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Title ORGANOCHLORINATED PESTICIDES LEVELS IN A REPRESENTATIVE SAMPLE OF THE SPANISH ADULT POPULATION: THE BIOAMBIENT.ES PROJECT

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Abstract

The present manuscript presents serum levels of most relevant organochlorinated pesticides (OCPs) on a representative sample of Spanish adults recruited in 2009 during their annual medical examination at work under the BIOAMBIENT.ES project, a nationwide human biomonitoring program promoted by Spanish Ministry of Agriculture, Food and Environment to estimate reference levels of environmental pollutants in the general population. Out of the thirteen OCPs investigated: Aldrin, endrin, dieldrin, heptachlor, heptachlor-epoxide, dichlorodiphenyldichloroethylenes (DDEs), hexachlorobenzene (HCB), dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), the highest concentrations were observed for DDE, HCB and β -HCH. The 95th percentile, in ng/g lipids, were: 717.7 for 4,4-DDE, 160.4 for HCB and 107.2 for β -HCH, whereas aldrin, endrin, heptachlor, -HCH, 2,4-DDT, 4,4-DDT and 2,4-DDE presented the lowest concentrations being detected in less than 10% of samples. Significant differences ($p < 0.05$) were found for DDE and HCB and seasonality, gender and age. The geographical distribution of these analytes showed the highest levels in people who lived in the north of Spain for HCB and in the center for 4,4-DDE. The influence of intake of several food groups in the OCP levels was also assessed. The results obtained showed a sharp decline in the OCP concentrations reported in the past in Spain and they were in the same range or lower than those reported in similar studies worldwide, except for HCHs and HCB that presented higher levels than in other European countries and much higher those observed in US and Canada. This study represents the first nationwide survey of exposure to OCPs in Spain and provides a background reference range for exposure to OCPs in the Spanish adult occupied population. These results will allow establishing reference values, observing temporal trends and identifying high exposure groups.

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Keywords Human Biomonitoring, Spain, Organochlorinated Pesticides, serum, DDE, DDT, β -HCH

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1 **ORGANOCHLORINATED PESTICIDES LEVELS IN A REPRESENTATIVE SAMPLE**
2 **OF THE SPANISH ADULT POPULATION: THE BIOAMBIENT.ES PROJECT**
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18 **Abstract**

19 **Keywords.** Human Biomonitoring, biomarkers, blood serum, organochlorinated pesticides,
20 Spain. DDE, DDT, β -HCH

22 1. Introduction

23 Organochlorine pesticides (OCPs) are synthetic chemicals with chlorine-substituted aliphatic or
24 aromatic cyclic rings industrially produced after the Second World War. Due to their high
25 effectiveness against insects and persistence in the environment, their widespread use in
26 agriculture and for pest control in cities for several decades, has resulted in their ubiquitousness
27 in various environments (Muñoz-Arnanz J and Jiménez B, 2011). Thereby, it is estimated that
28 from 1950 to 1980, 40,000 tonnes of dichlorodiphenyltrichloroethane (DDT) were used each
29 year worldwide (Geisz *et al.*, 2008). Nowadays, around 3,000 tonnes are still producing for the
30 control of malaria and visceral leishmaniasis (UNEP, 2010).

31 The production and use of most of these compounds were banned in the 70s and 80s in
32 developed countries, although because of their environmental persistence, bioaccumulation
33 and toxicity are still of environmental concern (ATSDR 2002). In addition, some of these
34 pollutants such as hexachlorobenzene (HCB), used in the past as fungicide for seed treatment,
35 can be produced unintentionally and released to the environment during industrial process
36 involving incomplete combustion of organic matter and chlorine (Richter and Steinhäuser,
37 2003).

38 Most organochlorine pesticides are known to be endocrine disruptors (Cohn *et al.*, 2007; Mrema
39 *et al.*, 2013), neurotoxicants, and carcinogens (IARC 1974; ATSDR, 2002). Recent
40 epidemiologic studies have shown that background exposure to organochlorine pesticides is
41 strongly associated with diabetes type 2 (Evangelou *et al.*, 2016) and disorders in the human
42 body as arthritis (Duk-Hee *et al.*, 2007). In addition, chronic low-dose human exposure to
43 organochlorine pesticides (p p- DDE, HCB and transnona-chlordane) have been associated with
44 a risk of cognitive impairment in later life (Duk-Hee *et al.*, 2016).

45 The primary source of exposure for humans is diet, mainly fatty food, such as meat, fish,
46 poultry and dairy products. In the general population, inhalation of OCPs present in ambient air
47 is insignificant compared with dietary intake (Peter, 2006).

48 Many OCPs are globally regulated by the Stockholm Convention on Persistent Organic
49 Pollutants (POPs) that prohibit the production and use of those compounds intentionally
50 produced, *i.e.* aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide, hexachlorobenzene
51 (HCB), dichlorodiphenyltrichloroethanes (DDTs) and hexachlorocyclohexanes (HCHs), as well
52 as reduce or eliminate releases from those unintentionally produced *i.e.* HCB. Stockholm
53 Convention also establishes the need of monitoring the presence, levels and trends of these
54 pollutants in humans ([UNEP, 2001](#)).

55 Human biomonitoring (HBM) allows temporal trends analysis and geographic pollutant
56 distribution, can identify new exposure sources, vulnerable groups or populations with higher
57 exposures. Nationwide HBM studies are useful tools to assess the effectiveness of policies to
58 reduce the chemical exposure as well as, to prioritize the design and implementation of new
59 measures (Angerer *et al.*, 2007). Most nationwide HBM programs included the analysis of
60 OCPs in human serum samples. Examples are the National Health and Nutrition Examination
61 Survey (NHANES) (CDC, 2009) in the US, the German Environmental Survey (GerES)
62 (Becker *et al.*, 2002), the Canadian Health Measures Survey (CHMS) (Health Canada, 2010) or
63 the French National Nutrition and Health Study (ENNS) (Saoudi *et al.*, 2014). These programs
64 have shown, in general, a decline of OCPs levels across the last two decades. However some
65 OCPs, *e.g.* p,p-DDE, the main metabolite of DDT, are still found at significant concentrations
66 in the general population.

67 In Spain, agriculture was the main economic sector up to the seventies. Almost half of the
68 occupied population was related with agricultural sector, leading to Spain to be an important
69 consumer and producer of OCPs in the past (Botella *et al.*, 2004; Fernández *et al.*, 2013).
70 Although OCPs human levels have been studied in Spain previously, these studies done at
71 regional level (Zumbado *et al.*, 2005; Porta *et al.*, 2010; Porta *et al.*, 2012; Jakszyn *et al.*, 2009)
72 or they were focused on specific population groups (López-Espinosa *et al.*, 2016).

73 The present work was carried out within the framework of the BIOAMBIENT.ES project,
74 funded in 2009 by the Spanish Ministry of Agriculture, Food and Environment, ~~this is~~ the first
75 national-level HBM survey on environmental pollutants carried out in Spain to estimate the
76 levels of heavy metals (Cañas et al 2012; López et al , 2015) and POPs (Huetos et al, 2014;
77 Bartolomé et al.2015) environmental pollutants in a representative sample of Spanish adult
78 population. The results provided will be useful to establish reference values, to assess exposure
79 factors related with the lifestyle and personal characteristic of participants.

80 **2. Materials and methods**

81 *2.1 Study design*

82 BIOAMBIENT.ES project is a nationwide cross-sectional epidemiological study carried out in
83 the adult general population. The details of this study have been previously described (Pérez-
84 Gómez *et al.*, 2013). In summary, participants were recruited in 2009 and 2010, through the
85 annual occupational medical check-up. The selection of volunteers was carried out through a
86 stratified cluster sampling which covered all geographical areas, gender, and activity sectors,
87 with the aim of obtain a representative sample of Spanish working population. To guarantee the
88 nationwide representativeness of the sample, 12 geographical areas were established: Northwest
89 I (Galicia), Northwest II (Asturias, Cantabria), North (Basque Country), Northeast I (Navarre,
90 La Rioja, Aragon), Northeast II (Catalonia), Central I (Castile y León), Central II (Madrid),
91 Central III (Castile-La Mancha, Extremadura), East (Valencia, Balearic Islands), South I
92 (Andalusia, Ceuta), South II (Murcia) and Canary Islands. A self-administered epidemiological
93 questionnaire was completed by all participants with the aim of compile individual information
94 on potential exposure factors related to the pollutants studied. The sample thus obtained had a
95 total number of 1880 men and women aged between 18 and 65. All participants spend at least 5
96 years living in Spain before sampling collection and 94.5% were born in Spain. Subsets for
97 OCP analysis were randomly selected, keeping ratios of genders, geographical areas,
98 economical sector and age.

99 2.2 Ethical approval

100 The study was performed in accordance with legal/ethical principles and regulations concerning
101 research involving individual information and biological samples, including “Organic Law
102 15/1999 on Personal Data protection and its Regulations”, “Law 41/2002, on Autonomy of
103 Patients and rights and obligations relating to health information and documentation” and
104 “General Health Law 14/1986”. Before the beginning of the survey, ethical approval from
105 *Comité Ético Científico* and the Legal Department of IBERMUTUAMUR was granted and, due
106 to blood samples collection, the principles of the Declaration of Helsinki and those contained in
107 the UNESCO Universal Declaration on the Human Genome and Human Rights have been
108 considered.

109 2.3 OCP Analysis

110 The samples were transported to the laboratory at 4 °C, centrifuged to obtain the serum and
111 stored at – 20 °C until chemical analysis (Esteban *et al.*, 2013). The sample preparation
112 approach was based on other describe previously (Lino *et al.*, 1998). Shortly, 1 mL of serum
113 sample was spiked with an internal standard solution (¹³C-labeled PCB 118) and deproteinized
114 with formic acid. The analytes were extracted using Florisil solid phase extraction cartridge
115 (SPE) and hexane:dichlorometane (1:1, v/v) as solvent extraction. The eluate once evaporated to
116 dryness was re-dissolved in 50 µL of hexane prior to be analysed by negative chemical
117 ionization gas chromatography coupled to mass spectrometry, GC-(NCI)MS.

118 In order to overcome the reproducibility problems detected during the chemical analysis of p,p-
119 DDT and HCB, due to the interactions with the matrix and loss in evaporation process
120 respectively, these analytes were processed separately from the rest of OCPs using ¹³C-HCB as
121 internal standard and an additional purification step based on multilayer column with modified
122 silica was included (Ramos JJ *et al.*, 2007).

123 Finally, a 2 μ L aliquot was inject in pulse-splitless mode. Inlet temperature was 275 $^{\circ}$ C, except
124 to HCB and DDT that was 220 $^{\circ}$ C in HCB and DDT analysis. Methane was used as reagent gas
125 and helium at 1 mL/min as carrier gas. Chromatographic separation was performance by DB-5-
126 MS with following temperature program: initial temperature 100 $^{\circ}$ C for 0.6 min, 40 $^{\circ}$ C/min to
127 200 $^{\circ}$ C held for 5 min, then 4.5 $^{\circ}$ C/min to 250 $^{\circ}$ C, held for 0.2 min, then 120 $^{\circ}$ C/min to 320 $^{\circ}$ C,
128 held for 2 min. In the DDT and HCB analysis, oven final temperature was 300 $^{\circ}$ C. The
129 temperatures of MS interface, quadrupole and source were set at 300 $^{\circ}$ C, 150 $^{\circ}$ C and 150 $^{\circ}$ C,
130 respectively.

131 For internal quality control purposes, blanks reagent, blanks rabbit serum and sample control,
132 *i.e.* rabbit serum spiked at known concentration, were included in every analytical series.
133 External quality assurance has been done by participation participating in the Arctic Monitoring
134 and Assessment Program (AMPA) and German External Quality Assessment Scheme (G-
135 EQUAS) during analyses period. The limit of quantification (LOQ) for selected OCP analytes
136 were 0.03 ng/ml of serum for aldrin, dieldrin, 4,4-DDE, 2,4-DDE, 4,4 DDT and heptachlor
137 epoxy; 0.06 ng/ml for 2,4 DDT, heptachlor, endrin, HCB; 0.13 ng/ml for β -HCH and LOQ of
138 0.24 ng/ml for γ -HCH (also known as Lindane) and α -HCH.

139 The OCP concentrations were individually lipid-adjusted. The total lipid content was calculated
140 with the following formula:

141 Total lipid= (2.27 * total cholesterol) + triglycerides + 62.3 mg/dl

142 ~~and therefore total~~ Both total cholesterol and triglycerides were quantifieddeterminate in all
143 serum samples using -an enzymatic method and further determination by spectrophotometry at
144 500 nm (Bernert *et al.*, 2007).

145 2.5 Statistical Analysis.

146 ~~A new set of weights has been computed to be used with this sub-sample, taking into account~~
147 ~~the probability of inclusion of each participant in it.~~

148 [A new set of weights has been computed to be used with this sub-sample, maintaining the](#)
149 [probability of inclusion of each participant in occupational sector, gender and geographic region](#)
150 [from the Spanish Active Population census of 2009 considered in the study design. Weights](#)
151 [were applied to all estimates presented herein.](#)

152 Descriptive analysis included the 10th, 25th, 50th, 75th, 90th and 95th percentiles and geometric
153 mean (GM), along with their 95 % confidence intervals (CI). Based on the symmetry in the
154 distribution of the natural logarithm of the variables we choose GM for analysis and their
155 Confidence intervals.

156 A threshold was set on the maximum number of samples under the LOQ: if it is 60 % or more
157 means will not be computed. Otherwise, samples under the LOQ will be imputed this limit
158 divided by the square root of two. Interval linear regression has been used for the study of
159 multiple regression. The level at which a test result was considered significant was 0.05.

160 All the statistical analyses were carried out using Stata 11 (StataCorp LP, USA).

161 **3. Results**

162 *3.1 Levels of organochlorinated pesticides in human serum. Descriptive analysis.*

163 Four of the thirteen OCPs studied, aldrin, endrin, heptachlor and α -HCH were not detected in
164 any samples whereas the detection frequencies were lower than 10% for 4,4-DDT, 2,4-DDT and
165 2,4-DDE (data not shown). Table 1 shows serum concentrations for γ -HCH, β -HCH, dieldrin
166 and heptachlor-epoxide according to the variables considered in the study. The pesticides
167 included in this second group were detected between 10% and 60% of the samples. The HCHs
168 concentration in men ~~concentration~~ were lower than in women (γ -HCH: 124.5 vs 49.31 ng/g
169 lipid; β -HCH: 100.6 vs 124.5 ng/g lipid), while no gender differences were obtained for
170 dieldrin and heptachlor-epoxide.

171 Table 2 shows the descriptive analysis with the selected variable for HCB. Concentration of
172 HCB was above the LOQ in 83.8 % of serum samples. Univariate analysis showed a significant
173 increase in HCB concentration with age ($p < 0.001$). Women showed higher HCB concentration

174 men ($p=0.016$) and no significant differences were found about working sector ($p=0.291$).
175 Basque Country presented significantly higher levels in relation to the national mean ($p=0.010$)
176 with levels more than two times higher than those found in Murcia, Canary Islands or Galicia.

177 Finally, the highest OCP levels were observed for 4,4-DDE (Table 3), virtually present in all
178 samples (99,5%). A positive association between 4,4-DDE levels and age was found ($p<0.001$,
179 in all age groups) while about gender, in contrast to HCB, women had significantly lower levels
180 (ng/g lipid) than men ($p<0.001$). Although services worker presented GM for 4,4-DDE slightly
181 higher than the other group studied, no significant differences were observed ($p=0.245$). In
182 relation to area of residence, three of the regions studied presented significant differences with
183 respect to the national mean reporting with a univariate analysis the highest 4,4-DDE levels in
184 Basque Country and Galicia ($p=0.001$) while Castile and Leon ($p=0.045$) presented significantly
185 lower values.

186 3.2.3 Multivariate analysis

187 The influence in the OCP levels of those variables collected by the BIOAMBIENT.ES
188 questionnaire was assessed using regression multiple approach. Due to the number of samples
189 below LOQ, only 4,4-DDE and HCB multivariate analysis was carried out (Table4). Significant
190 associations regarding age observed in descriptive analysis were confirmed for both
191 compounds. Thereby, 4,4-DDE and HCB levels in the older age group (50-65 years old) were
192 more than three and four times respectively of those found in the younger group (<30 years old).
193 The results showed significant lower levels in women, both HCB and 4,4-DDE, than in men
194 (12% for DDE, $p=0.004$; 38% for HCB $p<0.001$). [The Seasonality study of seasonal effect](#)
195 showed statistical differences, 4,4 DDE presented a reduction of 17% from June to September
196 ($p=0.030$) compared to the annual mean, whereas for HCB an increase of 28% was found for
197 the same period ($p=0.030$) reporting significantly lowest concentration in the fourth trimester
198 ($p=0.003$). No differences were found in pesticides levels by occupational sector, educational
199 level or smoking status ($P>0.05$). Therefore, age, gender and [trimester of sample collection](#)

200 ~~seasonality~~ were revealed by multivariable analysis as significant influences in the levels of the
201 pesticides studied in our sample.

202 Figure 1 show the geographic distribution of 4,4-DDE and HCB once adjusted the significant
203 variables. Significantly higher levels of HCB regarding national mean, were found in Basque
204 country, Madrid and Castile and Leon while the lowest concentrations were in Murcia, Canary
205 Islands and Galicia region. Although, 4,4-DDE serum levels were also high in the centre of the
206 country *i.e.* Madrid, Castile and Leon and Castile-La Mancha, and low in Galicia and Canary
207 Islands, in this case, unlike HCB distribution, the lowest levels were reported in the north *e.g.*
208 Basque Country ($p=0.047$) and higher in Murcia ($p<0.001$).

209 In order to assess potential exposure factors, mainly the influence of food consumption
210 frequency, a regression multiple model with all significant variables adjusted was applied (age,
211 gender and sampling trimester). Among the ten food groups considered (eggs, fruits, meat, cold
212 meat, bread and cereals, legumes, potatoes-rice and pasta, dairy, vegetables, fish and sweets)
213 significant associations were found in relation to fish consumption with higher 4,4-DDE and
214 HCB levels (Figure 2). In both analytes adjusted concentrations in participant who reported
215 daily fish intake, was around 80% higher than those obtained in people who reported lower fish
216 intake (< 1 one time per week). This association was confirmed both for consumer of white fish
217 and oily fish separately. Meat intake, presented significant association only for HCB ($p=0.005$)
218 with levels two and halftime higher than levels in low meat consumer.

219 Tobacco consumption, type of heating, time residence in Spain and environment (countryside,
220 urban or residential) were also evaluated. HCB concentration presented a positive relation with
221 years lived in Spain in all groups studied considering the group of more than 35 years as
222 reference, *i.e.* 26-35 years lived in Spain ($p=0.005$), 16-25 years: ($p<0.001$) and 5-15 years
223 ($p<0.001$). However, this tendency was not observed for 4,4-DDE. Even though the levels
224 obtained for HCB and 4,4-DDE were about 25% higher in participants who lived in an urban

225 environment than the others, although this differences were not statistically significant (data not
226 shown).

227 **Discussion**

228 The aim of the present study was to establish the baseline information about OCP exposure in
229 Spanish adults at national level. The study design omitted the assessment of unemployed adults,
230 retired and [non-active population homeworkers](#). At the time of the study, the unemployment rate
231 was 7.95 % (Instituto Nacional de Estadística, INE, 2009). Since we opted for efficiency, we
232 assumed the limitation that this part of the population would be out of our scope (Pérez-Gómez
233 *et al.*, 2013).

234 ~~In spite of the~~ ~~Although, there are~~ many studies focussed on the OCP levels in human serum
235 worldwide in the last three decades, up to now not many countries have developed HBM
236 programs to assess OCP exposure in general population at national level. Nevertheless, there are
237 some relevant studies on these chemicals such as those in US (CDC, 2009), Germany (Becker *et*
238 *al.*, 2003; Schettgen *et al.*, 2015), Canada (Health Canada, 2010) and France (Sauodi *et al.*,
239 2014). However, in many cases the results comparison among studies or countries is complex
240 since variables like the year of sampling, the target population, the sample size, the lipid
241 adjustment, and the analytical methodology must be taken into account for a proper comparison.

242 As previously shown in this study, no quantifiable concentrations of aldrin, endrin, heptachlor
243 and α -HCH were obtained while the isomers less abundant for DDT and DDE (*ortho*, *para*-
244 substituted) were detected in less than 5 % of the participants. Similar detection frequencies
245 were reported for adult population in US (CDC, 2009) and Canada (health Canada, 2010) for
246 these analytes whereas in France, where low LOD and LOQ were obtained using a sensible
247 micro-ECD detector, α -HCH was detected in more than 80% of participants, but at very low
248 levels (GM: 0.66 ng/g lipid). It is important to note that actually some of these pesticides tend to
249 be excluded in HBM studies once the baseline levels of the population is defined (Schettgen *et*
250 *al.*, 2015). A sharp decline concentration of these OCPs was observed compared to samples

251 collected in the 90s in Spain, [the same as happens](#) ~~and~~ in other neighbouring countries.
252 Regarding 4,4-DDT, the main component of commercial products of DDT, was measured in
253 less than 10% of the participants (LOQ: 0.04 ng/mL), older age groups showing the [lower](#)
254 [higher](#) frequency of detection (3.7% over LOQ from 16 to 29 years vs. 12.7% in 50-65 years
255 old). These results suggest that has not taken place recent human exposure to DDT and they are
256 in line with those reported in several regions of Spain (Jakszyn *et al.*, 2009), in Sweden (Glynn
257 *et al.*, 2000) or US (CDC, 2009), and lower than those obtained in Eastern Europe (Dirtu *et al.*,
258 2006).

259 Heptachlor epoxide and dieldrin were measured in 12.6% and 17.2% of the serum samples
260 respectively. The 95th percentile (P95) was 13.8~~3~~ ng/g lipid for heptachlor epoxide and 22.5~~5~~
261 ng/g lipid for dieldrin. These OCPs are not commonly included in HBM studies and the levels
262 were ~~too~~ similar to those reported for adult population in US (heptachlor epoxide P95: 20.6 ng/g
263 lipid and dieldrin P95: 20.3 ng/g (CDC. 2009) in 2003-2004. HCH isomers detected in this
264 study were γ -HCH in the 20% of participants and β -HCH about 15%. It is important to note the
265 relative high LOQ obtained for this analytes by GC-qMS, 0.13 and 0.24 ng/mL respectively.
266 Lipid adjusted P95 levels for γ -HCH in Spain (P95: 50.07 ng/g lipid) were clearly higher than in
267 US and Canada (all samples below LOD), in France (P95: 3.6 ng/g lipid) or in Germany that
268 reported a detection frequency of 5% in samples collected between 1997 and 1999 (Becker *et*
269 *al.*, 2003). Levels of β -HCH (P95: 107.2 ng/g lipid) were in the same range than those obtained
270 in Canada P95: 90.0~~4~~ ng/g lipid higher than US P95: 56.5 ng/g lipid but almost the half of
271 concentration found in France (P95: 193.6 ng/g lipid). The historical high levels of HCHs found
272 in Spain compared to other western countries can be explained since was the 9th consumer of
273 technical HCH mixture worldwide and a significant producer of Lindane in the past (Li and
274 Macdonald, 2005; Jakszyn *et al.*, 2009; Fernandez [et al.](#), 2013). However, when we compare
275 HCH levels with data from some years ago, a marked~~d~~ decline is observed (GM: 166 ng/g lipid
276 for β -HCH in 953 serum samples collected during 1992-1996 in Spain vs. P95: 107.2 in this
277 study (2009-2010) (Jakszyn *et al.*, 2009).

278 Studies on the background HCB levels in US and Canada show much lower levels than those
279 reported in this work (P95: 29 and 26.6 ng/g lipid respectively vs. 160.4 ng/g lipid) (CDC, 2009;
280 Health Canada, 2010). These differences were lower in a German HBM study with 2750
281 participants (Median: 0.09 ng/mL vs. 0.16 ng/mL serum in this study) (Schettgen *et al.*, 2015).
282 Our results were similar to those reported in 368 serum samples collected in France (GM: 24.3
283 vs. 28.50 ng/g lipid in this study) (Saoudi *et al.*, 2014) and Sweden (GM: 23 ng/g lipid) (Glynn
284 *et al.*, 2000) and they were lower than those reported in Czech Republic (NIPH, 2010).

285 As expected, DDE was the most predominant OCP detected in human serum (GM:158.5, P95
286 717.7). The P95 of DDE obtained in this study was lower than those found in the US and
287 Canada population (P95: 1,9989 and 1,0877 ng/g lipid, respectively). They were similar to
288 concentrations in French population (GM: 118, P95: 729 ng/g lipid) and higher than those found
289 recently in Germany (Median 0.36 ng/mL vs. 0.87 ng/mL in this study) and Sweden (GM: 88
290 ng/g lipid; (Glynn *et al.*, 2000).

291 In general, DDE and HCB levels were much lower than those found in the last two decades in
292 Spain (Porta *et al.*, 2010; Botella *et al.*, 2004, Jakszyn *et al.*, 2009) and in other countries,
293 confirming a significant decline in the internal body burden to these pollutants (Petrik *et al.*,
294 2006, Becker *et al.*, 2002; Cerna *et al.*, 2008). In Spain, Porta M. *et al.* (2012) observed a
295 decrease in HCB levels of almost 50% in five years in Barcelona city (GM: 227 ng/g lipid in
296 2002 to 106 ng/g lipid in 2006). The results found in 2,150 pregnant Spanish women recruited
297 by INMA project were GM: 119.1 for DDE and 43.4 ng/g lipid for HCB, in the same range that
298 those reported in our study (Forns *et al.*, 2012). Therefore, our data for HCHs and DDE, are in
299 agreement with the heavy use of pesticides in agriculture (EUROSTAT, 2012), whereas relative
300 high HCB level can be attributed to industrial sources emission as shown in the national
301 inventory of industrial emission produced by Spanish Ministry of Agriculture, Food and
302 Environment (MAGRAMA, 2015).

303 Regarding the variables included in this project, a significant increase was observed with age for
304 DDE and HCB, thus concentration in the youngest age group (16-29 years old) represented 17%
305 and 25% respectively of those observed in the oldest one (50-65 years old). This strong
306 association with age were also reported in most of the previous studies (CDC, 2009; Saoidu *et*
307 *al.*, 2014, Glynn *et al.*, 2000; Porta *et al.*, 2012; Botella *et al.*, 2004, Health Canada, 2010). High
308 past exposure, bioaccumulation and persistent character of these compounds can explain these
309 results. Although, many studies reported similar age differences regarding HCHs (Saoidu *et al.*,
310 2014; Becker *et al.*, 2002), no significant differences were obtained at 95th percentile in this
311 study, in line with Canadian HBM programs (P95: 98.01, group 20-39 year vs. 62.47 group 60-
312 79 year). Present exposures to HCHs, can justify this findings since contaminated sites are still
313 found Spain (Fernandez *et al.*, 2013). However, the limited number of sample quantized hinders
314 a suitable assessment.

315 The multivariate analysis shows the gender also as determinant of serum levels, with women
316 levels significantly higher than men for all OCP studied. DDE and HCB concentration in men
317 were 12% and 38% lower than in women, respectively. Our result was consistent with
318 numerous works previously published worldwide (Becker *et al.*, 2002; Petrik *et al.*, 2006, Porta
319 *et al.*, 2010; Saoudi *et al.*, 2014). The differences in toxicokinetics and distribution in the body
320 between men and women could contribute to explain these differences (Mergler, 2012).

321 [Trimester of sample collection](#)Seasonality influence was assessed considering four sampling
322 periods (Jan-Mar, Apr-Jun, Jul-Sep and Oct-Dic). DDE and HCB levels were affected in a
323 different way by seasonality whereas DDE was 17% lower in summer months compared to the
324 annual mean, in the case of for HCB were a 23% higher for the same period with a significant
325 decline in the fourth trimester (p=0.003). The inclusion of this variable is not as usual as age or
326 gender, although seasonal influences on human serum pesticide residue were reported many
327 years ago (Stehr-Green, 1989). [Since the primary source of OCPs human exposure is diet, its](#)
328 [seasonal differences could be due to dietary changes depending on the time of year. In order to](#)
329 [obtain a more accurate assessment of seasonal effect in human levels, a more detailed dietary](#)

330 ~~study should be conducted in the future. The higher levels for HCB in summer could be in part~~
331 ~~due to re-volatilization because of the low vapor pressure vapor of HCB. In case of DDE, the~~
332 ~~diet seems to be the main factor for its seasonal differences.~~

333 The geographic distribution observed for DDE and HCB by multivariate analysis shows
334 significant lower levels in Galicia, a 20% and 35% compared to the national mean([Fig 1](#)).
335 Higher levels were observed for both pollutants in Castile and Leon, Castile-La Mancha and
336 Madrid. However, relevant differences were found in some of the most industrialized areas *i.e.*
337 Basque Country and Asturias-Navarre, situated in the north, since HCB levels were between 40
338 and 50% higher than the mean whereas, DDE levels were between 10 and 20% lower than the
339 mean. These results could be reflecting the influence of industrial sources in the levels of the
340 Spanish population. Levels found in Murcia, in the southwest, were about 35% lower for HCB
341 and 50% higher than the mean for DDE. These results are in agreement with those observed in
342 previous studies and most likely are associated with the intensive use of pesticides in
343 agricultural applications (Jakszyn *et al.*, 2009). It is important to note that HCB geographical
344 distribution in Spain were similar to the ambient distribution the at national level reported by
345 Meteorological Synthesizing Centre-East (MSC-E), one of the international research Centres of
346 the European Monitoring and Evaluation Programme (EMEP), ~~2016~~. The distribution of this
347 pollutant in air, soil and vegetables presented the highest levels in the North and centre of Spain
348 (Asturias, Basque Country, Castile and Leon) while the lower levels were found in the south
349 and west of Spain ([EMEP, 2016](#)) ~~(see Supplementary Information, Table SII).~~

350 Finally, we assess the influence of other exposure factors such as dietary habits, tobacco, type of
351 heating, time residence in Spain and environment -by a multivariate approach adjusted to the
352 significant determinants (gender, aged and seasonality). Our results showed a positive
353 association of HCB and 4,4 DDE concentrations and fish consumption and meat [only](#) with
354 HCB. ~~In the case of DDE, the levels found regarding meat consumption were too similar in all~~
355 ~~frequency categories studied~~ (Figure 2).—Furthermore, information collected from
356 BIOAMBIEN.ES questionnaire provided a lineal significant increase in relation with the

357 frequency of consumption both white fish and oil fish. Therefore, the differences between
358 participant who reported high fish consumption (2-4 times per week) and those with sporadic
359 consumption were more marked for HCB, with twice the concentration in higher consumer than
360 sporadic, and 50% higher for DDE. A significant increase for both xenobiotic, around 40% were
361 observed for oil fish [in our study](#). Numerous authors found the consumption of foods of animal
362 origin, specially fish as determinant in the levels of OCPs (Glynn *et al.*, 2000; Jakszyn *et al.*,
363 2009; Falcó *et al.*, 2008; Boada *et al.*, 2014; Ibarluzea *et al.*, 2010, Saoudi *et al.*, 2014).
364 Nevertheless, we do not find significant associations with cold meat or dairy products as we
365 expected based on literature evidence (Saoudi *et al.*, 2014). The dietary questionnaire used in
366 our survey was not specific to assess OCP exposure so could be interesting in the future to
367 design a more detailed and specific food survey to assess the correlation between DDE and
368 HCB levels and the dietary intake (Martí-Cid *et al.* 2008).

369 5. Conclusions

370 This paper presents for the first time background levels for OCPs in a representative sample of
371 [adult](#) Spanish adult population. The results obtained were in general in the same range or lower
372 than those reported in comparable studies worldwide, except for HCHs and HCB that presented
373 higher levels than other European countries and much higher than observed in North America, in
374 line with previous studies conducted in Spain. Our data revealed a strong decline in the serum
375 levels of persistent organochlorine compounds in the Spanish population in the two last
376 decades. The exposure determinants ~~are~~ age, gender and [trimester of sample](#)
377 [collection seasonality](#). Although the geographical distribution showed higher levels in the
378 northwest and centre of Spain than in the south and east regions, differences between HCB and
379 DDE levels were found between a region predominantly industrial (Basque Country) and
380 agricultural (Murcia). The high frequency in the consumption of fish both oil and white [seem to](#)
381 [be related with higher background levels of](#) ~~were associated with elevated~~ DDE and HCB levels
382 in [human](#) serum.

383 The study presented here allow us to define the background OCP levels in adult general
384 population from Spain, and will serve in the future to evaluate temporal trends and the
385 effectiveness of environmental and health policies, allowing save and prioritize economic
386 resources on those biomarkers more relevant. ~~Therefore~~ future biomonitoring studies should
387 focus on assessing the exposure of those compounds released in industrial processes (HCB) and
388 HCHs analysis by a more ~~sensitive analytical~~ sensitive analytical method, due to its heavy use and production
389 of HCHs in Spain.

390

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397 PREVENCIÓN, and PREVIMAC.

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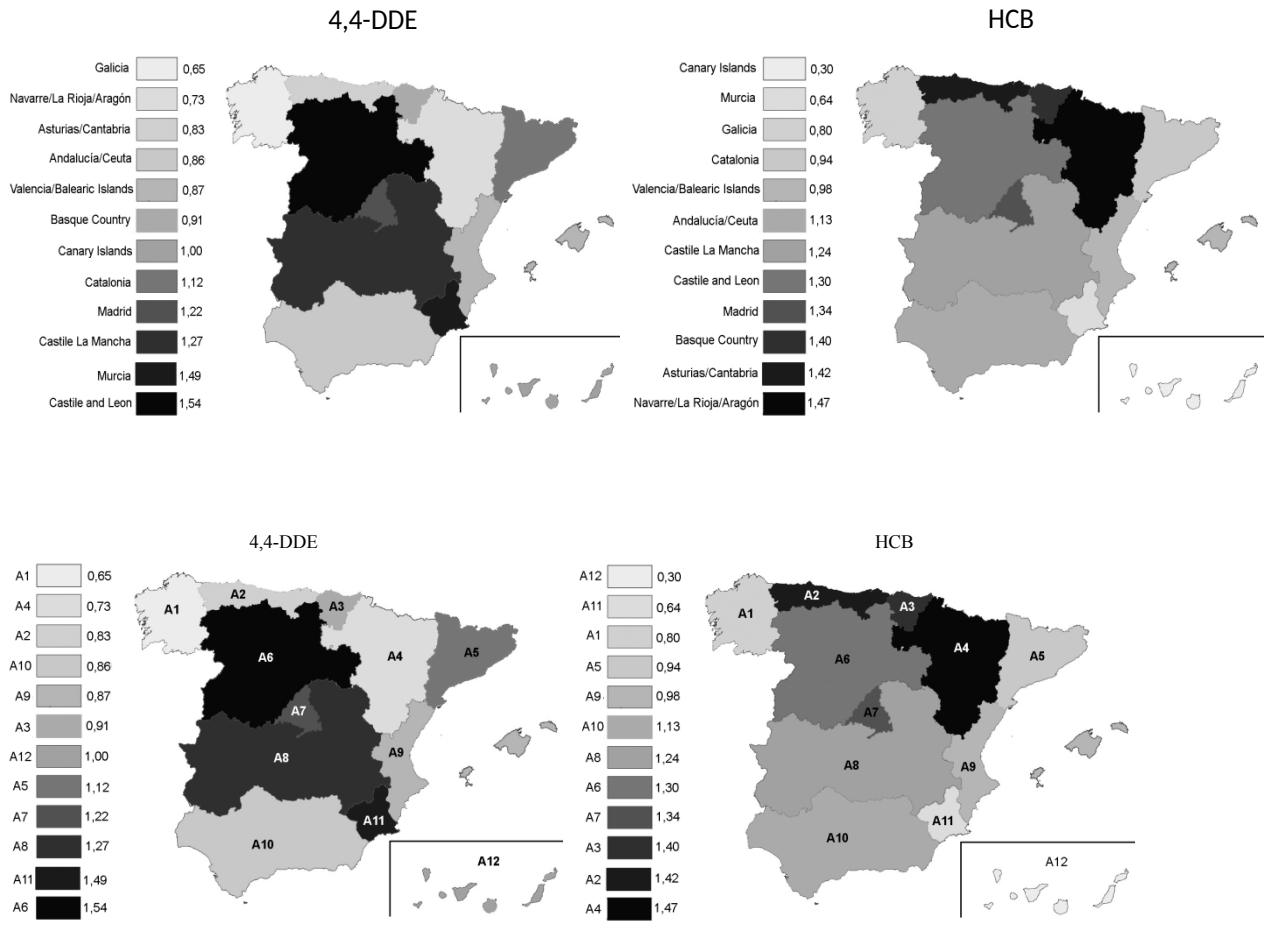
ORGANOCHLORINATED PESTICIDES LEVELS IN A REPRESENTATIVE SAMPLE OF THE SPANISH ADULT POPULATION: THE BIOAMBIENT.ES PROJECT

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Figure 1



Figures Legend

Figure 1. Geographical distribution of 4,4-DDE and HCB. [A.1 Galicia](#), [A.2 Asturias–Cantabria](#), [A.3 Basque Country](#), [A.4 Navarre–La Rioja–Aragon](#), [A.5 Catalonia](#), [A.6 Castile and Leon](#), [A.7 Madrid](#), [A.8 Castile la Mancha–Extremadura](#), [A.9 Valencia–Balearic Islands](#), [A.10 Andalusia–Ceuta](#), [A.11 Murcia](#), [A.12 Canary Islands](#). Results normalized considering national mean as reference level. Results adjusted to age, gender and [trimester of sample collectionseasonality](#).

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Figure 2

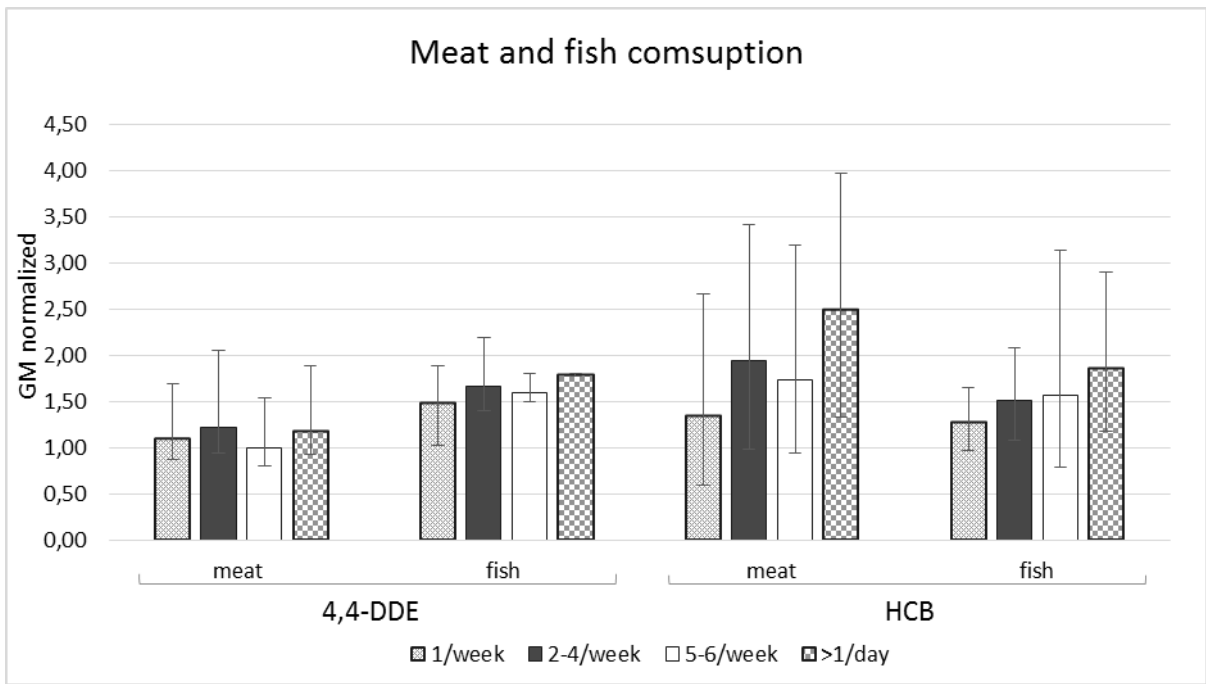
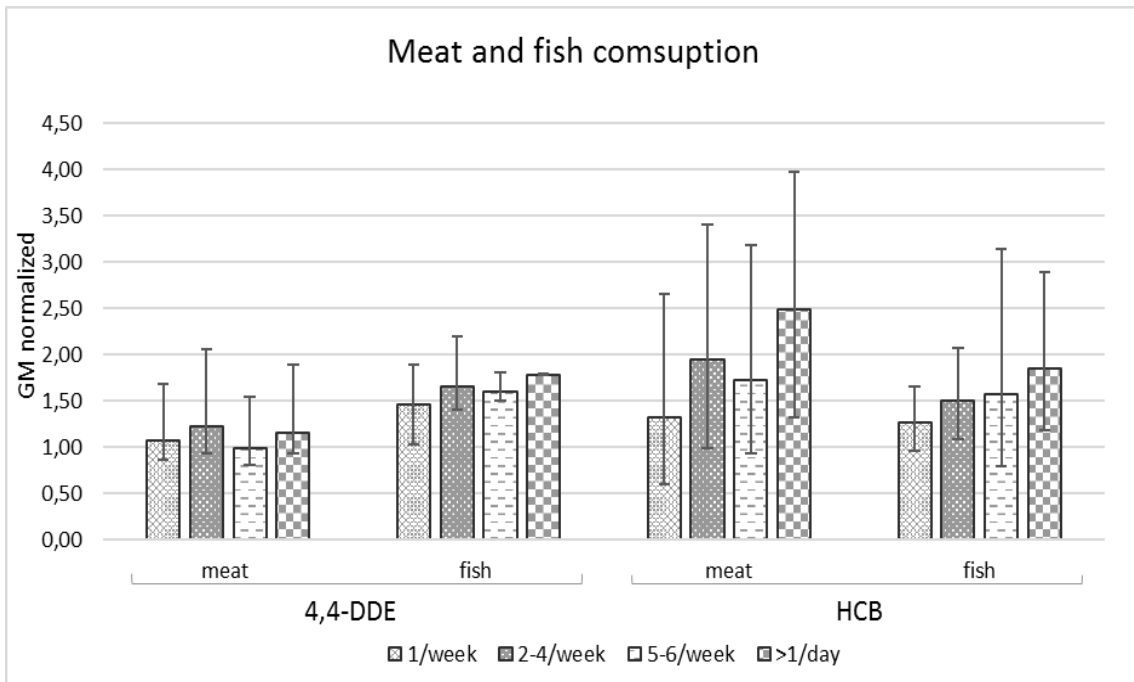


Figure legend

Figure 2. Relation between meat and fish frequency of consumption and HCB and 4,4-DDE levels. Results normalized considering GM of consumer of < 1 time per week as reference level. Results adjusted to age, gender and trimester of sample collectionseasonality.

ORGANOCHLORINATED PESTICIDES LEVELS IN A REPRESENTATIVE SAMPLE OF THE SPANISH ADULT POPULATION: THE BIOAMBIENT.ES PROJECT

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Table 1.

Variable	LevelsCate gory	N	γ -HCH		β -HCH		Dieldrin		Heptachlor- epoxide	
			95th	<LOQ %	95th	<LOQ %	95th	<LOQ %	95th	<LOQ %
Total	Total	934	50.07	79.8	107.2	85.3	22.55	82.8	13.83	88.4
Gender	Males	602	49.31	78.7	100.6	83.6	22.81	81.2	15.12	87.9
	Females	332	124.5	81.6	124.5	88.6	22.56	85.5	11.36	89.5
Working Sector	Services	570	48.38	80.4	120.7	85.4	22.81	81.2	13.10	88.6
	Others	364	96.06	78.8	96.06	85.2	22.01	85.2	15.05	88.2
Age groups	<29	221	56.90	79.2	98.67	86.4	22.21	85.1	15.23	86.4
	30-39	418	48.37	78.5	110.0	84.4	21.81	83.0	14.58	88.5
	40-49	212	52.53	83.5	98.0	86.8	22.08	83.5	14.63	89.6
	>50	80	48.13	78.8	131.6	82.5	31.10	73.8	12.79	90.0
Area of residence										
Galicia		73	63.24	79.5	150.1	80.8	18.26	86.3	16.49	89.0
Asturias-Cantabria		54	77.17	83.3	116.3	83.3	21.54	87.0	8.19	92.6
Basque Country		86	43.57	90.4	131.2	82.6	23.24	82.6	23.24	84.9
Navarre-La Rioja-Aragon		59	57.36	78.0	131.0	86.4	14.06	91.5	11.77	89.8
Catalonia		146	45.39	81.5	149.9	88.4	25.06	81.5	23.16	87.7
Castile and Leon		76	36.89	75.0	127.0	77.6	23.35	80.26	15.88	86.8
Madrid		108	59.14	67.6	113.3	77.8	20.30	88.9	21.07	83.3
Castile La Mancha-Extremadura		51	34.12	82.4	82.24	90.2	22.78	84.3	14.38	88.2
Valencia-Balearic I.		103	65.61	84.5	107.9	89.3	26.00	77.7	4.54	94.2
Andalucía-Ceuta		119	67.04	77.3	62.41	92.4	25.91	80.7	19.84	86.6
Murcia		30	40.90	83.3	259.2	83.3	23.14	86.7	14.90	93.3
Canary Islands		29	75.65	89.7	101.4	89.7	29.45	58.6	13.74	96.6

Table 2.

Variable	LevelsCategory	N	GM CI 95%	Selected percentile						<LOQ %
				10th	25th	50th	75th	90th	95th	
Total	Total	712	28.50 25.56-31.38	<LOQ	12.41	30.45	62.22	122.3	160.4	16.2
Gender	Males	426	24.53 21.91-28.39	<LOQ	11.25	23.80	54.10	115.4	147.9	18.1
	Females	286	33.42 27.68-39.42	<LOQ	20.91	36.90	73.55	128.6	182.3	13.3
Working Sector	Services	443	28.38 25.45-32.49	<LOQ	12.20	29.54	60.12	118.9	166.0	15.3
	Otros	269	25.98 21.98-30.29	<LOQ	9.85	26.87	48.25	108.6	178.4	17.5
Aged groups	<29	163	17.28 13.85-18.87	<LOQ	<LQ	19.98	33.52	66.81	72.51	29.5
	30-39	318	27.53 23.23-32.28	<LOQ	11.62	30.90	48.52	108.6	142.8	14.5
	40-49	165	38.79 31.85-47.79	<LOQ	19.82	39.85	80.52	164.3	232.9	10.3
	>50	63	62.80 46.75-82.33	14.36	29.98	67.94	126.98	230.4	413.9	3.2
Trimester Seasonality of sample collection	Jan-Mar	102	28.77 21.47-40.68	8.84	20.84	31.01	57.98	112.5	146.8	9.8
	Apr-Jun	283	28.39 24.67-32.28	<LOQ	88.90	29.51	56.98	110.3	142.5	16.3
	Jul-Sep	140	33.81 21.66-40.51	<LOQ	14.29	31.85	60.12	141.7	213.9	13.6
	Oct-Dic	176	23.69 20.30-28.85	<LOQ	13.63	21.48	53.25	112.5	164.2	22.2
<i>Area of residence</i>										
	Galicia	59	17.89 16.28-19.79	<LOQ	8.59	20.84	41.04	52.41	80.91	20.3
	Asturias-Cantabria	40	45.46 21.30-99.54	11.34	24.02	43.45	118.25	185.0	213.2	7.5
	Basque Country	59	46.69 30.40-72.52	12.31	25.50	44.54	106.9	226.8	254.8	1.7
	Navarre-La Rioja-Aragon	50	30.78 22.25-42.82	<LOQ	18.29	28.95	73.85	116.8	181.9	10.0
	Catalonia	115	24.71 20.63-30.38	<LOQ	10.29	26.58	63.18	94.21	128.9	20.9
	Castile and Leon	62	30.15 20.44-45.84	9.85	14.48	27.45	63.98	88.90	177.8	9.7
	Madrid	76	37.49 26.68-51.59	<LOQ	23.95	39.04	81.02	153.8	303.1	11.8
	Castile La Mancha-Extremadura	42	33.42 25.36-43.81	<LOQ	23.51	38.11	64.85	106.5	122.8	11.9
	Valencia-Balearic I.	74	24.66 16.099-35.72	<LOQ	11.14	29.12	52.58	116.9	144.8	18.9
	Andalucia-Cueta	91	24.99 19.05-32.36	<LOQ	10.85	31.21	54.05	111.3	162.2	23.1
	Murcia	22	18.76 16.22-17.18	<LOQ	<LQ	20.19	43.62	56.81	71.89	31.8
	Canary Islands	22	14.86 11.28-22.30	<LOQ	<LQ	17.82	29.93	41.42	41.42	36.4

Table 3.

Variable	LevelsCategory	N	GM CI 95%	Selected percentiles						<LOQ %
				10th	25th	50th	75th	90th	95th	
Total	Total	934	158.8 149.8-168.4	51.44	86.32	153.05	280.72	519.73	717.69	0.5
Gender	Males	602	172.4 160.3-185.5	59.33	91.57	162.08	310.29	602.28	824.94	0.2
	Females	332	136.7 124.3-150.2	40.67	73.34	141.45	243.89	432.75	595.02	1.2
Working Sector	Others	364	154.01 138.9-170.8	44.04	78.69	144.57	295.47	535.71	796.54	1.1
	Services	570	162.0 151.1-173.5	58.25	88.90	157.76	275.44	519.17	703.70	0.2
Aged groups	-29	212	112.8 101.1-125.9	40.67	68.19	107.65	178.99	345.07	449.44	0.5
	30-39	425	150.3 138.5-163.1	50.87	85.89	144.72	257.23	462.87	629.32	0.5
	40-49	210	190.5 169.2-214.5	57.77	108.77	182.52	335.98	609.35	822.26	0.5
	50-	87	310.0 252.5-380.7	88.83	156.11	299.01	641.68	1108.1	1783.82	0.1
Trimester of sample collectionSeasonality	Jan-Mar	143	160.9 146.0-177.3	54.49	86.30	161.25	327.51	557.44	868.89	0
	Apr-Jun	337	171.01 146.1-200.1	52.20	84.31	157.06	279.83	583.25	700.31	0.3
	Jul-Sep	202	149.0 131.9-168.3	44.90	85.04	148.88	264.78	504.88	641.11	1.5
	Oct-Dic	239	157.2 140.1-176.4	49.94	88.93	152.10	268.87	521.71	917.50	0.4
Area of residence										
	Galicia	73	196.1 165.0-233.1	39.31	58.91	101.63	217.15	325.35	361.84	1.4
	Asturias- Cantabria	54	151.6 118.5-193.9	40.59	76.99	123.13	329.18	499.61	711.71	1.9
	Basque Country	86	208.9 176.0-248.0	35.90	65.00	126.77	183.17	300.18	653.94	9
	Navarre-La Rioja-Aragon	59	242.3 180.2-325.9	51.49	88.93	137.95	210.20	331.03	596.13	9
	Catalonia	146	126.7 106.3-150.9	48.71	76.00	139.07	267.10	503.98	698.86	9
	Castile and Leon	76	142.6 122.8-165.6	50.27	71.09	125.46	191.14	329.17	483.65	9
	Madrid	108	105.7 87.4-127.7	63.04	117.23	180.51	312.54	643.64	923.94	1.9
	Castile La Mancha- Extremadura	51	178.4 141.5-224.8	83.65	120.60	237.09	425.50	773.63	2938.1	2.0
	Valencia- Balearic I.	103	145.1 121.2-173.8	70.51	106.48	217.79	420.15	637.33	848.46	0
	Andalucia-Cueta	119	192.6 162.6-228.2	65.00	95.40	203.11	352.92	699.81	1082.17	0
	Murcia	30	151.5 111.6-205.6	43.15	83.49	144.40	221.96	604.33	833.21	0
	Canary Islands	29	116.4 97.75-138.6	73.48	115.38	162.12	292.30	566.97	658.68	0

Table 4.

Variable	LevelsCategory	4.4-DDE		HCB	
		B CI 95%	P	B CI 95%	P
Working sector (ref. Services)	Others	0.60	0.311	0.91	0.376
		0.53-0.65		0.74-1.13	
Gender (ref. Females)	Males	0.88	0.004	0.62	<0.001
		0.58-1.12		0.49-0.79	
Age groups (ref. < 30 years)	30 - 39	1.40	0.024	1.68	<0.001
		1.36-1.47		1.42-1.98	
	40 - 49	1.80	<0.001	2.88	<0.001
		1.77-1.82		2.24-3.70	
50 -	3.26	<0.001	4.86	<0.001	
	2.65-3.70		3.26-7.24		
<u>Trimester of sample collection</u> (ref. mean)	Jan-Mar	1.03	0.659	1.01	0.912
	Apr-Jun	0.89-1.15		0.84-1.21	
		0.99	0.817	1.03	0.633
	Jul-Sep	0.92-1.06		0.91-1.17	
		0.83	0.030	1.23	0.030
Oct-Dic	1.01	0.742	0.78	0.003	
		0.92-1.08		0.67-0.91	

Table legends

Table 1. Descriptive analysis of those OCPs quantified in less than 60% of the studied population. Results of 95th percentile level in ng/g of lipid.

Table 2. Descriptive analysis of HCB for the studied population. Geometric mean and selected percentiles in ng/g of lipids.

Table 3. Descriptive analysis of 4,4-DDE for the studied population. Geometric mean and selected percentiles in ng/g of lipids.

Table 4. Serum levels of HCB and 4,4 DDE and their associations with selected covariates. Exponentiated coefficients of models are shown. Base reference levels are listed variable column in brackets. Significant are in bold.

ORGANOCHLORINATED PESTICIDES LEVELS IN A REPRESENTATIVE SAMPLE OF THE SPANISH ADULT POPULATION: THE BIOAMBIENT.ES PROJECT

a Juan José Ramos et al.

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Abstract

The present manuscript presents serum levels of most relevant organochlorinated pesticides (OCPs) on a representative sample of Spanish adults recruited in 2009 during their annual medical examination at work under the BIOAMBIENT.ES project, a nationwide human biomonitoring program promoted by Spanish Ministry of Agriculture, Food and Environment to estimate reference levels of environmental pollutants in the general population. Out of the thirteen OCPs investigated: Aldrin, endrin, dieldrin, heptachlor, heptachlor-epoxide, dichlorodipenyldichloroethylenes (DDEs), hexachlorobenzene (HCB), dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), —the highest concentrations were observed for DDE, HCB and β -HCH. The 95th percentile, in ng/g lipids, were: 717.7 for 4,4-DDE, 160.4 for HCB and 107.2 for β -HCH, whereas aldrin, endrin, heptachlor, α -HCH, 2,4-DDT, 4,4-DDT and 2,4-DDE presented the lowest concentrations being detected in less than 10% of samples. Significant differences ($p < 0.05$) were found for DDE and HCB and seasonality, gender and age. The geographical distribution of these analytes showed the highest levels in people who lived in the north of Spain for HCB and in the center for 4,4-DDE. The influence of intake of several food groups in the OCP levels was also assessed. The results obtained showed a sharp decline in the OCP concentrations reported in the past in Spain and they were in the same range or lower than those reported in similar studies worldwide, except for HCHs and HCB that presented higher levels than in other European countries and much higher those observed in US and Canada. This study represents the first nationwide survey of exposure to OCPs in Spain and provides a background reference range

for exposure to OCPs in the Spanish adult occupied population. These results will allow establishing reference values, observing temporal trends and identifying high exposure groups.

Human Biomonitoring, Spain, Organochlorinated Pesticides-, serum, DDT, DDE, HCB, β -HCH.