Exposure to hexachlorobenzene during pregnancy increases the risk of overweight in children aged 6 years

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INTRODUCTION

Organochlorine compounds (OCs) are persistent environmental contaminants that were intensively used in agriculture and industrial processes in the past (1). OCs are lipophilic chemicals which are resistant to degradation and bioaccumulate in the food chain (2,3). Therefore, they are difficult to eliminate from the environment. The toxicity of OCs was first established after two well-documented episodes of accidental exposure to polychlorinated biphenyls (PCBs) by contaminated cooking oil in Japan (4) and Taiwan (5).

Hexachlorobenzene (HCB) has extensively been used as a pesticide to protect the seeds of onions, wheat and other grains against fungus. It was also used to make fireworks, ammunition and synthetic rubber (6). Although restrictions were put on HCB use in the 1970s, it is still synthesized as a side product while producing other chlorinated solvents (7). It was used in closed systems, but it is still widespread in the environment. The main intake of HCB in humans is through diet and in unusual circumstances by inhalation (8). Newborns are exposed because of the HCB transport across the placenta and by breastfeeding due to their lipophilicity of HCB (9–11). Chronic exposure could cause porphyria, hepatotoxicity, immune suppression, neurotoxicity, cardiotoxicity, alterations in calcium metabolism and reproductive effects (6,12). As HCB is strictly banned from the United States and the concentrations are lower than in European countries (13), there are few child cohorts which evaluate the effects of prenatal and postnatal exposure to HCB in young children.

Several studies have shown an association between other OCs and body size. Patandin et al. and Karmaus et al. showed an association between maternal PCB concentration (respectively in plasma and serum) and birth weight and growth in children (14,15), but there are no conclusive answers regarding health effects caused by PCBs (16). Dichlorodiphenyl (p,p'-DDE) was associated with preterm birth, decreased birth weight (3) and a reduced height, specifically in females in early childhood (2,17). Information on HCB and body size is scarce. No animal studies found reduction of body weight as an effect of acute exposure to HCB (18). Intermediate and chronic duration experiments found reduction of body weight only in the presence of other adverse effects such as mortality, incidences of tumours or organ toxicity (19).

Due to the few existing data regarding exposure to HCB during the first stages of life and child growth, we aimed to assess the association between prenatal HCB exposure and its potential adverse effects on child’s weight and body mass.
index (BMI) in a general population with no local pollution sources.

**POPULATION AND METHODS**

**Study population**

This cohort was set up within the Asthma Multicenter Infants Cohort study in Menorca (a Balearic Island in the northwestern Mediterranean Sea, Spain) in 1997 and recruited all women presenting for antenatal care over 12 months starting in mid 1997. Subsequently 482 children (94% of those eligible) were enrolled. Umbilical cord could be obtained in 405 children (86%) and OCs were subsequently analysed. This study was approved by the ethics committee of the Institut Municipal d’ Investigació Mèdica and all mothers provided a signed informed consent.

**Study variables**

Concentrations of HCB, total PCBs (presented as the summation of the congeners: 25, 52 101, 118, 138, 153 and 180), \( p,p'-\text{DDE} \) and \( p,p'-\text{DDT} \) (2,2-bis(\( p \)-chlorophenyl)-1,1,1-trichloroethane) in serum were analysed by gas chromatography (GC) coupled to electron capture detection (Hewlett Packard 6890 N GC-ECD) as described elsewhere (20,21). Quantification was performed using external standards, with the PCB142 injection standard used to correct for volume. Tetrabromobenzene and PCB209 were added as recovery standards. Limits of detection were 0.2 ng/mL. A value of 0.01 ng/mL was given for the non-quantifiable concentrations. Serum samples were stored at \(-40^\circ\)C until analysis. All the analyses were carried out in the Department of Environmental Chemistry (IIQAB-CSIC) in Barcelona, Spain.

Children’s weight and height were specifically measured for the study by specially trained study personnel at age 6.5 years. Preterm births \( (n=23) \) were excluded, as catch-up growth patterns vary compared with term births (22). Child overweight/at-risk of overweight (hereafter ‘overweight’) growth patterns vary compared with term births (22). Child overweight and obesity prevalence were obtained by trained nurses at birth. Infant-feeding practices reported by mothers in interviews at 6 months and 1 year postpartum.

**Statistical analysis**

Initially, descriptive statistics were used to summarize the parameters. Because the OC distributions in cord blood and serum were skewed to the right, the natural logarithmic transformation was used in the analysis when necessary. HCB concentrations were divided into four categories according to the quartiles (cord blood: \(<0.46, 0.46-0.68, 0.68-1.03 \) and \( \geq 1.03 \) ng/mL).

The models were fitted to assess the association between levels of HCB in cord blood and weight and BMI at age 6.5 years. Coefficients and standard errors from these models reflect the mean difference in each outcome associated with the prenatal HCB exposure variable. Multivariate models were used to adjust for the statistically significant covariates reported from the literature. Models were as follows: (i) child age and sex, (ii) child age, sex, maternal age, height, pre-pregnancy overweight or obesity, education and parity, (iii) additionally adjusting for child’s birth weight. In supplementary models, we examined effects of adjusting for maternal diet and cigarette smoking during the first trimester of pregnancy. As these adjustments had no meaningful effect, they were not included in the main models.

Multivariate logistic regression was used to examine associations between prenatal exposure to HCB and child overweight and obesity at 6.5 years, adjusting in the same series of models as described above. Odds ratios (ORs) with 95% confidence intervals (CIs) are reported for each measure of HCB exposure (continuous and categorical).

All the analyses were repeated including normoweight mothers only (with a BMI between 18 and 25) adjusting by maternal BMI and the rest of covariates. We further adjusted the models for PCBs, \( p,p'-\text{DDE} \) and \( p,p'-\text{DDT} \). Interactions between the categorized HCB concentration, as well as the logarithmic transformed HCB concentration, and gender and maternal BMI were checked.

All statistical analyses were conducted with the SPSS version 12 and STATA 8.0 packages.

**RESULTS**

OCs were detected and quantified in all cord serum samples from 405 children at birth. \( p,p'-\text{DDE} \) had the highest median levels in cord blood and \( p,p'-\text{DDT} \) the lowest levels. The median and interquartile range for HCB was 0.68 ng/mL (0.46–1.03). Medians for DDT, DDE and PCBs were 0.08, 1.03 and 0.70 ng/mL, respectively.

There were no differences in the outcome variables and the studied covariates between children with or without organochlorine measurements (mean BMI at age 6.5 years with and without OCs measurements: 16.6 and 16.8 \( (p = 0.43) \), respectively, and prevalence of overweight/obese: 15.9/19.4 and 12.0/8.1 \( (p = 0.58) \), respectively).

Children in the group of the highest HCB levels \( (>1.03 \) ng/mL) in cord blood had a higher average of other OC levels \( (2.60 \) for \( p,p'-\text{DDE} \), 0.28 for \( p,p'-\text{DDT} \) and 0.98 ng/mL for PCBs) and their mothers were older and had a higher BMI. Children in the highest HCB had a higher weight at age 6.5 \( (\beta = 1.92 \text{ kg} (0.64)) \) and a higher BMI \( (\beta = 0.95 \text{ kg/m}^2 (0.31)) \). Child overweight and obesity prevalence was higher among children with higher HCB concentrations at birth \( (\chi^2 p < 0.05) \), with the highest prevalence (20% overweight and 17% obese) among children in the highest
categories of HCB exposure (Table S1 in Supplementary material online). Maternal age, maternal obesity and number of siblings were associated with child overweight.

The adjusted association between HCB in cord blood and BMI at age 6.5 is shown in Table 1. A statistically significant increase in BMI was found to be related to prenatal exposure to HCB. This increase was significant after adjusting for confounders. Children in the highest group of HCB concentration had an increase of 0.80 (0.34) points in the BMI compared to those with lower exposures. Similar results were obtained in linear models using BMI z-scores at 6.5 years as a continuous outcome (multivariate-adjusted $\beta \pm\ SE$ 0.18 ± 0.09, $p < 0.05$). Weight at 6.5 years was also associated with HCB at birth ($\beta = 1.14$ kg (0.38) per increase of log ng/mL HCB at birth). Height at 6.5 years was not associated with HCB. Birth weight did not modify the association.

Exposure to HCB was also associated with an increased risk of being overweight and obese at 6.5 years. The increased risk of being overweight was of 1.7 and of being obese was 2 (Table 2). Children in the higher exposure of HCB had an increased risk of 2.5 and 3 of being overweight and obese, respectively, but this association was only statistically significant for overweight. Further adjustments for birth weight did not modify the results.

The results with children from normoweight women only showed a reduced but statistically significant effect of HCB on BMI and weight at age 6 ($\beta = 0.39$ kg/m² (0.19) and 0.84 kg (0.38), respectively, per increase of log ng/mL HCB at birth).

There were no interactions between exposure to HCB and gender or maternal overweight. Further adjustment for other OCs did not modify the observed associations.

### DISCUSSION

Prenatal exposure to HCB is associated with an increase in BMI and weight at age 6.5. This association is independent of socio-economic status, maternal education, parity, maternal obesity and birth weight. The incidence of overweight and obesity at age 6.5 years is also related to the HCB exposure. All children in this population had detectable and quantifiable concentrations of HCB at birth, and the observed effect on BMI was for both continuous and categorical concentrations of HCB. Children in the higher exposure group of HCB ($>1.03$ ng/mL) had an increased risk of 2.5 and 3 of being overweight and obese. Maternal smoking during pregnancy was also associated with the risk of overweight at 6.5 years and stratification by maternal smoking showed that the HCB effect on BMI was stronger for children whose mothers had smoked while pregnant.

Few studies have investigated the association between prenatal HCB exposure and anthropometric measures at birth. A negative association between levels in cord blood and crown-heel length has been shown in the study of Ribas-Fito et al. (24) in a population with higher concentrations of HCB. No animal studies are performed regarding growth effects and prenatal exposure to HCB. There are intermediate and chronic duration experiments, which studied the main effects of HCB like porphyria and reproductive effects (25–27). These studies showed only a change in body weight in the presence of other adverse effects. Furthermore, the health effects, which are mentioned in these studies, are mainly caused by oral exposure to HCB postnatal and not by prenatal exposure through maternal blood.

Some studies found an association between exposure to other OCs, like PCB, DDE and DDT, and the effect on growth, but inconsistency about the results remains. Gladen et al. found that transplacental DDE exposure could increase the height and weight of boys at puberty (28) although the hypothesis was not confirmed in another study (29). Serum DDE concentrations at age 8 years were examined in relation to height among 175 boys and 125 girls, from birth to the age of 10 years (2). Higher exposures of DDE were associated with shorter height in girls, not in boys.

Besides the few existing data and the need to confirm the encountered results, it is difficult to hypothesize the

### Table 1: Associations between prenatal exposure to HCB and BMI at age 6.5 years

<table>
<thead>
<tr>
<th>Model</th>
<th>Adjusted for age (and sex)</th>
<th>Model 2 Multivariate*</th>
<th>Model 3 Multivariate + birth weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCB cord blood continuous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference*</td>
<td>15.34</td>
<td>14.74</td>
<td>14.48</td>
</tr>
<tr>
<td>HCB cord blood (log ng/mL)</td>
<td>0.55 (0.18)</td>
<td>0.49 (0.19)</td>
<td>0.51 (0.19)</td>
</tr>
<tr>
<td>HCB cord blood categorical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference*</td>
<td>15.35</td>
<td>14.78</td>
<td>14.53</td>
</tr>
<tr>
<td>0.46–0.67</td>
<td>0.28 (0.30)</td>
<td>0.09 (0.32)</td>
<td>0.10 (0.31)</td>
</tr>
<tr>
<td>0.68–1.03</td>
<td>0.41 (0.30)</td>
<td>0.47 (0.32)</td>
<td>0.50 (0.34)</td>
</tr>
<tr>
<td>&gt; 1.03</td>
<td>0.95 (0.31)</td>
<td>0.80 (0.34)</td>
<td>0.83 (0.33)</td>
</tr>
</tbody>
</table>

*Referent for all models is low exposure to HCB.

Multivariate models adjusted for maternal age, height, pre-pregnancy overweight or obesity, education, parity and child’s sex and current age.

### Table 2: Associations between prenatal exposure to HCB and overweight and obesity at age 6.5 years

<table>
<thead>
<tr>
<th>Model</th>
<th>Adjusted for age (and sex)</th>
<th>Model 2 Multivariate*</th>
<th>Model 3 Multivariate + birth weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overweight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference*</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HCB cord blood (log ng/mL)</td>
<td>1.77 (1.18–2.68)</td>
<td>1.67 (1.03–2.69)</td>
<td>1.69 (1.05–2.72)</td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference*</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HCB cord blood (log ng/mL)</td>
<td>2.12 (1.21–3.72)</td>
<td>2.06 (0.69–3.98)</td>
<td>2.02 (1.06–3.85)</td>
</tr>
</tbody>
</table>

*Referent for all models is low exposure to HCB.

Multivariate models adjusted for maternal age, height, pre-pregnancy overweight or obesity, education, parity and child’s sex and current age.
mechanisms through which in utero exposure to HCB may promote offspring overweight. A recent revision of the scientific evidence for prenatal programming of childhood overweight and obesity has concluded that the prenatal period constitutes an important window of vulnerability for adult obesity (30). The authors examined prenatal exposures to maternal diabetes, malnutrition and cigarette smoking and concluded that, although unknown, the mechanisms related to overweight programming could be associated with insulin, leptin and glucocorticoid resistance in utero. HCB has been reported to disrupt the gluconeogenetic pathways in animal models (31) and has been linked to diabetes in cross-sectional studies. Glynn et al. found that more than 20% of the variation of HCB concentrations in serum could be explained by diabetes, after adjustment for the other determining factors (32); Langer et al. reported higher proportions of impaired fasting glucose among subjects from high pollution areas with high serum concentrations of PCB, DDE and HCB (33) and Codru et al. (34) found that serum concentrations of HCB were positively associated with an elevated incidence of diabetes in an adult Native-American population. Although maternal diabetes was not taken into account in our study and no measures of fasting glucose were obtained, a potential mechanism of HCB as a cause of child obesity through maternal diabetes should not be discarded.

After correcting for the most plausible confounders the association between the HCB concentration in cord blood and BMI at age 6.5 still existed. In contrast to a cross-sectional study, this study indicates that prenatal exposure to HCB can influence the BMI after a few years and is not caused by postnatal exposure or by reverse causality. This study had some limitations such as the small cohort size. The data collection of this study was performed in 1997 and some now-known relevant variables were not included, like paternal height, weight and BMI, and improved diet variables. Information on cholesterol and triglycerides was not available at birth being impossible to adjust OC by lipids. This study is performed in a homogeneous cohort. The island does not have factories producing OCs and can be taken to be representative of the regular exposure in western countries to these pollutants.

The prevalence of obesity has increased at an alarming level of at least 500 million people worldwide (35,36). Additionally, other diseases like diabetes will increase in prevalence as well. Protection for this possible diabetes epidemic is needed. The risk on increased BMI at young age, caused by prenatal exposure to OCs like HCB, has to be minimized. Therefore, it is important that pregnant women are informed about the possible effects on prenatal exposure to HCB on the BMI of the child later in life.

In conclusion, high exposure of HCB prenatal (level in cord blood > 1.03 ng/mL) is associated with an increase of BMI and overweight in girls and boys at age 6.5 years. Additional studies are needed to assess directly whether HCB at current concentrations of exposure increases health risks on children such as obesity.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

There are no competing interests with authors and funding.

References


**Supporting Information**
The following supplementary material is available for this article:

**Table S1** Characteristics of the population for the extremes (<25th percentile and >75th percentile) of the HCB concentration (ng/mL) in cord serum

Additional Supporting Information may be found in the online version of this article.

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