In utero and post-natal accumulation of organochlorine compounds in children under different environmental conditions†

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A study of intake of organochlorine compounds (OCs) in children both *in utero* and at the age of four years has been performed in two cohorts. One encompasses children born in Menorca Island between 1997 and 1998, and the other those born between 1997–1999 in Ribera d'Ebre, an in-land industrial–agricultural area of Catalonia. Comparison of the OC concentrations in serum samples from both populations, including those obtained from cord blood and blood collected at four years, provides information on the influence of local pollution sources in the accumulation of these compounds. Statistically significant differences ($p < 0.01$) have been found for hexachlorobenzene, indicating that chronic airborne contamination to this compound in Ribera d'Ebre involved higher *in utero* exposure, which increased in the first four years of growth. Similarly, in the cohort of Menorca, higher *in utero* exposure to PCBs was observed and this increased subsequently in the first four years of growth. Other compounds encompassing temporal contamination episodes such as γ-HCH involved higher *in utero* exposure but strongly diminished in the first four years of growth. Overall, it can be concluded that local chronic pollution by OCs has a direct effect in children living in the surrounding areas. This influence is reflected in higher exposure, both *in utero* and in the first years of growth, that significantly stands out over the background contamination due to the ubiquity of these compounds.

1. Introduction

Organochlorine compounds (OCs) are the focus of extensive research by governmental, industrial and academic communities. Their widespread occurrence and accumulation is justified by their specific properties, such as chemical stability, persistence in the environment, semi-volatility, lipophilicity and subsequent biomagnification in the food chain.[1]Despite the restrictions on their production, use and fugitive emission over the recent decades, they are still found in human tissues.

There is concern about their health effects, even at the low levels reported for non-occupational exposure. Animal and *in vitro* experiments have shown that some of them exhibit teratogenicity, immunotoxicity, endocrine disruptive potential or carcinogenicity.[2] Studies combining epidemiology with direct measurements of body burden have provided mixed evidence of their role in the etiology of human cancers.[3–5] Polychlorobiphenyls (PCBs), hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), *p*,*p*′-DDT and other OCs accumulate in children *in utero* and through diet, namely by breastfeeding.[6–10] For all these reasons, up to twelve types of these compounds were banned by the Stockholm Convention.[11]

However, despite these regulations and the restrictions implemented in the early eighties, the incorporation of these compounds into children is still underway. Intake from breast milk continues to be a major determinant of the body burden of these compounds in children, even after several years of discontinuation of this feeding mode.[7,10,12,13] However, some studies have shown that environmental factors may also be relevant for the incorporation of OCs into children.[14,15]

According to these antecedents, the assessment of the health risks of these compounds requires the understanding of the processes leading to their accumulation into humans, particularly in the case of child intake. In this respect, there is a need for clarification of the relevance of local sources in the context of the widespread dispersion of OCs in the environment.[16,17] The implementation of adequate prevention and remediation strategies in the context of the Stockholm Convention[11] depends on having a strong background looking into this question.

In order to get progress on this problem, a study comparing the levels of OCs in two cohorts of children, Ribera d'Ebre and Menorca, is reported. The former is located in the interior of Catalonia, following the shore of the Ebro River. A significant proportion of the members of the cohort live in Flix, a village situated beside a chloro–alkali plant that receives the emissions of this factory, namely HCB. In addition, the area is

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characterized by agricultural activities related with limited water use, such as production of olive oil, wine and citrus fruits. The island of Menorca, a popular tourist destination in the northwestern Mediterranean Sea, does not contain any chemical plant related with the production of OCs. One of the main activities is cattle raising for milk production, which has involved an extensive use of the land for grass growth. The inhabitants also have easy access to fish and seafood, as a portion of them are fishermen. Both cohorts are composed of very stable populations. Most of the parents of the recruited children have been living in the area for more than twenty years.

The screening of human populations for OC exposure requires robust, cheap and efficient methods for analysis, as large numbers of samples must be processed to retain the statistical power required for elucidating the often weak relationship between intake and disease. Serum is the most convenient tissue for body burden assessment. It allows easier sampling and fulfilling of ethical constraints than other tissues. Adipose tissue, which represents the primary reservoir in the body of many persistent pollutants, approaches equilibrium with serum. Measurements in serum thus describe the legacy of uptake and depuration of OCs.[18]

2. Materials and methods

2.1 Cohorts selected for study

The two cohorts selected for study have different exposure scenarios, the Menorca cohort belongs to a rural environment lacking factories that manufactured OCs at present or in the past. The participant children constitute an example of the background exposure to these pollutants in western countries. The Ribera d'Ebre cohort is an area in which a chloro–alkali plant is located. This factory is situated beside the village of Flix and produced DDT and PCBs in the past. At present it is manufacturing organic solvents, which involves releases of HCB and pentachlorobenzene (PeCB) as byproducts.

The Ribera d'Ebre cohort recruited all singleton children of the area, born in the main hospital between March 1997 and December 1999, which included the village of Flix and all other towns from the same administrative health unit. 102 children were enrolled and 73 provided complete outcome data for the four year visit (71.5%), having been analyzed for OCs in cord serum (Table 1).

The Menorca cohort recruited all women presenting for antenatal care over 12 months starting in mid 1997. 482 children (94% of those eligible) were subsequently enrolled and 470 (97.5%) provided complete outcome data up to the four year visit. Among these, 410 (85%) had OCs measured in cord serum (Table 1).

In both cases, written consent for inclusion was obtained. Mean birth length (49 cm) and weight (3.2 kg) were the same in both populations. Mean gestational and maternal ages at delivery were also the same in both cases (40 weeks and 29–30 years, respectively). Mean ages at examination were virtually the same in both populations (4.3–4.4 years). Children from the Ribera d'Ebre cohort were more likely to be an only child.

Table 1 Characteristics of the populations from Ribera d'Ebre and Menorca

	Ribera d'Ebre	Menorca
Sample size at age 4	73	285
Recruitment period	1997–1999	1997–1998
Gender (%)		
Girls	54.9	48.5
Boys	45.1	51.5
Birth length/cm	49	49
Birth weight/g	3248	3186
Gestational age/weeks	40	40
Maternal age/yrs	30	29
Maternal social class (%)		
Professional	20.4	12.7
Skilled	15.3	51.4
Non-skilled	27.6	15.3
Unemployed, husband professional	6.1	2.4
Unemployed, husband skilled	7.1	14.8
Unemployed, husband non-skilled	23.5	3.4
Maternal education (%)		
High	0.0	13.1
Secondary	51.0	28.5
Primary	6.1	51.1
Less primary	42.9	7.3
Paternal education (%)		
High	14.3	8.3
Secondary	41.8	24.8
Primary	35.7	56.5
Less primary	8.2	10.4
Marital status (age 4)		
With a stable partner	97.6	91.0
Divorced or widow	2.4	9.0
Breastfeeding (%)		
<2 weeks	24.7	20.3
2–15.9 weeks	36.6	26.2
16–27.9 weeks	22.6	27.6
≥ 28 weeks	16.1	25.9
Age at examination/yrs	4.4	4.3

Breastfeeding in the Ribera d'Ebre and Menorca involved 75% and 80% of children, respectively.

2.2 Materials

Standards of tetrabromobenzene (TBB), PeCB, HCB, α-, β-, γ-, and δ-HCH, PCBs, *p*,*p*′-DDT and *p*,*p*′-DDE were purchased from Dr Ehrenstorfer, GmbH (Augsburg, Germany). Analytical grade concentrated sulfuric acid (conc.-H_2SO_4), acetonitrile (CH_3CN), iso-octane and *n*-hexane were all purchased from Merck (Darmstadt, Germany).

2.3 Serum extraction and clean up

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 s) and then centrifuged (*ca.* 1500 rpm, 10 min). The supernatant *n*-hexane layer was transferred 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 combined extracts 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 iso-octane; 10 μL) was added. Then, the sample was quantitatively transferred to GC vials using four 25 μL rinses of iso-octane. If emulsions were formed at any stage of the extraction they were broken by the addition of 10–15 drops of MilliQ water that were added before sample centrifugation.

2.4 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 the reagent gas (1.0 mL min^{-1}). 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/Agilent). Helium was the carrier gas (1.5 mL min^{-1}). In both cases, the temperature program was from 90 °C (held for 2 min) to 140 °C at 20 °C min^{-1}, then to 200 °C (held for 13 min) at 4 °C min^{-1} and finally to 310 °C (held for 10 min) at 4 °C min^{-1}. Injector, ion source and transfer line temperatures were 250 °C, 176 °C and 280 °C, respectively.

In both instruments, quantification was performed by external standards using PCB#142 injection standard to correct for volume. Recoveries of TBB and PCB#209 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.[19]

All experiments were performed in compliance with the relevant laws and institutional guidelines of the Institut Municipal d'Investigació Mèdica whose institutional committee approved the experiments. All mothers provided a signed informed consent.

3. Results

3.1 OC concentrations in cord serum

p,*p*′-DDE was the most abundant OC in the cord serum samples of the population of Menorca (1.6 ng mL^{-1}; Table 2) and the second most abundant in the population of Ribera d'Ebre (1.2 ng ml^{-1}; Table 2). These concentrations were lower than those reported in Norway[20] (3.0 ng mL^{-1}). However, the data from Norway was obtained in mid 1980, when body burdens for most OCs were much higher than at the end of the 1990s. The *p*,*p*′-DDE values in Ribera d'Ebre and Menorca were lower than in Canada[8] (0.4 ng mL^{-1}) or Catalonia[15] (0.83 ng mL^{-1}). Lower concentrations of *p*,*p*′-DDT than *p*,*p*′-DDE was observed (0.18 ng mL^{-1} and 0.13 ng mL^{-1} in Menorca and Ribera d'Ebre, respectively), likely reflecting that the mixtures of DDT metabolites correspond to old inputs, since a substantial amount of the *p*,*p*′-DDT initially introduced into the environment had already been transformed into *p*,*p*′-DDE.[21,22]

HCB was the major OC in Ribera d'Ebre (1.4 ng mL^{-1}; Table 2) and the second most abundant in Menorca (0.75 ng mL^{-1}; Table 2). This difference is consistent with the inclusion of Flix in the former, a village that, as mentioned in the introduction, is under the influence of the emissions from a chloro–alkali plant. The concentrations from Ribera d'Ebre were higher than those reported in Norway[20] (1.0 ng mL^{-1}), Catalonia[15] (1.2 ng mL^{-1}), Germany[22] between 1994–95 (0.61 ng mL^{-1}) and Canada[8] (0.04 ng mL^{-1}). Only the concentrations found in Germany[23] between 1984–85 (2.0 ng mL^{-1}) exhibited higher values. The concentrations in Menorca were lower than those in Norway,[20] Catalonia[15] and Germany[23] (1994–95 period). PeCB was found in low concentrations in Menorca (0.081 ng mL^{-1}, Table 2) and Ribera d'Ebre (0.024 ng mL^{-1}, Table 2).

Total concentrations of the ICES 7 PCB congeners were 0.68 ng mL^{-1} in Menorca and 0.53 ng mL^{-1} in the Ribera d'Ebre (Table 2). The PCB distributions in the former were

Table 2 Concentrations of the organochlorine compounds in cord serum from children from Menorca and Ribera d'Ebre

	Menorca (n = 410)		Ribera d'Ebre (n = 73)		
Compounds/ng mL^{-1}	Mean	Standard deviation	Mean	Standard deviation	t-sig.[a]
PeCB	0.081	0.27	0.024	0.069	**
HCB	0.75	0.79	1.4	0.95	**
β-HCH	0.21	0.42	0.67	0.71	**
γ-HCH	0.016	0.25	0.59	3.5	
PCB#28	0.014	0.070	0.015	0.027	
PCB#52	0.021	0.076	0.013	0.011	
PCB#101	0.032	0.097	0.018	0.021	*
p,*p*′-DDE	1.6	2.0	1.2	1.2	*
PCB#118	0.078	0.084	0.029	0.020	**
PCB#153	0.21	0.24	0.13	0.21	**
p,*p*′-DDT	0.18	0.27	0.13	0.32	
PCB#138	0.17	0.13	0.19	0.33	
PCB#180	0.23	0.67	0.14	0.25	
SumPCB[b]	0.68	0.71	0.54	0.64	

[a] *<0.05, **<0.01. [b] Sum of the seven individual PCB congeners analysed individually.

dominated by congener PCB#180 followed by PCB#153, but in the case of Ribera d'Ebre the congener distribution was dominated by congener PCB#138 followed by PCB#180. These concentrations were lower than those found in studies from Norway[20] (3.0 ng mL^{-1}), USA[24] (2.5 ng mL^{-1}), Faroe Islands[25] (1.1 ng mL^{-1}) and Germany[23] (0.96 ng mL^{-1} or 1.4 ng mL^{-1}) but higher than those reported in the Netherlands[6] (0.38 ng mL^{-1}), Canada[8] or Catalonia[15] (0.36 ng mL^{-1}). Comparison of these data must be done with caution because different PCB congeners were used for total PCB calculations in each study. However, the reported figures may vary by a factor of two at the most. In contrast to other studies, PCB congeners of higher volatility were also considered for quantification in this cohort.

α-, β-, γ- and δ-HCH isomers were analyzed. The α- and δ-isomers were only found above the quantification limit in less than 5% of total samples from Menorca and the Ribera d'Ebre. These compounds were therefore not included in the database. β-HCH was the highest HCH among the four isomers, being found at concentrations of 0.21 ng mL^{-1} in Menorca and 0.67 ng mL^{-1} in Ribera d'Ebre. In the last cohort, γ-HCH was detected with a mean concentration of 0.58 ng mL^{-1}.

3.2 OC concentrations in serum from four-year-old children

In general, the concentrations in serum at four years of age (Table 3) exhibit similar values as those found in cord serum (Table 2). Straightforward comparison of the OC concentrations in the two age groups shows specific changes between the two cohorts.

In the population of Ribera d'Ebre the major changes concern the increase in PeCB (from 0.024 ng mL^{-1} to 0.059 ng mL^{-1}), the reduction in p,p'-DDT (from 0.13 ng mL^{-1} to 0.064 ng mL^{-1}) and the strong decrease of γ-HCH (from 0.59 ng mL^{-1} to below limit of detection). In children from Menorca the main changes involve decreases in PeCB (from 0.081 ng mL^{-1} to 0.023 ng mL^{-1}), HCB (from 0.75 ng mL^{-1} to 0.42 ng mL^{-1}) and p,p'-DDT (from 0.18 ng mL^{-1} to 0.08 ng mL^{-1}). On the other hand, there is a significant increase in γ-HCH (from 0.016 ng mL^{-1} to 0.025 ng mL^{-1}) and in PCBs (from 0.68 ng mL^{-1} to 1.0 ng mL^{-1}).

These differences only refer to concentration changes. Estimation of the total blood volume in children[26] as 75 mL kg^{-1} and the weight increases between birth and four years of age, suggest an increase in the body burden of all these compounds in both cohorts. γ-HCH in the population of Ribera d'Ebre constitutes the only exception to this general trend (Table 2 and 3).

4. Discussion

4.1 OC levels and different exposure scenarios

The high levels of HCB in cord serum and serum collected at four years of age (1.4–1.5 ng mL^{-1}; Table 2 and 3) in the cohort of Ribera d'Ebre, reflect the inputs of HCB emitted to the atmosphere as a byproduct[27] by the electrochemical factory in Flix.

The concentrations of p,p'-DDE have a dual origin. Both in Menorca and Ribera d'Ebre they may reflect the past use of p,p'-DDT in agriculture. However, in the second case they could also reflect emissions from residues located in the surroundings of the factory of Flix, since the chloro–alkali plant was an important manufacturer of this insecticide in the past. However, the mean DDT : DDE ratios in newborns are about the same in both cohorts (0.11). In four-year-old children they are higher in Menorca than in Ribera d'Ebre, 0.051 and 0.038, respectively, showing that past production of DDT in the factory of Flix is not reflected in a higher body burden of this compound in the cohort of Ribera d'Ebre.

β-HCH is the most abundant HCH isomer in all samples. This is the case in human blood[7,9,10,15] due to the high stability of this isomer caused by the equatorial orientation of the chlorine atoms. The high concentrations of γ-HCH in cord serum of the population of Ribera d'Ebre constitute a singular feature. It may reflect the exposure of some individuals from this area to high levels of this pesticide that is still in use for some restricted agricultural applications.

Table 3 Concentrations of the organochlorine compounds in serum from four-year-old children from Menorca and Ribera d'Ebre

	Menorca (n = 285)		Ribera d'Ebre (n = 73)		
Compounds/ng mL^{-1}	Mean	Standard deviation	Mean	Standard deviation	t-sig[a]
PeCB	0.023	0.085	0.037	0.059	
HCB	0.42	0.43	1.5	1.5	**
β-HCH	0.29	0.43	0.46	0.45	**
γ-HCH	0.025	0.43	0	0	
PCB#28	0.024	0.33	0	0	
PCB#52	0.042	0.33	0	0	*
PCB#101	0.093	0.14	0	0	*
p,p'-DDE	1.6	3.2	1.7	2.8	
PCB#118	0.11	0.12	0.032	0.053	**
PCB#153	0.35	0.67	0.31	0.36	
p,p'-DDT	0.081	0.12	0.064	0.18	
PCB#138	0.25	0.53	0.061	0.20	**
PCB#180	0.21	0.48	0.24	0.56	
SumPCB[b]	1.0	2.5	0.65	1.0	*

[a] *$<$0.05, **$<$0.01. [b] Sum of the seven individual PCB congeners analyzed individually.

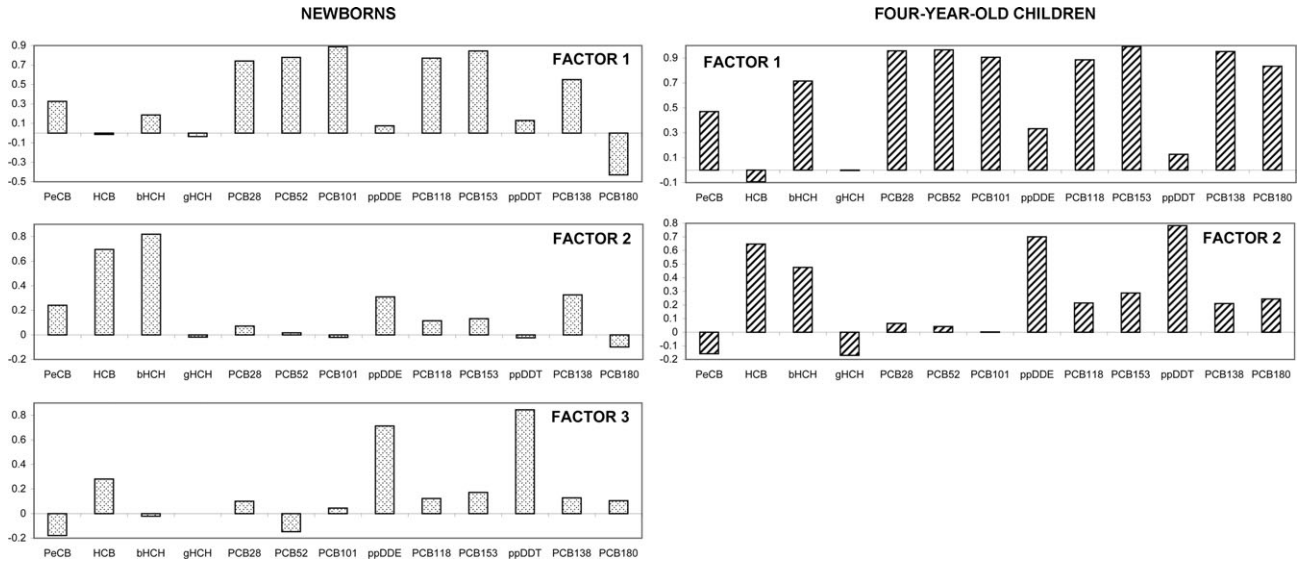

Fig. 1 Factor loadings of the OC concentrations in the pooled populations of Ribera d'Ebre and Menorca at birth and at four years of age.

Cross-comparison of the mean OC concentrations between the two cohorts (Table 2 and 3) indicates which compounds exhibit significant differences between the two populations. The cord serum concentration means are significantly different for PeCB, HCB, β-HCH, PCB#118 and PCB#153 ($p < 0.01$) and p,p'-DDE and PCB#118 ($p < 0.05$). In all these significant cases, except β-HCH and HCB, the concentrations are higher in the cohort of Menorca. The higher concentrations of PCBs in this population may reflect the higher proportion of fish consumption in the diet of the islanders. Furthermore, the higher concentration of p,p'-DDE may reflect important intake due to past agricultural activities.

On the other hand, the higher concentrations of HCB in Ribera d'Ebre reflect the specific influence of the emissions from the chloro–alkali plant that involve *in utero* exposure to this compound. In this respect, it would be expected that PeCB would also be found in higher concentrations in the cohort of Ribera d'Ebre, since it is also a byproduct of the production of chlorinated solvents. However, the concentrations of this compound are found in significantly higher concentrations in the cohort of Menorca.

The mean concentrations in sera of the four-year-old children from both populations shows significant differences for HCB, β-HCH, PCB#118 and PCB#138 ($p < 0.01$) and PCB#52, PCB#101 and total PCBs ($p < 0.01$). The differences exhibit, in general, the same pattern as that observed in cord serum. The higher concentrations of PCBs are found again and define a specific feature of the population from Menorca. Conversely, the higher concentrations of HCB and β-HCH in the cohort of Ribera d'Ebre also record a singular exposure to these OCs in this area.

4.2 Multivariate analysis

Pooled analysis of the OC concentrations in the cohorts from Ribera d'Ebre and Menorca shows several common trends in both newborns and four-year-old children. Compilation of the correlation matrices for these two age groups and subsequent factor analysis shows that the factor determining most of the variance (55% in both cases) is composed by all the PCB congeners (Fig. 1). This main factor has also some weight of PeCB and β-HCH, particularly in the OC database of four-year-old children.

Two additional factors are significant in the OC database of newborns. Firstly, factor 2 (11% of total variance), is highly loaded by HCB and β-HCH and secondly, factor 3 (9% of total variance), is dominated by p,p'-DDE and p,p'-DDT (Fig. 1). The three factors of the newborn database are consistent with the three main distinct sources of OCs that are generally encountered in the environment, *e.g.* industrial residues, pesticides and byproducts from the synthesis of organochlorine components. The correspondence between compound grouping of these three factors and known OC sources indicates that the contributions from these distinct origins can be identified within the pooled database of newborns from Menorca and Ribera d'Ebre.

In the cohort of four-year-old children, a second factor representing 12% of total variance can be identified (Fig. 1). In this case, the factor is equally loaded by HCB, β-HCH, p,p-DDE and p,p'-DDT, showing that in this database the sources leading to the variations of these OCs are highly correlated.

Representation of the sample scores for the factor loadings shown in Fig. 1 exhibit a different behaviour for the two age populations (Fig. 2). The samples from four-year-old children

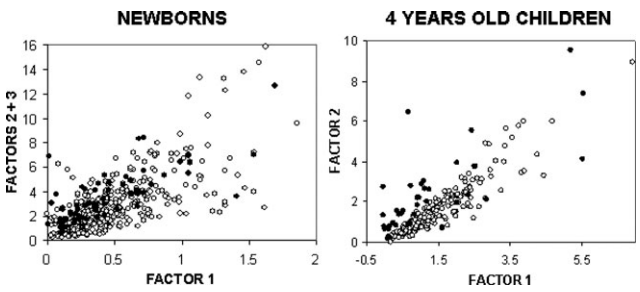

Fig. 2 Sample scores of the factors shown in Fig. 1.

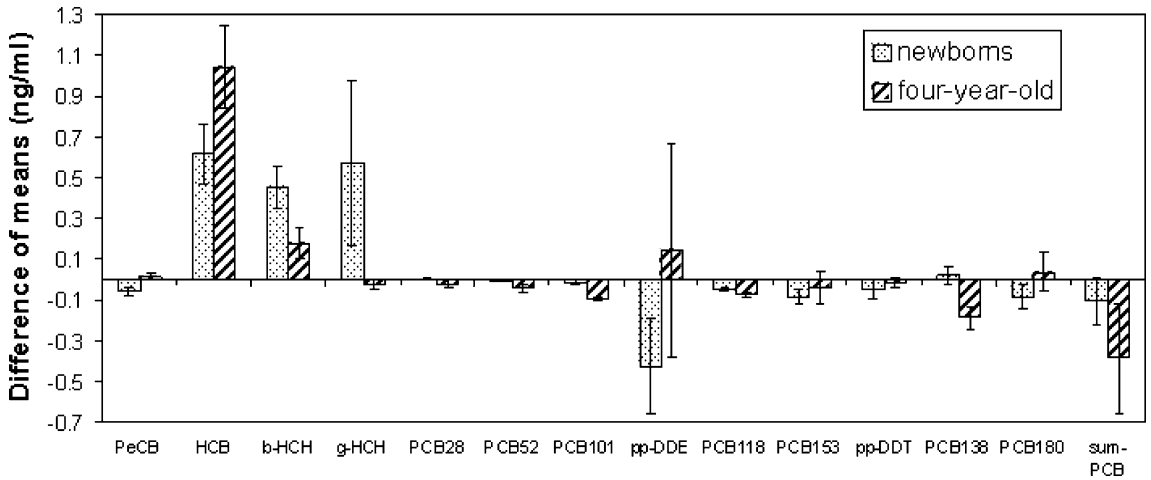

Fig. 3 Differences in mean concentrations between the populations of children studied in Ribera d'Ebre and Menorca. Positive values indicate higher concentrations in the former rather than in the latter. Intervals represent standard error (<0.005).

in Ribera d'Ebre exhibit a higher enrichment in *p*,*p*′-DDE, *p*,*p*′-DDT, HCB and *β*-HCH in relation to the PCB burden than those from Menorca (Fig. 2). In this comparison the scores of factors 2 and 3 for the cohort of newborns have been plotted together in order to have comparable plots for the two ages.

The score plots from Fig. 2 show that the mean ratio between factor 2–3 : factor 1 for newborns is 1.3 times higher in Ribera d'Ebre than Menorca. In contrast, the mean ratio between factor 2 : factor 1 for the four-year-old children in Ribera d'Ebre is 1.5 times higher than in Menorca. The difference is consistent with the above reported higher increase of HCB, *β*-HCH, *p*,*p*′-DDT and *p*,*p*′-DDE in the cohort of Ribera d'Ebre than in Menorca.

4.3 Temporal trends of OC accumulation

Both in the cohorts of Ribera d'Ebre and Menorca the populations of children examined at birth and at four years of age correspond to the same individuals. Thus, comparison of the two age periods provides an estimate of the temporal evolution of the accumulation of these compounds.

Previous studies have shown that among breastfed children lactation is the main determinant of the total body burden of OCs at the age of four years.[7,12,13] In the cohorts of Ribera d'Ebre and Menorca, the proportion of children undergoing breastfeeding was 75% and 80%, respectively. This feeding mode is therefore a main determinant of OC intake in both cases. In addition, specific studies comparing the distributions of OCs in newborns and one and four-year-old children from the populations of Ribera d'Ebre[9] and Menorca,[10] respectively, showed that lactation is the main process leading to accumulation of these compounds in the first period of growth. Therefore, mothers act as carriers of external pollutants to their children.

Subtraction of the two age group means calculated for each cohort enhances the differences in the accumulation of OCs between birth and four years (Fig. 3). In the case of HCB, the differences between the two cohorts increase at four years of age. The increase in children from Ribera d'Ebre is consistent with the occurrence of the specific pollution process introducing this compound in the surroundings of the village of Flix. The differences from the cohort of Menorca are significant ($p < 0.01$; Table 2 and 3).

Conversely, PCBs exhibit an increasing difference between the two cohorts that involve higher concentrations at four years of age than at birth in Menorca. This difference indicates that the chronic exposure to PCBs in the population of Menorca gives rise to enhancement of the concentrations of these compounds in children as they grow. Again the differences between the two cohorts are significant ($p < 0.05$; Table 2 and 3).

p,*p*′-DDE exhibits a different pattern (Fig. 3). Thus, it shows higher concentrations in Menorca among newborns and higher concentrations in Ribera d'Ebre among four-year-old children. However, the differences between the two populations are not significant. This trend probably reflects that this pollutant is incorporated into the diet of children in both populations.

γ-HCH shows a strong difference between the two cohorts among newborns ($p < 0.01$) that disappears when comparing the OC composition in children after four years. This evolution likely responds to a temporal contamination by this compound in part of the population of Ribera d'Ebre but not to chronic exposure.

β-HCH exhibits higher concentrations in the population of Ribera d'Ebre, both among newborns and four-year-old children. The differences between both populations are significant ($p < 0.01$). However, they are smaller among four-year-old children. This pattern may correspond to a situation of chronic pollution by this compound in Ribera d'Ebre. However, previous studies have shown that the incorporation of this compound through breastfeeding is low,[10] which could also explain the different accumulation ratio when comparing to HCB.

Abbreviations

Pentachlorobenzene (PeCB), hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), polychlorobiphenyl (PCB),

dichlorodiphenyltrichloroethane (*p*,*p*′-DDT), dichlorodiphenyldichloroethylene (*p*,*p*′-DDE),

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