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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 4 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 4 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 4 years are much higher 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 and total PCBs 1.4 vs 0.52 ng/ml. Comparison of gender differences in 4 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 and total PCBs 1.6 vs 1.0 ng/ml for breastfed children and β -HCH 0.23 vs 0.19 ng/ml, total DDTs 0.59 vs 0.48 ng/ml and total PCBs 0.58 vs 0.45 ng/ml for formula fed children. Gender comparison of the body burden between children fed with breastmilk or formula also shows higher concentrations in females than in males, e.g. β -HCH 0.47 vs 0.35 μ g, total DDTs 3.0 vs 1.8 μ g and total PCBs 1.9 vs 1.2 μ g for breastfed children, and β -HCH 0.39 vs 0.17 μ g, total DDTs 0.48 vs 0.27 μ g and total PCBs 0.66 vs 0.55 μ g for formula fed children. The results may suggest a higher capacity in female children for the retention of OCs incorporated through breastfeeding. 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.

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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; Rhoads 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 favour their accumulation in human fat. These properties are those characteristic of persistent organic pollutants (POPs) and define a group that encompasses those mentioned above and others such as hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs) and 4,4'-DDT, involving up to 12 types of organochlorine compounds (OCs) that have been banned by the Stockholm agreement (Stockholm Convention on Persistent Organic Pollutants, 2005).

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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 activities (e.g. Ribas-Fito et al., 2005; Sala et al., 2001).

In contrast, little attention has been paid to the importance of the personal 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 gender differences in the accumulation of these compounds in newborns and children at the age of 4 years. This goal has been addressed by considering whether children were fed with breastmilk 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 4 years.

2. Materials and Methods

2.1. 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. Ehrenstorfer (Augsburg, Germany). Analytical grade concentrated sulphuric acid (conc. H₂SO₄), isooctane and *n*-hexane were all purchased from Merck (Darmstadt, Germany).

2.2. 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. H₂SO₄ and 3 mL of *n*-hexane were added, mixed in a vortex (ca. 1500 rpm, 30 s) and then centrifuged (ca. 1500 rpm, 10 min). 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 H₂SO₄/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. H₂SO₄ 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 μ L) was added. Then, the sample was quantitatively transferred to gas chromatographic vials using four 25 μ L 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.

2.3. Composition of organochlorine compounds

Selection of OCs 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 found above quantification limit only 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, the significance of the statistical tests or the correlation analyses discussed below.

2.4. Instrumental determinations

A gas chromatograph with electron capture detection (Hewlett Packard 6890N 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 ionization mode using ammonia as reagent gas (1.0 mL/min). In both instruments samples were injected (2 μ L) 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 \pm standard error = 102 \pm 4.2 and 93 \pm 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 Program (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.

2.5. 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, which were evaluated according to the *t* test.

3. Results

3.1. 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 the children were breastfed. Duration of lactation encompassed from very short periods (2 months or less) to large periods (more than 1 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 and 0.42 ng/mL in cord and 4 years sera, respectively (Table 2).

Table 1
Characteristics of the population under study.

	Number of individuals	%
Participants		
At birth (at 4 years) ^a	410 (285) ^a	
Sex		
Male	202 (136) ^a	49 (48) ^a
Female	208 (148)	51 (52)
Feeding practice		
Breast	339 (235) ^a	83 (83) ^a
Formula	71 (49)	17 (17)
Duration of lactation (weeks)		
0.3–10 (0.3–12) ^a	85 (59) ^a	25 (25) ^a
10–20 (12–21.5)	85 (59)	25 (25)
20–28 (21.5–28)	85 (59)	25 (25)
28–100 (28–96)	84 (59)	25 (25)
Parity		
1	112	27
2	225	55
3	64	16
4	9	2.2

^a Subset of the same individuals participating in the study at 4 years old.

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