Hexachlorobenzene is associated with low birth weight among Norwegian Mothers

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Introduction

Hexachlorobenzene (HCB) has been associated with spontaneous abortion,[1] skin diseases,[2] and developmental abnormalities such as low birth weight and low birth length,[3,4] although conflicting results have been reported.[5] Studies have mainly been performed in populations thought to be among the most highly exposed to HCB. In the present study we explore whether an association could be found between HCB levels and birth weight, in a general population moderately exposed to HCB.

Materials and Methods

HUMIS is a prospective birth cohort at the Norwegian Institute of Public Health. Mother and child pairs are consecutively recruited after birth and maternal milk samples collected when the child is between 2 weeks and 2 months old. All details on how and when the milk was sampled are registered by a sampling scheme. The containers have been thoroughly washed and the absence of toxicants in its empty and washed state checked. When the container is filled it is posted by the mothers, and stored at –20 degrees in a Biobank of the Norwegian Institute of Public Health upon arrival. Information on health outcomes is obtained by questionnaires sent to the families when the child is 1, 6, 12 and 24 months and 7 years of age. The recruitment started in 2003 and provided sufficient funding we plan to include a total of 6000 mother/child pairs enabling future nested case-control studies on different health outcomes. By 2005, 1200 mother/child pairs have been recruited.

In the first phase of the study the level of polychlorinated biphenyls (PCBs), chlorinated pesticides and brominated flame retardants (BFRs) will be determined in 400 of the human milk samples and related to common outcomes. We are here reporting preliminary results from the 222 samples analyzed so far for chlorinated pesticides and PCBs. Among these, dioxins and furans have been determined in 25 samples.

Concentrations of HCB, β-betahexachlorocyclohexane (β-HCH) , p,p'-DDE, the sum of 10 indicator PCB congeners, IUPAC # : 28, 52, 74, 99, 101, 138, 153, 170, 180, 194 and 8 mono-ortho PCB congners IUPAC # : 105, 114, 118, 123, 156, 157, 167, 189 were measured at the Norwegian School of Veterinary Science. The extraction and lipid clean-up were done according to methods described earlier.[6] Prior to extraction the internal standards PCBs 29 and 112 were added. The lipid concentration of the milk was determined gravimetrically. Details for determination of organochlorine pesticides and PCBs on a GC-ECD were described earlier.[7] PCDDs and PCDFs were analyzed by slightly modified from the method described earlier.[8]

Statistical analysis was performed using SPSS 12 software. In bivariate analysis, means are given and non-parametric tests (Kruskal Wallis) were used to test for significance due to a skewed distribution. Linear regression analysis was used to study the association between birth weight and HCB, adjusted for potential confounders. The adequacy of the final linear regression model was tested by checking whether the assumptions of the model: linear effects and constant variance (homoscedastic) were met by plotting residuals versus predicted values. Inclusion of gestational length as a square term in the model, improved the model and was therefore used in the regression analysis. Furthermore, we checked the model for co linearity and for points with high influence. The association between HCB and other POPs were checked by means of Spearman correlations.

Results and Discussion

HCB values

The concentration of HCB has been determined in 222 milk samples, their mean and median being 12.2 and 11.5 µg/kg fat, respectively, and with a minimum and maximum value of 3.6 and 35.2 µg/kg fat , respectively.

Potential confounders

In order to map potential confounders, we first studied factors that were associated with HCB levels in the milk samples. In unadjusted analysis, using nonparametric tests the level of HCB in human milk was significantly influenced by maternal age (p=0.010), first born child (p=0.035) and maternal bodyweight at the start of the pregnancy (p=0.036). There was a non-significant tendency for higher milk fat levels among daily smokers, while no apparent differences in HCB levels were shown between different counties in Norway, child gender and maternal height.

Association between HCB and other POPs

We also studied the correlation between HCB and β-HCH, p, p'-DDE and specific PCBs. Significant correlations were observed between HCB and the other POPs with only few exceptions. There were significant and moderate correlations between HCB and β-HCH (r=0.648 p<0.000) and p, p'-DDE (r=0.550 p<0.000). Correlations between HCB and specific PCBs were mostly between r>0.5 and r< 0.7, with very few congeners showing no correlations or higher correlations.

TEQ values

The total TEQ was calculated for PCDDs/PCDFs and dioxin-like PCBs according to acknowledged standards in 25 samples. It has recently been proposed that HCB should be given a TEF factor of 0.0001.[9] Using this TEF value, HCB contributed approximately 10% to the total TEQ in the milk samples. In a linear regression model total TEQ was not significantly associated with birth weight. However, since total TEQ was only analyzed in 25 samples, we may not have enough strength to detect an effect of total TEQ on birth weight (type II error), and further statistical analysis will be performed when 60 milk samples have been analyzed.

HCB and birth weight

A non-significant association was seen between HCB and birth weight in the crude analysis (Regression coefficient (b) = -7.2 95 CI -29 to 15). However, a significant association was seen between HCB and birth weight adjusted for gestational length (linear regression model: B-14.9 CI -30 to -0.005, p=.05).

In a multivariate model adjusting also for maternal age, parity, child gender, maternal weight at start of pregnancy and maternal smoking, the effect of HCB on birth weight was further strengthened (Table 1). Stratified analysis on gender did not reveal any interaction between HCB and gender on birth weight. Including specific PCBs, ppDDE or sum of PCBs only strengthened the association between HCB and birth weight, adjusted for gestational length.

As can be seen from the Table, the final model estimates a 20 gram decrease in birth weight per µg/kg increase in HCB concentration. For a 10 unit increase in HCB concentration the corresponding estimate is an expected 200g decrease in birth weight. However, as reflected by the wide confidence intervals, there is large uncertainty to this estimate, and the results needs to be confirmed and further explored in larger population for a more precise estimate.

Birthweight is considered an indicator of prenatal conditions and a predictor of subsequent health. Factors that are associated with reduced birth weight are, independently of the mechanism by which they work, generally considered to be risk factors for subsequent reduced health quality. The result of the present study indicates that even moderate gestational HCB exposure may have a biological effect on the fetus, manifesting itself as a substantial reduction in birth weight. Although the biological mechanisms are not clear, the reduction in birth weight may be associated with adverse effects on child health and development. Further studies on the effect of HCB on child health seem clearly warranted. However, since environmental toxicants coexist in exposure sources, and adjustment for other toxicants can only partly be done, we can not excluded the possibility of confounding by other as yet unmeasured POPs that are strongly correlated to HCB exposure.

Table 1. Birth weight according to HCB levels in human milk (µg/kg milkfat).

The unadjusted analysis shows number (N), mean birth weight (g) and p-

value
for differences between categories.
The adjusted analysis shows estimated decrease (B) in birth weight pr µg/kg
increase in HCB, with confidence intervals (CI), based on linear regression.

	Bivariate (unadjusted)			Adjusted coefficients		
		Birth weight (g)				
	N	Mean	p	B	(95% CI)	p
All	222	3578				
HCB concentration in tertiles			*0.28*			
3.6 to 10.1 µg/kg milkfat	74	3665				
10.2 to 12.7 µg/kg milkfat	75	3461				
12.8 to 35 µg/kg milkfat	73	3610				
Per µg/kg increase in HCB in milkfat				-20	(-35 to -4.6)	*0.01*

Adjusted for gestational length, maternal age, maternal parity, maternal weight at start of pregnancy, maternal height, smoking (yes/no), child sex. 10 children not included in ajusted analysis due to missing values.

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