (HP 5975A). The source was set to operate in negative-ion chemical ionization mode (GC-MS/NCI), with methane as reagent gas and helium as carrier gas, both of high-purity grade (Air Liquide, Madrid, Spain). Samples were analysed using a DB-5-ms (or similar) capillary column (Agilent; 5% diphenyl, 95% dimethylpolysiloxane column, 15 m x 0.25 mm id. x 0.25 µm stationary phase). Temperatures were programmed as follows: MS interface temperature 270ºC; oven temperature 100 ºC for 4.2 min, 40 ºC/min to 200 ºC, then 7 ºC/min to 219 ºC, held for 0.2 min, then 56ºC/min to 275 ºC, held for 3 min to a final temperature of 300ºC, held for 3 min. The total runtime was about 17.24 min.

The PCB concentrations obtained were individually lipid-adjusted and total cholesterol and triglycerides were also measured (Bernert et al., 2007). PCBs with concentrations below the LOQ were reported as LOQ/2. Limits of quantification (LOQ) expressed in ng/mL and in ng/g lipid for serum samples can be found in Table 1.

External quality assurance during our analysis was guaranteed by means of participation in intercomparison exercises: in Round 3 of 2010 and Round 2 and 3 of 2011 of the AMAP Ring Test (National Institute of Public Health, Quebec, Canada), with a z-score of between 1.11 and -0.70, and in G-EQUAS 46 and 47 from 2010 and 2011, respectively (German External Quality Assessment Scheme, Erlangen, Germany). All values were within the range of tolerance.

2.5 Statistical analysis

Because of the complex survey design (multi-stage stratified cluster sampling) and the different selection probabilities of study participants (small geographical areas, non-service economic sectors, and women were over-sampled) (Perez-Gómez et al., 2013), all analyses were weighted to the distribution of the employed Spanish population in 2009 (National Institute of Statistics, 2009) and taking the effect of stratification and clustering on point and interval estimates into account.

Basic descriptive statistics were calculated for PCBs 138, 153 and 180 individually and for their sum $\sum PCBs_{138,153,180}$ (from this point forward we will use ∑PCBs to refer to the sum of these 3 PCB congeners). Statistics include the arithmetic mean (AM), geometric mean (GM) and their confidence intervals (95%), as well as percentiles 10, 25, 50, 75, 90 and 95 for the entire study population. In a second step, the same statistics were calculated for the following subgroups: men and women, economic activity sector (service activities and farming, industry and construction activities), geographical area (Northwest I (Galicia), Northwest II (Asturias, Cantabria), North (Basque Country), Northeast I (Navarre, La Rioja, Aragon), Northeast II (Catalonia), Central I (Castile and Leon), Central II (Madrid), Central III (Castile-La Mancha, Extremadura), East (Valencian Community, Balearic Islands), South I (Andalusia, Ceuta), South II (Murcia) and Canary Islands), sampling quarter (January–March, April–June, July–September, and October–December), age groups (≤29, 30-39, 40-49, ≥50 years), and fish and shellfish consumption (<once per week, once per week, 2/4 times per week, ≥5 times per week).

Multivariate linear regression models were fitted using log-transformed ∑PCBs levels as the dependent variable, and including age, sex (reference category was women), economic sector (reference category was services) fish and shellfish consumption (reference category was once per week), geographical area and sampling quarter. The exponentiated beta coefficients of the models represent the proportional change in the geometric mean of ∑PCB concentration for the different levels of the variables studied, compared to a reference category as independent variables.

Statistical analyses were carried out using survey modules for complex design studies (STATAv11.0), and maps were prepared using R and ArcView using the ratio between the geometric mean in each variable category and the geometric mean for all participants. Statistical significance was established at $p<0.05$.

3. Results

PCBs 138, 153 and 180, the main PCB markers of environmental pollution and those with the longest half-lives (penta- to heptachlorinated biphenyls), were quantified in at least >90% of the samples analyzed. As a result, a new variable was computed as the sum of PCBs 138, 153 and 180 (∑PCB). The median value of ∑PCB is 143.6 ng/g lipid, the geometric mean (GM) is 135.4 ng/g lipid (121.3–151.2 ng/g lipid) and the 95th percentile is 482.2. Samples below LOQ for PCBs 138, 153 and 180 were 0.88%, 0.57% and 0% respectively.

Congeners 28 and 52, which undergo fast enzymatic degradation in animals and humans, were detected in 1% of samples. PCB 28 was detected in only one person, with a value of around 7 ng/mL serum. PCB 101 was detected in just 6% of participants. As a result, these three PCB congeners were not included in the statistical analyses.

Table 2a shows the descriptive statistics for ΣPCBs in serum for all participants, whereas Tables 2b and 2c present the descriptive data for men and women separately. Men have slightly higher serum

PCB levels than women, with geometric means of 139.9 (95% CI: 124.3–157.3) and 129.9 ng/g lipid (95% CI: 129.9–147.1) respectively.

The highest contribution to the PCB load was found for PCB 180, with a GM of 55.97 ng/g lipid (95% CI: 49.5–63.2), followed by PCB 153, with a GM of 43.61 ng/g lipid (95% CI: 38.85–48.95), and PCB 138, with a GM of 31.89 ng/g lipid (95% CI: 28.81–35.30; Table 3).

As is generally the case for pollutants that bioaccumulate over time, an increase in the concentration of PCBs with age was found. Thus, PCB levels increase by approximately 56% per 10 years of age, and are slightly higher in men than in women, except for the older groups. On average, PCBs in men are 9% higher than those in women. However, there is a statistically significant interaction between sex and age, and these differences are mainly due to the contrast between PCB levels in the over-50 years age group (Figure 1).

No statistically significant differences were observed by economic sector, with the values only being significantly higher in workers who do not belong to the service sector in the case of the oldest group. Irrespective of the occupational sector to which they belong, men always have higher PCB levels than women.

As regards geographic differences, the concentrations of ΣPCB congeners in samples from Northeast and Northern Spain were higher than those reported in the South of Spain, with the GMs for Asturias-Cantabria and the Basque Country being 45% and 88% higher than the Spanish average, respectively. The lowest concentrations were found for the Canary Islands, with a GM of 78.9 ng/g lipid. Figure 2 presents the ratio of GMs for the serum ΣPCB concentration for different geographic regions.

As far as the distribution of PCB levels per sampling quarter is concerned, these levels were higher in the samples collected during the second quarter of the year (April–June), whereas the lowest GM was found for the last quarter (September–December). The biggest differences were found for PCB 180, with the value for the second quarter being almost twice that for the fourth.

Serum PCB levels increased with the frequency of weekly fish consumption. Thus, 44% of participants ate fish between two and four times a week, and 13% more often than that, with women consuming 10% more fish than men. People who reported eating fish less than once or once per week (12% and 31% of the participants, respectively) had lower PCB levels than those eating fish two or more times per week (57%). This increase is more evident in the youngest group. For a similar frequency of fish consumption, men have somewhat higher serum PCB levels than women, although the differences do not reach statistical significance. We did not find significant differences in serum PCBs among those subjects who reported eating fish more than twice per week and those eating fish four or more times per week (Figure 3).

The results of the multivariate analysis are presented in Table 4 for the whole population studied and for men and women separately. These results are consistent with the patterns of the basic descriptive statistics shown in Tables 2a to 2c, although significant differences become evident for some determinants. Thus, women had 9% lower serum PCB levels than men, and the oldest group (>50) had three times more PCBs in serum than the youngest group (<29 years) after adjusting for other factors. Body mass index (BMI) was not included in the models since it does not seem to significantly affect PCB levels.

4. Discussion

The work presented here offers the first data on body burden for the most relevant NDL-PCBs in Spanish adults.

As reported for many other industrialized countries and in previous studies in some Spanish regions, PCBs 138, 153 and 180 were detected in more than 90% of our population (Agudo et al., 2009; Bates et al., 2004; CDC, 2009, 2005; Cerna et al., 2008; De Felip et al., 2008; Glynn et al., 2000; Heudorf et al., 2002; Longnecker et al., 2000; Park et al., 2007; Patterson et al., 2009; Porta et al., 2012; Turyk et al., 2006; Zubero et al., 2009).

As is the case in many other countries, PCBs 180 and 153 can be assumed to be major contributors to the PCB load in the Spanish population, although the predominance of each congener varies between studies. The major contributor in BIOAMBIENT.ES was found to be PCB 180, whereas Zubero and co-workers found that PCB 153 was most prevalent in volunteers resident in the Basque Country, followed by PCB 180 and 138 (Zubero et al. 2009). A similar predominance was also found in a multiregional study conducted in five Spanish regions in 1992-1996 (Agudo et al., 2009), as well as in the Canary Islands in 1998 (Henriquez et al. 2011) and in Catalonia in 2002 (Porta et al 2010).

Also in accordance with other authors (Cerna et al., 2012), PCB congeners 28, 52, and 101 were, in general, below the limit of quantification (LOQ) in the general population except in the case of specific exposure to indoor air contamination (Kohler et al., 2005; Wingfors et al., 2006). Gabrio et al. (2000) and Schwenk et al. (2002) have reported elevated indoor PCB air concentrations (congeners 28, 52 and 101) in schools containing PCB caulking materials and Fitzgerald et al. have reported statistically significant associations between indoor-air and serum concentrations for PCB 28 (Fitzgerald et al., 2011).

PCB concentrations in humans are known to be significantly associated with different factors, the most significant of which is age due to their bioaccumulative character. A positive correlation between age and serum PCB levels was also observed in our study. Thus, the GM for $\sum PCB_{138,153,180}$ concentrations was almost three times higher in the oldest group (250.5 ng/g lipid) than in the youngest (72.0 ng/g lipid) as a result of past cumulative exposures and the long half-life of these pollutants (De Felip et al 2008; Porta et al., 2010, 2008; Wolff et al., 2007). In addition, environmental exposure has decreased with time since PCB production was banned. Older participants were born at a time when they were exposed to higher background levels and therefore they are likely to have accumulated a higher body burden throughout their lives (Collins et al., 2007). It has also been suggested that this correlation with age may be related to differences in PCB metabolism, which is lower in elderly people (Bates et al., 2004; Fangstrom et al., 2005; Park et al., 2007; Schulz et al. 2009; Wolff et al., 2007).

As regards the presence of PCBs in men and women, our results are consistent with the available literature (Agudo et al., 2009; Apostoli et al., 2005; Burns et al., 2009; Cerna et al., 2008; Henríquez-Hernández et al., 2011; Park et al., 2007; Petrik et al., 2006; Zubero et al., 2009) in which the sum of PCB congeners tended to be higher in men than in women. Some exceptions to this have been reported, such as the study by Porta and co-workers in Catalonia, in which women presented a statistically significantly higher median concentration of the most prevalent PCBs than men after adjusting for age and BMI (Porta et al., 2012).

There is no clear explanation for these gender-related differences in PCB levels.. The major determinants of human polychlorinated biphenyl (PCB) body burden include a mixture of external and internal factors, including source, route and timing of exposure along with individual determinants such as uptake, metabolism and clearance (De Caprio et al., 2005, Gladen et al., 2003; James et al., 2002). Some authors have justified the higher concentrations of PCBs in men than in women on the basis of differences in diet, occupational exposures or metabolism (Bates et al., 2004; Glynn et al., 2003; Patterson et al., 2009; Porta et al., 2010; Schaeffer et al., 2006; Wolff et al., 2007). In our study we observed much higher age-related increases for PCBs in men than in women, with women older than 50 presenting three times more PCBs than the youngest participating females and men in the oldest group presenting four times more than the youngest group after adjusting for other factors. The highest difference was found for PCB 180. Many factors may contribute to these differences. Metabolic differences between genders could be one of them. Breastfeeding could also be an additional route by which these compounds are eliminated therefore women who have had children and have breastfed may have lower concentrations of these compounds.

Diet may be an important source of PCBs in humans. In our population, fish consumption, which forms an essential part of the Spanish diet, has a clear influence on the increase of PCB levels in the youngest group of volunteers (<29 years), whereas this influence was not so evident in older groups (Figure 3). High levels due to contaminated food consumption have also been reported in other European studies (Abad et al., 2002; Zuccato et al., 2008). Agudo et al. (2009) explored the association between dietary factors and serum PCB concentrations. These authors selected five food groups, all of them of animal origin (meat, fish, seafood, dairy products (without milk) and fat and oils) and found that the serum PCB concentration was strongly associated with fish intake. Other studies have also shown that the major source of exposure to these compounds is the diet, particularly the consumption of fatty fish (Bocio et al., 2007; Bordajandi et al., 2006, 2003). However, fish consumption cannot justify the different PCB levels found for participants from different Spanish regions per se.

The geographical differences found in our results could be associated with the use of PCB-containing equipment in the different regions. The results of the multivariate analysis showed that the geographical differences in serum PCB concentrations persisted after adjusting for age, sex, economic sector, sampling quarter and fish consumption. In our study serum PCB concentrations were markedly higher in participants living and working in northern regions. These results are consistent with data provided by the Spanish Ministry of Agriculture, Food and the Environment, which show that the highest number of inventoried PCB-containing devices are found in the Basque Country (17925.46 tonnes as of 31 December 2010) versus the 1267.58 tonnes for the Canary Islands (MAGRAMA, 2013). The wide range of levels found across regions highlights the importance of studying local sources of exposure.

Our results confirm the findings of previous Spanish studies. Thus, over four years (1992-1996) Agudo and co-workers recruited volunteers aged 35-64 years in five Spanish regions, three from the North (Asturias, Guipuzcoa and Navarra) and two from the South (Granada and Murcia) and found that the values for Guipuzcoa (in the Basque Country) were around 35% higher than those found for Granada (South of Spain) (Agudo et al., 2009). Henriquez and co-workers also found the lowest levels of PCB exposure in inhabitants of the Canary Islands (Henriquez et al., 2011). In addition to having the lowest quantity of PCB-containing equipment, their inhabitants eat the lowest amounts of fish in the country according to a recent report from the Spanish Ministry of Agriculture, Food and the Environment (MAGRAMA, 2011).

We found seasonal differences in PCB levels, with the highest levels being found in April-June and the lowest in October-December. Given that the frequency of fish consumption, age and other confounders have all been considered in the multivariate analysis model, we have no clear explanation for this phenomenon. It could be that some variations in food habits, associated with seasonality, or interferences with the geographic zone could explain the observed data. Although the geographical distribution was included in the multivariate data-analysis model, the fact is that most volunteers from the Basque country (the highest concentrations in Spain) were recruited in the first two quarters whereas volunteers from the Canary Islands (with the lowest concentrations in Spain) were predominantly recruited in the second half of the year. In light of this, more detailed studies should be performed to confirm the season variability of PCB levels.

With respect to BMI, although the lack of an association (Baris et al., 2000; Schaeffer et al., 2006) or a positive association (Fernandez et al., 2008) with BMI have been reported, most studies have found a negative association, i.e. PCB levels in circulating blood decrease with increasing BMI (Agudo et al., 2009; Bachelet et al., 2011; Cerna et al., 2008; Glynn et al., 2003; Porta et al., 2012; Wolff et al., 2007). A similar pattern was observed with our data, with PCB concentrations decreasing with increasing BMI (Table 2a). Accumulation in body fat deposits in those subjects with a higher BMI is one of the likely causes for this negative association (Dhooge et al., 2010).

One of our critical concerns is to determine how much human serum PCB levels have decreased since the ban. The overall trend is that serum PCB levels have been decreasing since the 1980s. In the study carried out by Agudo (Agudo et al., 2009), although samples were collected over a relatively short period, the PCB concentration was, on average, 12% higher in samples from 1993 than in those from 1995, and between two and three times higher than the PCBs levels found in our study. A similar situation was observed by Porta and co-workers, in Barcelona, in 2002 and 2006, where $\sum PCB_{138,153,180}$ in serum decreased by around 30% and by almost 50% in the youngest age groups (Porta et al.,2012 and 2010). The ∑PCB values found by BIOAMBIENT.ES in Catalonia (GM 170.3 ng/g lipid) are much lower than those found in 2002 (GM 229.8 ng/g lipid) and very similar to the results for 2006 (166.4 ng/g lipid; Porta et al., 2012, 2010). Our $\sum PCB_{138,153,180}$ values for samples from the Basque Country (GM of 277.3 ng/g lipid) are in the same range as those obtained in a study conducted in 2006 in the same region (GM 232.94 ng/g lipid; Zubero et al., 2009). This suggests that the PCB ban in Spain has been more effective in some regions than in others.

Effectiveness is obviously greater as the observance period increases. Thus, a 33% reduction in PCB 153 levels was reported over a 10-year period (1991-2001) in Sweden (Hagmar 2006). The PCB levels in Spanish samples have dropped considerably since 1988, and between 2002 and 2004 the levels were two times lower (Gomara et al., 2011; Bordajandi et al., 2008).

A significant decrease in PCB levels in human milk between 1984 and 2003 was also reported by Wilhelm et al. (2007) in Germany and by Schuhmacher et al., (2013) between 1998 and 2012, in Catalonia, with the decrease reaching almost 70%. This downward trend is more prominent for PCB 153 than for PCB 180 (about seven times versus five times lower, respectively) when comparing breast milk samples from 1984 to 2003 (Wilhelm et al 2007) and with serum samples from 1991 and 2001 (Hagmar 2006) and from 2000 to 2005 in Romania (Dirtu et al, 2006). This finding could explain the variation in predominance found in our results when compared with previous Spanish studies in which PCB 153 was found in the highest concentrations (Herriquez-Hernández et al., 2011; Porta et al., 2012, 2010; Zubero et al., 2009).

At a European level, the PCB concentrations found in our study are lower than those reported from Germany (GerESIII; Becker et al., 2002), although the surveys were performed almost 10 years apart (GerESIII in 1998 and BIOAMBIENT.ES in 2009-2010). Taking this fact into account, our values are almost half those for PCBs 138 and 153, whereas the values for PCB 180 (the most persistent congener) are similar (Table 5).

In contrast, it is clear that the Spanish population is less exposed to PCBs than the French (Fréry et al., 2012). Thus, the levels for PCBs 138 and 153 in a survey from 2006-2007 were similar to those found in the German population from 1998 and therefore up to twofold higher than ours in 2009 and the GM for $\sum PCB_{138,153,180}$. The concentration obtained for PCB 180 in the French study was higher than both the German values and ours (Table 5).

The values obtained in a study conducted in five urban areas in the Czech Republic in 2006 (Cerna et al., 2008) were between six and ten times higher than those found by Bioambient.es (Table 5), whereas the levels found in Slovakia in 2001 (Petrik et al., 2006) were between four and five times higher than those found in our population (Table 5).

A recent study in volunteers aged between 18 and 64 years from urban and rural areas around Naples (Italy) found similar levels for the three main PCB congeners to those found in our study (Esposito et al., 2013) and similar to those found in Greece by Leondiadis and co-workers (Leondiadis et al., 2004) (Table 5). In general, similar results were found in southern European countries and lower than those for central Europe (Table 5).

Over the past 20 years, PCB 153 has been found to be the major contributor in the serum of populations from most countries (Frery et al., 2013; CDC 2009; Becker et al, 2002). As shown in several studies (Hagmar et al. 2006; Dirtu et al. 2006; Wilhelm et al. 2007; Esposito et al. 2013), the levels of this PCB have decreased to a greater extent than those of PCB180, which contains a higher number of chlorinated positions. We have observed a similar effect in our samples, in which PCB 180 is the main contributor to the PCB body burden.

Spanish levels are still higher than those found in the general population of the USA, Canada and New Zealand even though sampling in these populations was conducted some years ago (Bates et al., 2004; CDC 2009, 2005; Haines et al., 2012; Health Canada, 2010) (Table 5). In the USA study, PCBs levels decreased by approximately 20% between 2002 and 2004.

5. Conclusion

This paper presents the first data regarding PCB levels in a representative sample of the Spanish working population. As the participants in our study were not occupationally exposed, in practical terms the sample can be considered to be representative of the general population and therefore the levels can be interpreted as a reference for Spanish adults at a national level. The few comparisons we have been able to make with available local studies confirm the reduction in PCB levels in some regions of Spain.

In our volunteers we have found a change in the abundance of PCB congeners with respect to previous Spanish studies, with PCB 180 being the largest contributor to PCB burden in volunteers from all Spanish regions and all age groups. As such, we propose to use this PCB as a marker to follow trends in future variations in the body burden of PCBs in the general population and to check the implementation of environmental POP reduction policies.

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	LOQ (ng/mL)	LOQ (ng/g lipid)
PCB28	1	58.94
PCB52	1	58.94
PCB101	0.2	11.79
PCB138	0.01	0.59
PCB153	0.01	0.59
PCB180	0.005	0.29

Table 1: Limits of quantification (LOQ) for each PCB congener expressed in ng/mL and ng/g lipid in serum samples.

Table 2a
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TOTAL

Variables	N	GM*	95% CI			AM*	95% CI			P10	P25	P50	P75	P90	P95
Total															
	1880	135.4	121.3	-	151.2	187.5	162.4	-	212.7	48.9	81.9	143.6	230.1	359.4	482.2
Sex															
Men	963	139.9	124.3	-	157.3	191.7	169.6	-	213.9	49.8	85.0	147.2	237.5	374.6	556.0
Women	917	129.9	114.8	-	147.1	182.1	150.5	-	213.7	46.6	79.3	137.5	218.2	342.4	437.9
Age															
≤29 years	372	72.0	63.3	-	81.8	97.8	84.2	-	111.4	25.7	47.9	73.1	119.4	189.3	244.8
30-39 years	762	120.7	107.0	-	136.2	153.6	132.0	-	175.3	51.1	81.3	128.0	188.2	275.4	365.9
40-49 years	466	188.1	166.3	-	212.8	243.2	200.9	-	285.6	74.0	125.9	189.9	280.3	394.4	599.1
≥50 years	268	250.5	226.3	-	277.4	309.9	275.2	-	344.5	91.6	150.3	254.9	364.5	561.5	705.2
Economic sector															
Other sectors	657	134.5	115.5	-	156.8	191.9	164.8	-	219.0	46.3	81.2	151.4	232.6	382.7	524.7
Services	1223	135.8	119.6	-	154.1	185.8	156.3	-	215.3	50.9	82.3	140.6	227.9	352.9	465.5
Geographical area															
Galicia	146	128.4	107.2	-	153.8	168.1	149.7	-	186.4	52.3	80.8	147.8	204.0	306.5	377.4
Asturias, Cantabria	99	208.8	150.1	-	290.5	266.8	190.9	-	342.7	73.1	115.6	218.4	351.3	542.0	631.6
Basque Country	148	277.3	244.5	-	314.5	340.6	310.4	-	370.7	117.9	180.9	267.1	430.4	637.0	830.8
Navarre, La Rioja, Aragon	145	118.1	96.1	-	145.1	168.5	135.2	-	201.9	41.8	74.0	136.1	219.1	341.7	432.2
Catalonia	248	170.3	112.4	-	258.0	237.7	106.7	-	368.7	65.3	104.4	168.9	261.8	470.5	642.8
Castile and Leon	154	102.5	80.6	-	130.2	138.9	114.4	-	163.3	33.2	58.8	113.1	183.1	278.5	383.5
Madrid	197	125.9	89.5	-	177.1	166.5	121.9	-	211.2	42.9	76.7	145.7	218.2	326.7	359.4
Castile-La Mancha, Extremadura	150	136.5	116.9	-	159.5	183.3	144.0	-	222.7	53.7	78.8	138.1	229.1	336.9	524.7
Valencian Community, Balearic Islands	206	146.6	104.0	-	206.6	192.2	145.6	-	238.7	52.8	100.4	163.2	262.0	366.5	475.7
Andalusia, Ceuta	240	102.6	77.3	-	136.2	139.6	94.5	-	184.7	39.8	64.8	111.8	175.6	260.0	338.7
Murcia	97	126.0	98.4	-	161.5	176.8	114.3	-	239.4	37.7	78.1	133.1	221.4	371.2	460.2
Canary Islands	50	78.9	73.9	-	84.3	88.3	83.6	-	93.0	38.8	59.3	86.1	112.2	129.3	171.7
Sampling Quarter															
January - March	349	145.9	131.1	-	162.3	186.1	167.3	-	205.0	59.0	101.7	156.7	240.4	339.1	399.7
April - June	562	171.3	141.5	-	207.4	229.8	166.4	-	293.2	67.8	104.0	176.6	275.4	425.6	596.8
July - August	375	134.0	107.7	-	166.7	186.8	142.1	-	231.4	51.4	81.2	138.4	236.6	374.0	565.3
September - December	559	101.8	85.9	-	120.7	144.3	119.5	-	169.1	36.0	62.0	119.2	190.1	305.5	358.1
Frequency fish consumption															
<Once per week	212	91.2	76.7	-	108.4	126.5	109.4	-	143.6	29.1	57.1	109.4	179.5	275.8	364.6
Once per week	574	117.5	102.3	-	134.8	158.0	137.7	-	178.2	44.9	73.3	122.9	201.2	321.5	412.6
2/4 times per week	835	158.5	144.0	-	174.6	212.7	187.3	-	238.0	57.5	98.8	163.5	267.6	387.9	524.7
≥5 times per week	221	157.5	125.3	-	198.0	225.9	157.3	-	294.6	51.4	91.5	159.0	261.0	476.7	596.8
BMI															
Normal (<25)	1489	137.0	121.0	-	155.0	190.6	161.3	-	219.9	51.1	83.1	144.0	232.4	365.2	493.4
Overweight (25-30)	289	129.7	116.0	-	145.0	176.7	157.7	-	195.7	44.1	79.3	143.8	227.4	344.9	407.6
Obesity (>30)	60	131.3	103.0	-	167.3	172.1	127.6	-	216.6	45.8	80.8	152.5	190.1	326.7	460.2

Table 2a: PCBs descriptive analysis statistic for all participants by sex, age, economic activity sector, Autonomous Community, sampling quarter, frequency of fish consumption and Body Mass Index. Geometric mean (GM*), arithmetic mean (AM*) and their confidence intervals (95%) of the ΣPCB 138, 153 and 180 expressed in ng/g lipid, as well as percentiles 10, 25, 50, 75, 90 and 95.

Table 2b
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Variables	N	GM*	95% CI			AM*	95% CI			P10	P25	P50	P75	P90	P95
Men															
	963	139.9	124.3	-	157.3	191.7	169.6	-	213.9	49.8	85.0	147.2	237.5	374.6	556.0
Age															
≤29 years	178	66.8	56.6	-	78.9	88.5	76.3	-	100.7	28.3	44.9	71.1	112.1	159.1	227.4
30-39 years	377	123.0	106.9	-	141.7	157.0	130.7	-	183.2	51.4	80.3	127.3	192.1	281.3	380.5
40-49 years	256	185.7	166.2	-	207.5	231.3	206.1	-	256.4	72.6	121.8	185.2	271.3	395.5	617.6
≥50 years	142	283.0	251.7	-	318.1	334.6	294.1	-	375.1	98.9	166.2	284.8	374.9	596.8	711.7
Economic sector															
Other sectors	411	135.9	116.8	-	158.0	193.9	167.3	-	220.4	46.5	81.2	154.4	235.4	380.5	525.2
Services	552	142.7	124.7	-	163.3	190.3	164.5	-	216.0	51.4	86.0	144.4	242.7	370.9	569.1
Geographical area															
Galicia	74	135.3	104.8	-	174.7	167.2	131.1	-	203.3	55.6	91.1	152.9	204.0	334.8	377.4
Asturias, Cantabria	49	226.0	164.6	-	310.3	285.8	214.7	-	357.0	103.3	159.7	233.1	351.3	525.2	663.1
Basque Country	74	268.1	252.1	-	285.2	337.8	330.0	-	345.6	105.9	165.5	267.1	509.6	637.0	743.8
Navarre, La Rioja, Aragon	78	115.1	80.5	-	164.6	180.5	128.8	-	232.2	33.8	73.8	122.4	243.0	380.5	502.6
Catalonia	123	171.6	103.8	-	283.7	233.5	118.1	-	348.9	51.9	104.4	183.5	290.7	569.1	642.8
Castile and Leon	78	107.3	84.3	-	136.7	138.8	120.2	-	157.4	44.1	58.8	117.5	189.6	278.5	335.5
Madrid	92	133.6	101.9	-	175.2	173.9	143.5	-	204.2	47.9	78.4	148.7	235.4	339.1	364.5
Castile-La Mancha, Extremadura	80	161.4	143.3	-	181.8	218.9	181.0	-	256.8	59.0	90.1	169.0	292.8	407.6	565.3
Valencian Community, Balearic Islands	110	147.8	97.2	-	224.8	198.8	142.6	-	255.0	54.6	104.0	163.2	251.4	375.1	477.4
Andalusia, Ceuta	122	106.4	84.2	-	134.3	136.6	104.5	-	168.6	47.9	65.5	107.6	176.6	269.1	338.7
Murcia	59	125.8	79.4	-	199.4	184.9	73.6	-	296.2	37.7	82.8	129.3	221.4	394.4	614.8
Canary Islands	24	79.1	77.7	-	80.5	87.1	85.8	-	88.4	38.8	60.7	86.9	114.4	129.3	135.9
Sampling quarter															
January - March	159	151.8	128.1	-	179.9	201.7	169.0	-	234.5	51.7	98.9	159.6	242.7	349.4	476.7
April - June	310	180.0	149.8	-	216.4	234.3	185.7	-	282.9	71.1	104.4	189.6	292.8	461.2	625.6
July - August	180	131.1	101.1	-	170.0	191.1	142.7	-	239.6	47.9	75.3	135.9	225.2	421.8	595.4
September - December	299	109.5	91.7	-	130.8	147.6	126.2	-	169.1	43.7	68.5	127.1	192.2	310.6	347.2
Fish and shellfish consumption															
<Once per week	123	97.9	80.4	-	119.2	132.1	112.8	-	151.4	39.0	62.1	123.1	191.6	280.3	364.5
Once per week	322	119.2	100.7	-	141.1	162.1	136.4	-	187.8	44.9	73.6	128.0	206.6	335.4	432.7
2/4 times per week	390	170.2	152.8	-	189.6	222.7	201.0	-	244.5	59.4	102.8	171.3	298.5	425.6	612.0
≥5 times per week	106	166.9	166.9	-	216.4	235.8	164.9	-	306.6	51.4	91.1	166.6	284.8	556.0	596.8
BMI															
Normal (<25)	680	141.5	124.0	-	161.4	195.0	168.5	-	221.5	51.9	85.7	144.8	241.0	391.8	564.3
Overweight (25-30)	218	137.6	120.5	-	157.1	185.5	164.9	-	206.1	46.1	84.6	159.1	236.4	358.1	407.6
Obesity (>30)	43	129.7	102.6	-	164.0	171.2	121.7	-	220.6	55.6	76.9	158.6	190.1	326.7	460.2

Table 2b: PCBs levels for men in according to age groups, economic activity sector, geographical area, sampling trimester, fish and shellfish consumption and Body Mass Index. Geometric mean (GM*), arithmetic mean (AM*) and their confidence intervals (95%), of the ΣPCB 138, 153 and 180 expressed in ng/g lipid, as well as percentiles 10, 25, 50, 75, 90 and 95.

Table 2c
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Variables	N	GM*	95% CI			AM*	95% CI			P10	P25	P50	P75	P90	P95
Women															
	917	129.9	114.8	-	147.1	182.1	150.5	-	213.7	46.6	79.3	137.5	218.2	342.4	437.9
Age															
≤29 years	194	77.8	64.6	-	93.5	107.5	84.5	-	130.4	25.3	51.1	76.3	124.9	217.6	280.9
30-39 years	385	118.1	105.0	-	132.7	149.7	130.3	-	169.0	47.3	84.1	128.9	186.3	265.6	346.1
40-49 years	210	191.9	157.7	-	233.4	261.4	173.2	-	349.6	75.3	128.9	196.5	294.4	381.1	566.9
≥50 years	126	208.6	184.5	-	235.7	272.5	228.0	-	317.1	87.9	113.6	210.8	306.5	444.3	612.4
Economic sector															
Other sectors	246	129.0	103.1	-	161.3	183.5	142.4	-	224.6	36.2	75.5	139.3	220.5	399.7	482.2
Services	671	130.1	112.8	-	149.9	181.9	146.6	-	217.2	48.2	79.3	137.5	218.2	338.6	437.4
Geographical area															
Galicia	72	120.7	99.0	-	147.2	169.1	147.7	-	190.5	47.8	79.0	125.3	196.5	306.5	493.4
Asturias, Cantabria	50	189.3	132.3	-	270.8	243.1	158.5	-	327.7	64.7	103.0	193.3	365.2	542.0	599.1
Basque Country	74	289.2	232.7	-	359.5	344.0	270.7	-	417.3	156.1	217.6	268.0	390.4	628.2	1029.6
Navarre, La Rioja, Aragon	67	122.1	114.6	-	130.1	152.9	129.9	-	175.9	47.3	90.8	137.4	188.2	259.0	350.5
Catalonia	125	168.8	113.2	-	251.7	242.7	80.8	-	404.7	71.4	101.7	156.1	241.7	443.3	576.1
Castile and Leon	76	95.9	70.1	-	131.3	138.9	97.6	-	180.3	24.0	59.9	107.3	164.6	345.8	449.0
Madrid	105	117.3	70.0	-	196.4	157.7	88.7	-	226.7	41.3	74.7	129.7	204.8	280.4	355.4
Castile-La Mancha, Extremadura	70	104.4	81.7	-	133.3	126.1	92.4	-	159.8	44.3	67.6	97.8	167.9	258.8	301.5
Valencian Community, Balearic Islands	96	145.0	112.0	-	187.8	183.7	149.0	-	218.4	52.2	98.8	163.9	263.4	356.2	381.1
Andalusia, Ceuta	118	97.5	67.2	-	141.3	144.0	77.0	-	211.0	22.6	64.0	114.7	175.6	247.4	342.0
Murcia	38	126.4	122.4	-	130.5	166.2	166.2	-	166.3	46.4	78.1	135.4	211.4	333.9	373.2
Canary Islands	26	78.8	70.3	-	88.3	89.5	78.5	-	100.5	32.4	59.3	83.8	105.3	171.7	182.6
Quarter															
January - March	190	140.7	128.8	-	153.6	171.9	159.2	-	184.7	65.7	101.7	156.1	232.9	324.2	390.4
April - June	252	159.5	124.9	-	203.8	223.4	133.4	-	313.4	66.5	100.7	167.9	258.8	383.5	518.6
July - August	195	137.7	112.1	-	169.0	181.4	135.8	-	227.0	56.7	89.9	147.7	246.3	365.2	458.6
September - December	260	91.3	71.7	-	116.3	139.4	100.9	-	177.8	25.3	57.1	110.1	186.9	302.9	416.9
Fish and shellfish consumption															
<Once per week	89	81.6	63.1	-	105.7	117.8	87.8	-	147.9	25.1	52.2	95.3	150.2	275.8	364.6
Once per week	252	114.6	101.8	-	128.9	150.9	134.6	-	167.2	44.3	72.1	116.4	189.7	305.5	381.1
2/4 times per week	445	147.4	128.9	-	168.6	202.3	164.8	-	239.9	55.3	93.5	156.8	237.1	358.4	444.3
≥5 times per week	115	147.7	113.3	-	192.5	215.0	139.2	-	290.9	48.5	93.4	151.0	234.8	368.1	576.1
BMI															
Normal (<25)	809	132.5	115.6	-	151.7	186.0	151.1	-	220.9	48.2	80.5	140.3	220.9	345.8	443.3
Overweight (25-30)	71	106.5	89.1	-	127.3	147.2	110.9	-	183.5	36.2	66.2	100.7	167.1	338.6	391.3
Obesity (>30)	17	136.9	83.6	-	224.2	175.3	100.4	-	250.2	42.9	87.2	134.8	214.3	357.4	416.9

Table 2c: PCBs levels for women according to age groups, economic activity sector, geographical area, sampling trimester, fish and shellfish consumption and Body Mass Index. Geometric mean (GM*) arithmetic mean (AM*) and their confidence intervals (95%) of the ΣPCB 138, 153 and 180 expressed in ng/g lipid, as well as percentiles 10, 25, 50, 75, 90 and 95.

Table 3
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						Percentiles					<LOQ		
	N	GM	95% CI	AM	95% CI	P10	P25	P50	P75	P90	N	%	95% CI
PCB 138													
Men	963	31.92	28.51 - 35.73	45.77	41.07 - 50.47	11.18	20.06	35.25	57.09	93.05	14	1.36	0.40 - 2.32
Women	917	31.85	28.27 - 35.89	47.14	37.69 - 56.59	11.35	20.05	34.31	55.76	86.74	2	0.21	0.00 - 0.52
PCB 153													
Men	963	44.23	39.22 - 49.88	62.49	54.88 - 70.10	16.51	27.18	47.78	79.00	125.75	9	0.88	0.13 - 1.63
Women	917	42.82	37.56 - 48.82	60.33	50.09 - 70.56	15.37	26.40	45.57	72.97	118.03	2	0.17	0.00 - 0.51
PCB 180													
Men	963	59.12	51.80 - 67.46	83.47	72.23 - 94.72	20.37	33.15	62.97	106.48	171.49	1	0.10	0.00 - 0.32
Women	917	52.17	45.56 - 59.75	74.63	61.07 - 88.19	18.46	31.01	55.06	90.38	139.47	0	-	- -

Table 3: Contribution of individual PCB congeners for both men and women. The geometric mean (GM), arithmetic mean (AM) and their confidence intervals (95%), percentiles 10, 25, 50, 75 and 90 of the PCB 138, 153 and 180 expressed in ng/g lipid, as well as the percentage of samples below Limit of Quantification (LOQ) .

Table 4
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	TOTAL				Men				Women			
	exp(b)	[95% Conf.Interval]		P	exp(b)	[95% Conf. Interval]		P	exp(b)	[95% Conf. Interval]		P
Sex												
Women	1											
Men	1.09	1.02	1.15	0.01								
Age*												
≤29 years	0.50	0.47	0.53	0.00	0.47	0.41	0.52	0.00	0.56	0.50	0.62	0.00
30-39 years	0.83	0.79	0.87	0.00	0.83	0.77	0.90	0.00	0.83	0.76	0.90	0.00
40-49 years	1.31	1.24	1.39	0.00	1.29	1.19	1.40	0.00	1.37	1.26	1.49	0.00
≥50 years	1.83	1.73	1.95	0.00	2.01	1.89	2.12	0.00	1.58	1.42	1.76	0.00
Economic sector												
Services	1				1				1			
Other sectors	0.89	0.79	1.00	0.05	0.88	0.77	1.01	0.07	0.96	0.77	1.20	0.70
Geographical area*												
Galicia	0.97	0.87	1.08	0.57	0.99	0.88	1.12	0.88	0.96	0.86	1.07	0.40
Asturias, Cantabria	1.45	1.04	2.01	0.03	1.50	1.10	2.03	0.01	1.37	0.95	1.97	0.09
Basque Country	1.88	1.47	2.41	0.00	1.91	1.58	2.29	0.00	1.86	1.33	2.59	0.00
Navarre, La Rioja, Aragon	0.99	0.75	1.31	0.95	0.95	0.72	1.26	0.72	1.08	0.89	1.31	0.43
Catalonia	1.18	0.90	1.56	0.22	1.19	0.94	1.52	0.15	1.17	0.85	1.61	0.31
Castile and Leon	0.91	0.80	1.04	0.17	0.86	0.71	1.03	0.11	0.99	0.88	1.12	0.89
Madrid	0.91	0.83	1.01	0.08	0.94	0.82	1.07	0.31	0.87	0.72	1.05	0.13
Castile-La Mancha, Extremadura	0.92	0.83	1.01	0.09	0.95	0.85	1.06	0.34	0.83	0.70	0.98	0.03
Valencian Community, Balearic Islands	1.04	0.90	1.21	0.56	0.99	0.78	1.26	0.94	1.11	1.00	1.23	0.05
Andalusia, Ceuta	0.91	0.75	1.11	0.32	0.90	0.74	1.08	0.25	0.93	0.73	1.19	0.56
Murcia	1.10	0.94	1.29	0.25	1.10	0.82	1.47	0.50	1.06	0.98	1.15	0.14
Canary Islands	0.40	0.33	0.49	0.00	0.42	0.33	0.54	0.00	0.42	0.34	0.51	0.00
Sampling quarter*												
January - March	1.04	0.92	1.17	0.53	1.05	0.91	1.22	0.47	1.04	0.91	1.18	0.57
April - June	1.23	1.10	1.38	0.00	1.21	1.07	1.38	0.00	1.25	1.10	1.42	0.00
July - August	1.00	0.89	1.13	0.99	0.98	0.83	1.15	0.79	1.04	0.90	1.19	0.59
September - December	0.78	0.73	0.84	0.00	0.80	0.72	0.89	0.00	0.74	0.67	0.82	0.00
Frequency fish consumption												
<1 per week	1				1				1			
1 per week	1.25	1.15	1.35	0.00	1.22	1.04	1.43	0.02	1.28	1.11	1.49	0.00
2/4 per week	1.48	1.31	1.67	0.00	1.48	1.22	1.78	0.00	1.50	1.28	1.74	0.00
≥5 per week	1.43	1.16	1.76	0.00	1.41	1.09	1.83	0.01	1.48	1.17	1.87	0.00

* Reference: Geometric mean for all participants

Table 4: Multivariate analysis for the whole population and for men and women separately. Multivariate linear regression models were fitted using log-transfomed ∑PCBs levels as the dependent variable, and including the following independent variables: age, sex (reference category was women), economic sector (reference category was services) fish and shellfish consumption (reference category was once per week), geographical area and sampling trimester.

Table 5 2nd rev
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PCB	COUNTRY	YEARS	RANGE OF POPULATION AGE	N	GM (ng/g lipid)	GM (ng/L)	AM (ng/g lipid)
PCB 138	Spain (Our study)	2009-2010	16 - 65	1880	31.89	286.00	46.37
	Germany (GerESIII, Becker 2002)	1998	18 - 69	2823		420.00	
	France (ENNS, Fréry 2013)	2006 - 2007	18 - 74	386	70.80	457.40	
	Czech Republic (Cerna 2008)	2006	> 18	202	186.00		
	Slovakia (Petrik et al., 2006)	2001	> 18	2047	138^{+163}: 165.00		
	Italy (Esposito 2014)	2011-2012	18 - 64	58			36.88
	Greece (Leondiadis et al., 2004)	2002 - 2004		40			32.20
	United States (NHANES, CDC, 2009)	2001 - 2002	> 20	2293	138^{+158}: 19.90		
		2003 - 2004	> 20	1896	138^{+158}: 15.10		
	Canada (Health 2010)	2007 - 2009	20 - 79	1668	10.13	60.00	15.11
	New Zealand (Bates 2004)	1996 - 1997	> 15	60 pools	138^{+158}: 15.30		
PCB 153	Spain (Our study)	2009-2010	16 - 65	1880	43.61	377.00	61.54
	Germany (GerESIII, Becker 2002)	1998	18 - 69	2823		680.00	
	France (ENNS, Fréry 2013)	2006 - 2007	18 - 74	386	113.30	731.80	
	Czech Republic (Cerna 2008)	2006	> 18	202	423.00		
	Slovakia (Petrik et al., 2006)	2001	> 18	2047	267.00		
	Italy (Esposito 2014)	2011-2012	18 - 64	58			56.31
	Greece (Leondiadis et al., 2004)	2002 - 2004		40			58.60
	United States (NHANES, CDC, 2009)	2001 - 2002	> 20	2306	27.20		
		2003 - 2004	> 20	1896	19.80		
	Canada (Health 2010)	2007 - 2009	20 - 79	1668	18.31	110.00	28.62
	New Zealand (Bates 2004)	1996 - 1997	> 15	60 pools	23.70		
PCB 180	Spain (Our study)	2009-2010	16 - 65	1880	55.97	490.00	79.60
	Germany (GerESIII, Becker 2002)	1998	18 - 69	2823		440.00	
	France (ENNS, Fréry 2013)	2006 - 2007	18 - 74	386	93.70	605.10	
	Czech Republic (Cerna 2008)	2006	> 18	202	374.00		

	Slovakia (Petrik et al., 2006)	2001	> 18	2047	246.00		
	Italy (Esposito 2014)	2011-2012	18 - 64	58			72.81
	Greece (Leondiadis et al., 2004)	2002 - 2004		40			58.30
	United States (NHANES, CDC, 2009)	2001 - 2002	> 20	2302	19.20		
		2003 - 2004	> 20	1896	15.10		
	Canada (Health 2010)	2007 - 2009	20 - 79	1668	15.21	90.00	25.74
	New Zealand (Bates 2004)	1996 - 1997	> 15	60 pools	20.30		

Table 5: Serum levels of the main PCB contributors reported in different European countries compared to those found in our study in Spain. The geometric mean (GM) expressed in ng/g lipid and in ng per L of serum as well as arithmetic mean (AM) of PCB 138, 153 and 180.

Figure 1
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Figure 1

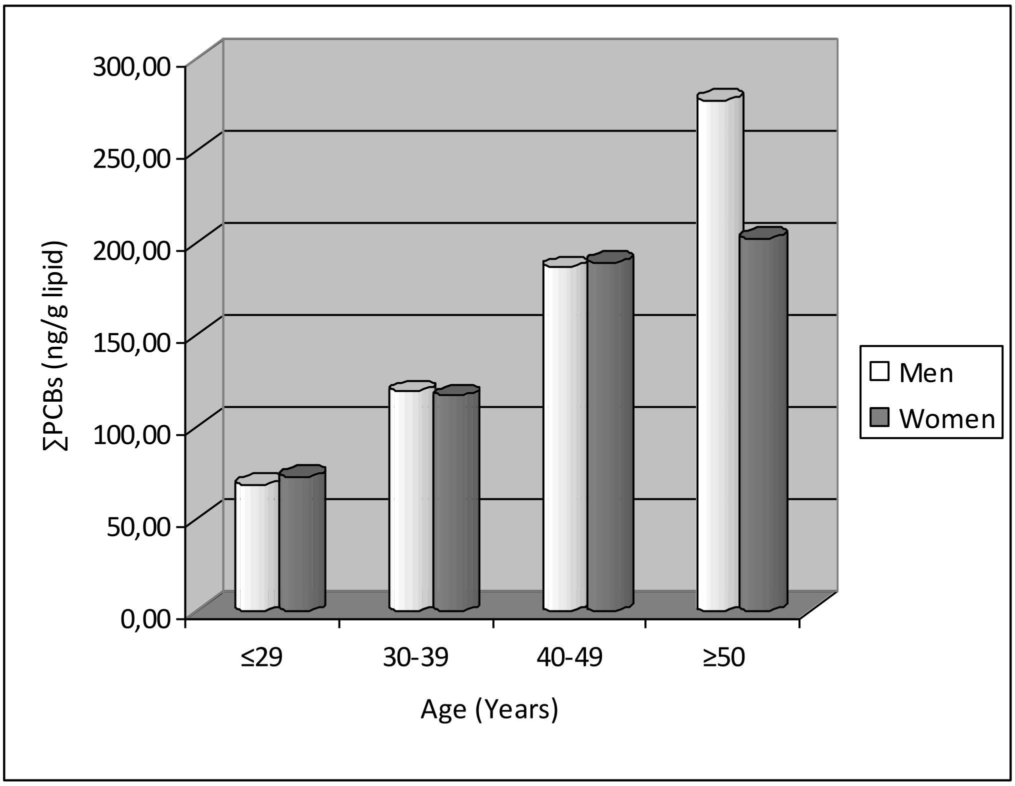

Figure 2
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Figure 2

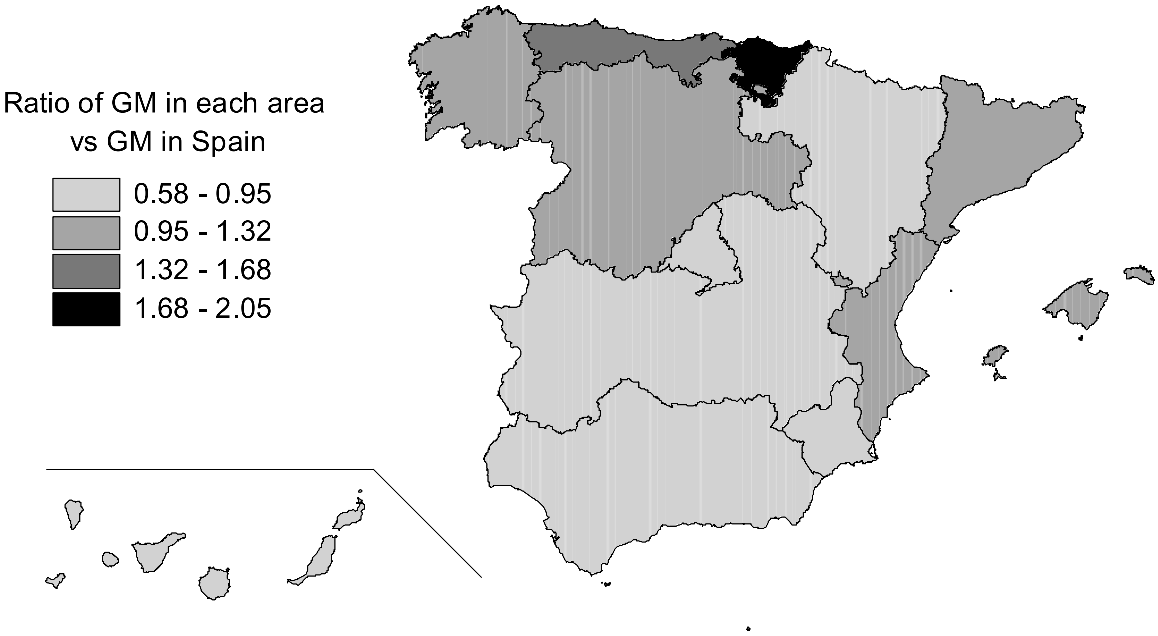

Figure 3
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Figure 3

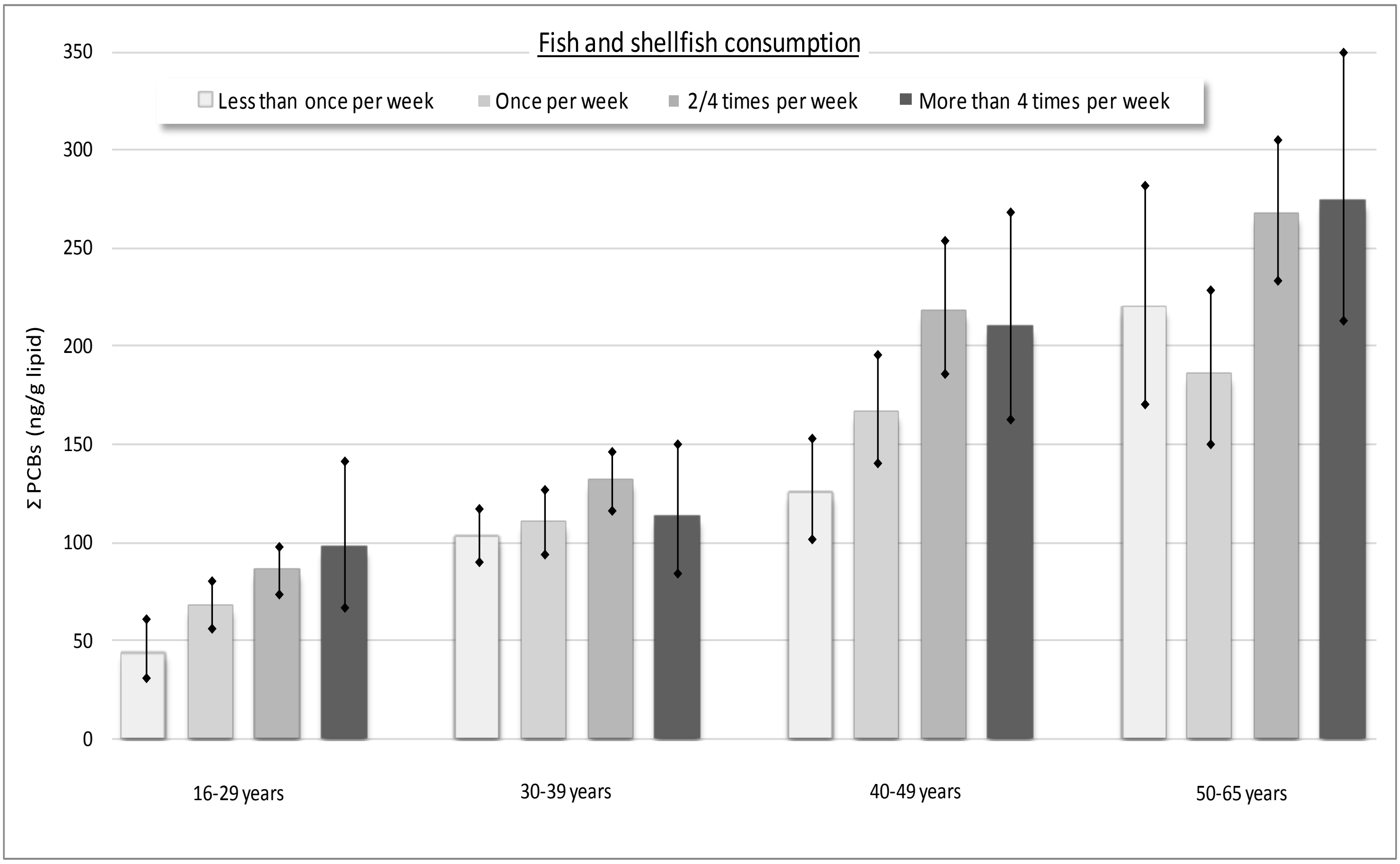

Figure 1: Increase in $\Sigma PCB_{138,153,180}$ levels with the age. PCB levels increase by approximately 56% per 10 years of age, being higher in men than in women and with statistically significant differences ($p<0.05$) found in the oldest group (>50 years old).

Figure 2: Ratio of the geometric means (GM) of the serum $\Sigma PCB_{138,153,180}$ concentrations for different geographical regions referred to the GM for all participants in Spain. The concentrations of ΣPCB congeners in samples from Northeast and Northern Spain were higher than those reported in the South. The lowest concentrations were found for the Canary Islands.

Figure 3: Increase in serum $\Sigma PCB_{138,153,180}$ levels with frequency of weekly fish consumption and age. Bars represent the geometric mean of ∑PCB in each category, and lines represent 95% confidence interval.

***Conflict of interest**

The authors declare that they have no competing financial interests.