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An evaluation of the sexual differences in the accumulation of organochlorine compounds in children at birth and at the age of four years

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Abstract

This study of the body burden and serum concentrations, of organochlorine compounds (OCs), represents a general population in a cohort from Menorca Island (birth 1997-1998) of children at birth and at four years of age; the study has shown that the concentrations of hexachlorobenzene (HCB), 4,4'-DDE, 4,4'-DDT, polychlorobiphenyl (PCB) congeners #153, #138 and #180 and total PCBs in sera collected at four years exhibit much higher values in breastfed children than in those fed with formula, e.g. HCB 0.48 vs 0.21 ng/ml, β-HCH 0.32 vs 0.24 ng/ml, total DDTs 2.2 vs 0.57 ng/ml, total PCBs 1.4 vs 0.52 ng/ml, respectively. Comparison of gender differences in four years old children shows higher concentrations of all examined OCs in females than in males with the exception of HCB and PeCB in breastfed children which are higher in males than in females, e.g. β-HCH 0.34 vs 0.28 ng/ml, total DDTs 2.6 vs 1.7 ng/ml, total PCBs 1.6 vs 1.0 ng/ml, respectively, for breastfed children and e.g. β-HCH 0.23 vs 0.19 ng/ml, total DDTs 0.59 vs 0.48 ng/ml, total PCBs 0.58 vs 0.45 ng/ml, respectively, for formula fed children. Gender comparison of the body burden between children fed with breast milk or formula also shows higher concentrations in females than males, e.g. β-HCH 0.47 vs 0.35 μg, total DDTs 3.0 vs 1.8 μg, total PCBs 1.9 vs 1.2 μg, respectively, for breastfed children, and β-HCH 0.39 vs 0.17 μg, total DDTs 0.48 vs 0.27 μg, total PCBs 0.66 vs 0.55 μg, respectively, for formula fed children. The results may suggest a higher capacity in female children for the retention of OC incorporated through breast feeding. However, these results should be taken with caution because the differences of the gender averages have low statistically significance when evaluated with the Student test.

Key words: Organochlorine compounds, persistent organic pollutants, breastfeeding, polychlorobiphenyls, 4,4'-DDE, pollutants in cord serum, background concentrations of organochlorine compounds in children sera, newborns

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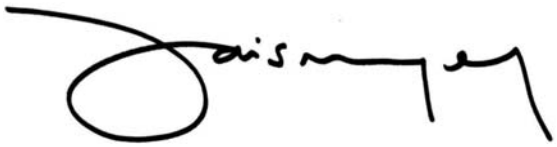
This study was approved by the ethics committee of the Institut Municipal d'Investigació Mèdica and all mothers provided a signed informed consent.

Yours sincerely,



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1. Introduction

4,4'-DDE and polychlorobiphenyls (PCBs) are incorporated into children *in utero* and through diet, namely breastfeeding (Huisman et al. 1995; Karmaus et al. 2001; Rhainds et al. 1999; Ribas-Fito et al. 2003; 2005; Carrizo et al., 2006). The lipophilic properties and high stability to chemical degradation of these compounds favours their accumulation in human fat. These properties are those characteristics of persistent organic pollutants (POPs) and define a group that encompasses those mentioned above and others such as hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), 4,4'-DDT, involving up to twelve types of organochlorine compounds (OCs) that have been banned by the Stockholm agreement (Stockholm Convention on Persistent Organic Pollutants, 2005).

One key aspect needed for the assessment of the risks of these compounds into human health is the understanding of the processes leading to their accumulation into humans, namely in the first period of growth. Several studies have documented the significance of intake through breastmilk in children that is a major determinant of their body burden, even after several years of discontinuation of this feeding mode (Lanting et al., 1998; Jacobson et al., 1989; Karmaus et al., 2001; Carrizo et al., 2006). Other studies have also shown the influence of environmental exposure in relation to some specific activies (e.g. Ribas-Fito et al., 2005; Sala et al., 2001).

In contrast, little attention has been paid to the importance of the own characteristics of the exposed individuals in OC intake. In this respect, sex is one of the most obvious aspects to consider. Accordingly, the present paper reports a study devoted to assessing the gender differences in the accumulation of these compounds in newborns and children at the age of four years. This goal has been addressed by considering whether children were fed with breast milk or formula; since, as mentioned above, these two feeding practices are major determinants of the accumulation of these compounds in the early stages of life.

The population selected for this study is from Menorca, one of the Balearic Islands in the northwest Mediterranean Sea. The island does not have factories producing OCs. DDT was used for agriculture in the past. Thus, the participating children were exposed to background POP levels and can be taken as examples of the regular exposure to these pollutants in western countries. A general population birth cohort was set up in 1997 within the Asthma Multicenter Infants Cohort study (AMICS) (Polk et al. 2004). The cohort recruited all women presenting for antenatal care over 12 months starting in mid 1997. 482 children were subsequently enrolled and 470 (97.5%) provided complete outcome data up to the fourth year visit. Among these 410 (85%) had OCs measured in cord serum and 285 (59%) in serum collected at four years.

2. Materials and Methods

Materials

Standards of tetrabromobenzene (TBB), pentachlorobenzene (PeCB), HCB, α-, β-, γ-, and δ-HCH, PCBs, 4,4'-DDT and 4,4'-DDE were purchased from Dr. Ehrenstoffer (Augsburg, Germany). Analytical grade concentrated sulphuric acid (conc.-H_2SO_4), iso-octane and *n*-hexane were all purchased from Merck (Darmstadt, Germany).

Sample extraction and clean up

Samples were obtained in the context of the procedures defined by the INMA Project (Fernandez et al., 2007). Serum samples (0.5 mL) were introduced into 10 mL centrifuge tubes and TBB and PCB 209 were added as recovery standards. Two mL of conc.-H_2SO_4 and 3 mL of *n*-hexane were added, mixed in a vortex (*ca.* 1500 rpm, 30 seconds) and then centrifuged (*ca.* 1500 rpm, 10 minutes). The supernatant *n*-hexane layer was aspirated into a second centrifuge tube using

a Pasteur pipette. Further, *n*-hexane (2 mL) was added to the first tube containing the H_2SO_4 / serum, stirred (vortex *ca.* 1500 rpm, 30 s) and then centrifuged (*ca.* 1500 rpm, 10 min). This last step was repeated, yielding a combined extract of 7 mL of *n*-hexane, to which 2 mL conc-H_2SO_4 was added, the sample mixed (vortex mixer, *ca.* 1500 rpm, 90 s), centrifuged as before, and the supernatant transferred to a conical bottomed, graduated tube. The combined extracts were then reduced to near dryness under a gentle stream of nitrogen and an injection standard (PCB 142 in isooctane; 10 µL) was added. Then, the sample was quantitatively transferred to gas chromatographic vials using four 25 µL rinses of isooctane. If an emulsion was formed at any stage of the extraction, 10 – 15 drops of MilliQ water were added before sample centrifugation.

Composition of organochlorine compounds

Selection of OC for analysis was based on a literature search of commonly-studied contaminants and included representatives of industrial and agrochemical products. Four HCH isomers were analyzed (α-, β-, γ- and δ-). The α-, γ- and δ-isomers were only found above quantification limit in less than 5% of total samples. Therefore, these compounds were not included in the database of the study. Among the large family of PCB congeners, those often referred to as the "ICES 7" (from International Council for the Exploration of the Sea) were selected: PCBs #28, #52, #101, #118, #138, #153 and #180. These congeners are frequently found at high concentrations in humans and wildlife. In all cases, when the concentrations were below the limit of detection or quantification (Table 2), the value of 0 was introduced. Substitution of these values by half of the limit of quantification (Table 2) did not change the results or the significance of the statistical tests nor the correlation analyses discussed below.

Instrumental determinations

A gas chromatograph with electron capture detection (Hewlett Packard 6890*N* GC-ECD) was used to quantify PeCB and HCB, PCB congeners #28, #52, #101, #118, #138, #153, #180, *p,p'*-DDT and *p,p'*-DDE. α-, β-, γ-, and δ-HCH were quantified with GC-MS (HP 5973 MSD) in negative chemical ionisation mode using ammonia as reagent gas (1.0 mL/min). In both instruments samples were injected (2 μL) in splitless mode onto a 60 m, DB-5 column with a retention gap (both from J&W and Agilent). Helium was the carrier gas (1.5 mL/min). The temperature program was: 90°C 2 min, 20°C/min to 140°C, 4°C/min to 200°C held for 13 min, 4°C/min to 310°C held for 10 min.

In both instruments, quantification was performed by external standards using PCB 142 injection standard to correct for volume. Recoveries of TBB and PCB 209 (mean ± standard error = 102 ± 4.2 and 93 ± 1.8, respectively) were used to correct results. Limits of detection (LOD) and quantification (LOQ) were calculated from blanks (LOD = mean of all blanks plus three times the standard deviation, LOQ = mean plus five times the standard deviation) or from instrumental LOD using diluted standards if the compound was absent from the blanks.

This method performed satisfactorily in repeated international intercalibration exercises within the Arctic Monitoring and Assessment Programme (AMAP 2004).

This study was approved by the ethics committee of the Institut Municipal d'Investigació Mèdica of Barcelona and all mothers provided a signed informed consent.

Statistical analyses

Means and standard deviations of the OC concentrations or body burden in the diverse groups of children, e.g. breastfeeding and formula fed, males and females, were used for comparison of their differences with were evaluated according to the t test.

3. Results

Characteristics of the population under study

Sex, feeding practices, and duration of lactation of children in the population studied are shown in Table 1. 83% of children were breastfed. Duration of lactation encompassed from very short periods (2 months or less) to large periods (more than one year). No significant selection biases between the group of participants at birth (n = 410) and 4 years later (n = 285) are observed. No significant differences in feeding practices, either maternal or formula, or duration of lactation are found between the two gender groups of this population.

Over the whole population included in the study, 4,4'-DDE is the most abundant OC, in both cord serum and serum collected at 4 years (average 1.6 ng/ml in both cases; Table 2). The lower concentrations of 4,4'-DDT (0.18 ng/ml and 0.073 ng/ml, respectively) than 4,4'-DDE likely reflect that the whole mixture of DDT metabolites correspond to past exposure of this pesticide because a substantial amount of the 4,4'-DDT initially introduced into the environment has already been transformed into 4,4'-DDE (Wedemeyer, 1967; Aguilar, 1984).

HCB is the second major OC involving average values of 0.75 ng/ml and 0.42 ng/ml in cord and four years sera, respectively (Table 2). Total concentrations of the ICES 7 PCB congeners are 0.70 ng/ml and 1 ng/ml, respectively. The PCB distributions are dominated by congener #153 in both types of samples. β-HCH is found at concentrations of 0.21 ng/ml and 0.28 ng/ml in cord and four years sera, respectively. PeBC is the OC found in lowest concentration, 0.081 ng/ml and 0.023 ng/ml, in serum collected at birth and at four years, respectively, among those included in the present study.

Gender differences

A summary of the gender differences in the populations of newborns and 4 year-old children is represented in Fig. 1. The results are in agreement with those discussed above and summarized in Table 2. However, small gender differences can be observed. Thus, in newborns slightly higher concentrations of HCB and 4,4'-DDE are observed in male whereas PCBs are found in higher concentrations in female (Fig. 1). In contrast, in the examined population of children at the age of 4 years, 4,4'-DDE, PCBs and β-HCH are present in higher concentrations in females than in males.

Influence of breastfeeding

The average concentrations of HCB, 4,4'-DDE, 4,4'-DDT, PCB congeners #153, #138 and #180 and total PCBs in serum collected at four years exhibit significant higher values in breastfed children than in those fed with formula (Table 3). The degree of statistical probability of the dissimilar averages is very high ($p < 0.0001$) for HCB and most hydrophobic compounds. Therefore, breastfeeding is a key factor for the presence of these OC in children at age four even after 3.5-2.3 years that this practice was discontinued. A full discussion of the maternal aspects such as age, body mass index (BMI) and period of lactation that are correlated to OC body burden in children at four years of age is given elsewhere (Carrizo et al., 2006). None of those was statistically significantly correlated with the gender differences examined in the present study.

The gender averages of OCs in the population of four years old children fed with breastmilk show higher concentrations in female than male for all OCs except PeCB and HCB (Table 3). Formula fed children also show higher concentrations in female than male but the gender differences are higher in breasted than formula fed children (Table 3). These gender differences are statistically significant in the case of 4,4'-DDT among breastfed children ($p < 0.1$; Table 3). Neither BMI nor age of the mother were statistically significantly correlated with the gender differences examined in the present study.

Calculation of the concentration differences in serum OC at four years and at birth shows a general increase for breastfed children (Table 4). This increase is observed for all OCs with the exception of HCB and a small decrease found in the case of PeCB, 4,4'-DDT and PCB-180. In contrast, formula fed children show small variations and those involving more than 0.1 ng/ml, HCB, 4,4'-DDE, 4,4'-DDT, PCB-180 and total PCBs, exhibited decreases (Table 4). The differences between breast and formula fed children are statistically significant for HCB, 4,4'-DDE, 4,4'-DDT and most of the hydrophobic PCBs.

Comparison of the concentration changes in breastfed children after grouping by gender shows, for all OCs except PeCB and HCB, higher concentration increases, or lower concentration decreases, in female than male (Table 4). The differences are statistically significant in the case of 4,4'-DDE ($p < 0.1$; Table 4). Gender comparison of the concentration changes in formula fed children also shows, for all OCs except HCB, PCB-28 and PCB-52, higher increases, or lower decreases, in female than male children.

Straightforward comparison of the concentrations at birth and at four years overlooks the changes in blood volume due to children growth. Calculation of OC body burdens allows the inclusion of this aspect in the calculations.

Body burden

Transformation of the measured concentrations at birth and at the age of four years into body burden using the estimated total amount of serum from weight, *ca.* 0.075 L/kg, allows the calculation of the increase in OC amounts in each individual between these two periods (Table 5). The average values show that there is an OC increase between the two ages for all compounds. Except in the case of PeCB, the magnitude of the increase is higher among breastfed than formula fed children which is consistent with the above mentioned concentration differences. The OC body burden differences are statistically significant for HCB and all the most hydrophobic OC (Table 5).

Examination of the gender averages of this body burden data shows again a higher intake in female than male children. The differences are about the same as those previously discussed on concentrations.

4. Discussion

OC concentrations in children from Menorca and other populations

The OC concentrations found in the children from the cohort of Menorca correspond to background values of general population. The observed average concentrations of total PCB in cord serum, 0.68 ng/ml, are lower than those found in studies from Norway (3.0 ng/ml, Skaare et al. 1988), USA (2.5 ng/ml, Schwartz et al. 1983), Faroe Islands (1.1 ng/ml, Grandjean et al. 1997) and Germany (0.96 ng/ml or 1.4 ng/ml, Lackmann et al. 1996). On the other hand, they are higher than those reported in the Netherlands (0.38 ng/ml, Huisman et al. 1995), Canada (0.50 ng/ml, Rhainds et al. 1999) or Catalonia (0.36 ng/ml, Sala et al. 2001). Comparison of these data must be done with caution because different PCB congeners were used for the calculation of total PCBs in each study. However, the reported figures may vary by a factor of two at the most. In this cohort the PCB congeners of higher volatility were also considered for quantification which is not usually performed in other studies.

The concentrations of HCB in cord serum from these Menorcan children, 0.75 ng/ml, are lower than those reported in Norway (1.0 ng/ml, Skaare et al, 1988), Germany between 1984-85 (2.0 ng/ml, Lackmann et al. 1996) or Catalonia (1.2 ng/ml, Sala et al., 2001) but higher than the average concentrations found in Germany between 1994-95 (0.61 ng/ml, Lackmann et al. 1996) or Canada (0.04 ng/ml, Rhainds et al. 1999). The concentrations of 4,4'-DDE, 1.6 ng/ml, are lower than those reported in Norway (3.0 ng/ml, Skaare et al. 1988) and higher than the average

283 measurements of Canada (0.4 ng/ml, Rhainds et al. 1999) or Catalonia (0.83 ng/ml, Sala et al.
284 2001). Previous studies have outlined the capacity of these compounds to cross the placenta leading
285 to *in utero* exposure (DeKoning and Karmaus, 2000).
286
287 *OCs in male and female children*
288 The cord serum OC concentrations show higher concentrations of 4,4'-DDE and HCB in
289 male than female newborns, about the same concentrations of PeCB and β-HCH in both genders
290 and higher PCB concentrations in female than male (Fig. 1) but the differences are not statistically
291 significant ($p < 0.1$). These differences change when comparing the same children at age 4. Now,
292 the concentrations of all OC except PeCB and HCB are higher in female than male breastfed
293 children but again the differences are only statistically significant for 4,4'-DDT ($p < 0.1$; Table 3).
294 These differences are not related with different BMI of the two gender groups since both of them
295 have the same average value, 16.1 kg/m^2 (standard deviation 1.4 kg/m^2). In formula fed children all
296 OC concentrations are higher in female than male (Table 3). Examination of the BMI of both
297 gender groups show higher average values in male, 17.3 kg/m^2 (standard deviation 2.5 kg/m^2), than
298 female, 16.3 kg/m^2 (standard deviation 2.6 kg/m^2). The differences are not statistically significant
299 but, in any case, the higher BMI values are found in male children (the group in which lower OC
300 concentrations were found). Thus, the OC differences in formula fed children cannot be attributed
301 to BMI differences. In a previous study, on children at 7 years of ages from the Federal State of
302 Hessen (Germany), the concentrations of 4,4'-DDE, β-HCH and HCB in both genders were
303 practically the same but total PCBs were significantly higher in male than female ($p < 0.001$)
304 (Karmaus et al. 2001). Higher levels of PCBs were also found in male than female children, at age
305 four, from Michigan (USA) but the differences were small (Jacobson et al. 1989).
306
307 *Breastfeeding*

As shown in Table 3, breast feeding is a major way of incorporation of OCs into children of the population of Menorca, which is in agreement with previous observations for some OCs in other studies, e.g. 4,4'-DDE (Karmaus et al. 2001), 4,4'-DDT (Jacobson et al. 1989), β-HCH (Karmaus et al. 2001), HCB (Karmaus et al. 2001) and PCB mixtures encompassing diverse congeners with more than five chlorine substitutents (Jacobson et al. 1989; Lanting et al. 1998; Karmaus et al. 2001). A full account of the maternal characteristics influencing the intake of OCs through breastfeeding is given in Carrizo et al. (2006).

Examination of the gender differences between breast and formula feeders shows higher contrasts in breast fed female children. Thus, all OC exhibit higher concentrations in female than male children (Table 3). The dependence between breastfeeding and gender differentiation is also illustrated in Fig. 2 where the average composition of the main OC groups is represented after grouping by gender and feeding practices. As shown in this figure higher duration of breastfeeding involves higher gender differentiation of the average values but the coefficients of variation are also larger. These differences suggest that female children could retain more efficiently OC incorporated through breastmilk. In this context, the parity differences between the two groups have also to be considered. For breastfed children they are 1.1 (standard deviation 0.74) and 0.8 (standard deviation 0.74) for males and females, respectively, and for formula fed children they are 1.2 (standard deviation 0.97) and 1.1 (standard deviation 0.76), respectively. These differences are small and not statistically significant.

Further insight into this retention capacity can be evaluated from the comparison of the concentration changes of these OC in children between birth and at the age of four years (Table 4). These data show a general concentration increase in breastfed female children (all OC except PeCB, HCB, 4,4'-DDT and PCB-180). In male children there is also a concentration increase but with more exceptions (all OC except PeCB, HCB, PCB-28, 4,4'-DDE, 4,4'-DDT and PCB-180). On the other hand, all OC except PeCB and HCB exhibit higher concentration increases (or lower

333 concentration decreases) in breastfed female than male children (Table 4). Formula fed children
334 show a predominance of OC concentration decreases in both female and male children (Table 4).
335
336 *Changes in body burden of OCs between birth and four years*
337 Examination of the OC intakes between four years and birth in each individual by
338 subtraction of the body burden of these compounds at four years and at birth confirms that
339 breastfeeding is a major way of incorporation of these compounds in the first years of children
340 growth (Table 5). Higher gender differentiation involving higher accumulation in female children is
341 observed for all PCBs. These observations give more ground to a possible higher retention capacity
342 of female children in the retention of OC incorporated through breastfeeding. Nevertheless, the high
343 standard deviations resulting from these body burden calculations give rise to lack of statistical
344 significance to the observed gender average differences.
345
346
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353
354
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410
411 **FIGURE CAPTIONS**
412
413 Fig. 1. Average concentrations of organochlorine compounds in cord blood and sera collected at
414 four years of female  and male  children. The intervals represent standard error ($p <$
415 0.05).
416
417 Fig. 2. Average concentrations and coefficients of variation of diverse gender and feeding practice
418 groups of children included in the cohort of Menorca. BF: breastfeeding. Long BF > 3 months,
419 short BF ≤ 3 months. F: female. M: male. As shown in the plots, the OC concentrations at four
420 years of age are strongly influenced by breastfeeding and its duration at the early stages of life.
421 Female accumulate larger concentrations of OC except HCB but the differences with respect to
422 male are not statistically significant.
423