Cover Page for Supporting Information

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Maternal origin and other determinants of cord serum organochlorine compound concentrations in infants from general population

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Summary:

SI-1. Further description of the analytical methods

SI-1.1. Materials

Standards of tetrabromobenze (TBB), PCB209 and PCB142 were purchased from Dr Ehrenstofer (Wesel, Alemanya). α-HCH, β-HCH, γ-HCH, δ-HCH, HCB, PeCB, 2,4'-DDT, 4,4'-DDT, 2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, PCB28, PCB52, PCB101, PCB118, PCB153, PCB138 and PCB180 were also purchased from Dr Ehrenstofer (Wesel, Alemanya). Isooctane for trace organic analyses, analytical grade concentrated sulfuric acid and *n*-hexane were purchased from Merck (Darmstadt, Germany).

SI-1.2. Instrumental analysis.

OC concentrations were determined by GC with electron capture detection (GC-ECD) using an Agilent 6890N GC with a Micro-ECD (Agilent Technologies, Palo Alto, CA, USA) equipped with a DB-5 fused silica capillary column (60 m long, 0.25 mm internal diameter and 0.10 μm film thickness; J&W Scientific, Folsom, Ca, USA). The oven temperature was programmed from 80ºC (holding time 2 min) to 150ºC at 15ºC/min and finally to 280ºC at 4ºC/min, keeping the final temperature for 10 min. Injector and detector temperatures were 270ºC and 310ºC, respectively. Injection was performed in splitless mode, keeping the split valve closed for 35 s. Helium was the carrier gas (50 cm/s).

Selected samples were analysed by NICI GC-MS with a GC from Agilent Technologies 6890A (USA) coupled to an MS detector 5973N for confirmation of the qualitative and quantitative results. The samples were injected in split/splitless mode (48 s) at 280°C and data acquisition started after a solvent delay of 4 min. Source temperature was 150°C. Ammonia was used as reagent gas. The chromatographic conditions were the same as described above. Ion source pressure (currently 1.6 Torr) was adjusted to maximise the perfluorotributylamine ions (m/z 312, 452, 633 and 671). Ion repeller was 1.5 V.

OC identification was based on retention time and mass spectral information. Quantification was performed by reference to linear calibration lines and correction by the surrogate and injection standards (26). Procedural blanks were included with each sample

batch. Percent recoveries ranged between 70% and 130% and detection limits were between 0.001 and 0.03 ng/ml depending on the OCs. A value of half detection limit was assigned when measurable analyte concentrations were not found. Reference materials obtained from the Arctic Monitoring and Assessment Program (AMAP) were used to assess precision and accuracy. The laboratory is in compliance with the AMAP Ring Test Proficiency Program for persistent organic pollutants in human serum (Centre de Toxicologie Institut National de Santé Publique du Québec, Québec, Canada).

SI-2. Variables included in the calculations of the multiple linear regression models.

For the normally-distributed contaminants, multivariate linear regression was performed to examine trends between contributions and explanatory variables. First, we analyzed each predictor separately in bivariate analysis taking the organochlorine compounds of interest as outcome

4,4'-DDE

Bivariate analysis. Variables included ($p<0.2$): maternal age, maternal origin, education level, pre-pregnancy body mass index (BMI) and total cord serum lipids. Variables excluded ($p>0.1$): Parity and site of residence. The final model is shown in Table S1. Age, total lipids in cord serum and BMI were significantly ($p<0.05$) directly correlated with prenatal 4,4'-DDE concentrations: 0.044, 0.002 and 0.021 per increased unit, respectively. Age was the maternal determinant involving the highest effect. Maternal education levels were also directly associated to 4,4'-DDE concentrations in newborns but without statistical significance (Table S1).

Interactions assessed between maternal determinants: maternal age*pre-pregnancy BMI, maternal age*education level and maternal origin*pre-pregnancy BMI. Maternal age and pre-pregnancy BMI was found significant ($p=0.027$). Accordingly, the influence of age and BMI on 4,4'-DDE concentrations was evaluated through stratified analysis. Age effect was

higher among mothers older (n=280) than younger (n=219) than 30 years. In the first group, 4,4'-DDE prenatal levels increased 0.033 units (p=0.006) per BMI unit and 0.056 units (p<0.001) per year. In the second group (women younger than 30 years old) the association was also positive but the effect on 4,4'-DDE concentrations was small and did not reach significance.

PCBs

Bivariate analysis. Variables included (p<0.2): maternal age, maternal origin and total cord serum lipids. Variables excluded (p>0.1): Education level, pre-pregnancy BMI and weeks of lactation. The final model is shown in Table S2. Age and total lipids in cord serum were significantly (p<0.05) directly correlated with PCB concentrations in newborns, 0.029 and 0.001 per increased unit, respectively.

4,4'-DDE and PCBs

The contribution of each variable was evaluated with the partial F test and r^2 changes. Analyses of the model assumptions were performed among the independent variables, e.g. linearity, normality, homoscedasticity, independence of the residuals, absence of outliers, and multicollinearity. Statistical significance was fixed at p<0.05 (two sided).

Table S1. Associations between 4,4´-DDE levels in cord serum and related maternal determinants.

	β	p	95%CI
Maternal origin			
Spain (R)	--	--	--
South America	0.728	<0.001	0.460-0.996
Other European Countries	-0.318	0.185	-0.788-0.152
Pre-pregnancy BMI[a]	0.021	0.009	0.005-0.037
Total Lipds (mg/dL)	0.002	0.008	0.000-0.003
Maternal age	0.044	<0.001	0.028-0.061
Educational Level			
Up to primary school (R)	--	--	--
Primary school	0.284	0.227	-0.178-0.747
Media studies	0.395	0.092	-0.064-0.854
University	0.358	0.136	-0.113-0.829

[a] Body mass index

Table S2. Associations between ΣPCBs[a] levels in cord serum and related maternal determinants.

	β	p	95%CI
Maternal origin			
Spain (R)	--	--	--
South America	-.0510	<0.001	-0.664-(-0.356)
Other European Countries	0.011	0.937	-0.282-0.260
Total Lipds (mg/dL)	0.001	0.001	0.000-0.002
Maternal age	0.029	<0.001	0.020-0.039

[a]ΣPCBs= Sum of PCB congeners 28, 52, 101, 118, 138, 153, 180.

Table S3. Diet groups of mothers from different origins

First trimester of pregnancy	Dairy products	Meat	Fish	Green vegetables
Spain (n=438)	458.4(242)	134.1(51.8)	68.7(37.9)	203.4(110.6)
South America (n=42)	499.3(271)	126.7(69.2)	50.2(30.3)	229.9(110.0)
Other European Countries (n=13)	616.9(292)	140.6(46.4)	83.3(48.6)	279.2(174.5)
Kruskal Wallis test	0.10	0.41	**0.004**	**0.043**

Third trimester of pregnancy	Dairy products	Meat	Fish	Green vegetables
Spain (n=438)	504.3(251.9)	121.8(48.0)	64.8(38.6)	207.5(126.9)
South America (n=42)	533.4(290.7)	121.2(52.2)	58.0(36.9)	263.6(137.9)
Other European Countries (n=13)	696.5(298.1)	132.6(56.6)	98.8(70.6)	226.7 (85.0)
Kruskal Wallis test	**0.021**	0.898	**0.050**	**0.016**

Fig S1. Descriptive information (not for statistical evaluation) on 4,4'-DDE (a) and ΣPCBs (b) concentrations in cord serum by maternal country of origin