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Association of Hexachlorobenzene and Other Organochlorine Compounds with Anthropometric Measures at Birth

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ABSTRACT

The aim of the present study was to assess the association of prenatal exposure to hexachlorobenzene (HCB) and other organochlorine compounds with anthropometric measures at birth. A total of 98 mother-infant pairs (83% of all children born in a specific area polluted with HCB in the period 1997–1999) were recruited after giving written consent. Levels of organochlorine compounds were measured in 72 maternal serum samples at delivery and in 70 cord serum samples. Of the organochlorines measured in cord serum, median levels of HCB were higher than for the other compounds (median of HCB = 1.13 ng/mL, median of dichlorodiphenyl dichloroethylene = 0.85 ng/mL, and median of total polychlorinated biphenyls = 0.27 ng/mL). Premature newborns had higher concentrations of HCB [1.94 ng/mL among prematures *versus* 1.10 among nonprematures ($p < 0.10$)], dichlorodiphenyl dichloroethylene [2.40 *versus* 0.80 ($p < 0.05$)], and polychlorinated biphenyls in cord serum [0.70 *versus* 0.14 ($p < 0.10$)]. Those infants born with a small length for gestational age had higher levels of HCB in cord serum than those with an adequate length for gestational age [1.64 ng/mL *versus* 1.00 ng/mL ($p < 0.05$)]. In addition, HCB cord serum levels were negatively associated in a dose-response way with crown-heel length [for each doubling of the dose there was a decrease of 0.46 (SE = 0.22) cm] after adjusting for smoking, gestational age, and other organochlorine compounds. The associations of dichlorodiphenyl dichloroethylene and polychlorinated biphenyls with length were not significant. The results did not vary when stratified for prematurity. These data suggest that HCB reduces intrauterine physical linear growth. **(*Pediatr Res* 52: 163–167, 2002)**

Abbreviations

HCB, hexachlorobenzene
p,p'-DDE, dichlorodiphenyl dichloroethylene
β-HCH, β-hexachlorocyclohexane
PCB, polychlorinated biphenyls

Whether exposure to persistent organochlorine pollutants has an adverse effect on children's health is a matter of worldwide concern (1). Production and intensive use of organochlorine chemicals like HCB in agriculture and industrial processes in the past have led to a widespread contamination of the environment. At present, HCB is still formed as a by-product during the manufacture of some chlorinated solvents. The main intake of organochlorine compounds in humans is through diet and, in unusual circumstances, by inhalation (2). Newborns are exposed across the placenta and by breast-feeding (3, 4).

Exposure to some organochlorine compounds, such as PCB, has been associated with fetal growth alterations such as a decrease of birth weight and birth length (5). Specific effects of HCB on birth weight have been also found in animals (6), but little information is available about the effects on humans.

In a rural village of 5000 inhabitants located in the vicinity of an electrochemical factory (Flix, Catalonia, Spain), unusually high atmospheric levels of HCB were detected. Adult inhabitants studied in 1994 had the highest serum HCB levels ever found (7), and levels of HCB in the cord serum of newborns from this population are among the highest ever reported (8). A general population birth cohort was set up in this area to assess the effects of prenatal and postnatal HCB exposure on growth and neurologic development of infants.

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The aim of the present study was to assess the association of prenatal exposure to hexachlorobenzene and other organochlorine compounds with anthropometric measurements.

SUBJECTS AND METHODS

Subjects. All children born in the main hospital of the study area during the period March 1997 to December 1999 were selected. The study area included the village of Flix and all other towns of the same administrative health area (12,000 inhabitants). A total of 110 children were born in this hospital (93% of all children born in the study area). The children had no congenital anomalies or diseases. Two non-Caucasian infants and two twins were excluded. The mothers of 98 infants (93% of the eligible cases, 83% of all children born in the area) gave written consent. This study was approved by the ethical committee of the Institut Municipal d'Investigació Mèdica.

Exposure measures. Organochlorine compounds were measured in 72 maternal serum samples at delivery and in 70 cord serum samples. From the remaining mothers and newborns, no information was available due to the small volume of the obtained samples. Serum samples were stored at −40°C until analysis. Organochlorine compounds in sera were analyzed by gas chromatography (GC) with electron capture detection and GC coupled to chemical ionization negative-ion mass spectrometry. All the analyses were carried out in the Department of Environmental Chemistry (CID-CSIC). Details of the methodology have been reported elsewhere (9). We present results for the most prevalent compounds found in sera samples, HCB, p,p'-DDE, and PCB, which we present as the summation of the individual congeners 28, 52, 101, 118, 138, 153, and 180, and HCH. Because the sum of PCB 118, 138, 153, and 180 represented 91% and 98% of total PCBs in cord serum and in maternal serum, respectively, we also provide results for these four congeners. Detection limits for HCB, β-HCH, and p,p'-DDE were 0.03, 0.15, and 0.09, respectively, and for the individual PCB congeners 28, 52, 101, 118, 138, 153, and 180 were 0.17, 0.15, 0.09, 0.11, 0.15, 0.12, and 0.10 ng/mL, respectively. A value of 0.01 ng/mL was given for the nondetectable levels and a value of 0.05 ng/mL for those detectable but not quantifiable.

The between-assay coefficient of variation for the assays was 6.4% for HCB, 11.5% for β-HCH, 8.6% for p,p'-DDE, and 9.5%, 7.1%, 6%, 11%, 8.4%, 7.1%, and 8.9% for the individual PCB congeners 28, 52, 101, 118, 138, 153, and 180, respectively.

Outcome measures. Anthropometric measures at birth were obtained from the hospital delivery record. All measures were taken in the delivery room with the same device. Gestational age was based on the mother's report of her last menstrual period.

The outcome variables were birth weight (in grams), crown-heel length (in centimeters), head circumference (in centimeters), prematurity (≤37 wk), small weight for gestational age (defined as birth weight below the 10th percentile for gestational age) and small length for gestational age (defined as birth length below the 10th percentile for gestational age) (10).

Other variables. Information on socioeconomic background, maternal history, parity, gender, and fetal exposure to alcohol and cigarette smoking was obtained with a questionnaire that was administered in person. Active prenatal smoking was defined as smoking at least one cigarette per day during the entire gestation period based on self-report. Passive smoking was defined as exposure to any smoker either at home or at work.

Statistics and data analysis. Cord serum organochlorine levels were normalized by base 2 logarithmic transformation because their distribution was skewed to the right. Two sets of multiple linear regression analyses were used to study the associations between prenatal organochlorine exposure on birth weight, crown-heel length, and head circumference. In the first set of models, the dependent variable (either birth weight, crown-heel length, or head circumference) was examined in relation to level of organochlorines and other variables. Both continuous and categorized levels of HCB, p,p'-DDE, β-HCH, and total PCB, were used in the regression analyses. Models for the most frequent PCB congeners (118, 138, 153, and 180) were also fitted. PCB congeners were only analyzed as categorical variables given the high proportion of nonquantifiable values. In the second set of models, the dependent variable (organochlorine level) was examined in relation to prematurity, small weight, length for gestational age, and other variables. Potential confounding variables, such as sex, maternal tobacco and/or alcohol consumption during pregnancy, gestational diabetes, parity, maternal height, weight and body mass index, maternal and paternal education, and maternal and paternal social class, were considered for inclusion in the regression based on the literature. Variables were included in the final regression model if their association with the outcome had a p value < 0.2. All statistical analyses were conducted with the SPSS and STATA statistical software packages (SPSS Inc., Chicago, IL, U.S.A.). A p value <0.05 was considered statistically significant.

RESULTS

There were no significant differences in the outcome variables or covariates between those mother-infant pairs with biologic samples and those without [means (SE) and percentages among those mother-infant pairs with samples: birth weight = 3244.6 (488.78) g; crown-heel length = 49.4 (2.19) cm; head circumference = 34.4 (1.65) cm; premature = 6%; small weight for gestational age = 10%; and small length for gestational age = 17%]. The highest organochlorine levels were found for HCB followed by p,p'-DDE, β-HCH, and, finally, by the sum of seven PCB congeners both in cord serum (Table 1) and in maternal serum (data not shown). Levels of HCB and total PCB in cord serum were quantified in 100% of the samples, and levels of p,p'-DDE in 97%. Among the PCB congeners, PCB 118 was not quantifiable in any of the samples. HCB cord serum levels were similar in children whose mother were smokers (median = 1.03 ng/mL, $n = 23$) and nonsmokers (1.21 ng/mL, $n = 47$) ($p = 0.3$), as were p,p'-DDE levels (0.67 and 0.92, respectively) and total PCB (0.23 and 0.31, respec-

Table 1. *Levels of organochlorine compounds in cord serum (ng/mL) (n = 70)*

	Detectable levels (%)	Quantifiable levels (%)	Percentiles				
			5	25	50	75	95
HCB	100	100	0.33	0.79	1.13	1.72	3.14
p,p′-DDE	100	97	0.22	0.49	0.85	1.69	3.21
β-HCH	87	69	0.01	0.05	0.54	1.08	2.53
PCB138	91	27	0.01	0.05	0.05	0.19	1.01
PCB180	90	23	0.01	0.05	0.05	0.05	0.65
PCB153	87	21	0.01	0.05	0.05	0.05	0.63
PCB118	49	0	0.01	0.01	0.01	0.05	0.05
All PCB	100	100	0.11	0.19	0.27	0.58	2.03

tively). The results did not vary when nonsmokers were categorized as exposed or nonexposed to passive smoking.

HCB cord serum levels were inversely associated with crown-heel length, even adjusting for smoking and the other potential confounding variables such as parents' education (Table 2). The associations had a dose-response relationship (decreased length with increasing HCB tertiles) and were statistically significant for the highest level of exposure. When maternal serum levels at delivery were analyzed instead of cord serum levels, an inverse association was also observed [coefficient (SE) for the third tertile of maternal HCB exposure = 1.02 (0.48) cm ($p = 0.04$)].

Neither birth weight nor head circumference (data not shown) were associated with HCB. Inverse associations with crown-heel length were also found for the other organochlorine compounds but these were not statistically significant (Table 3). The association of HCB with length was not modified after adjusting for the other organochlorine compounds. Smoking was negatively associated with birth weight and crown-heel length (Table 2). No interaction between HCB levels and maternal smoking was found.

Newborns with a small length for gestational age had higher levels of all organochlorine compounds, but were only statistically significantly different for HCB (Table 4). No differences in organochlorine compounds were encountered between those newborns with and without small weight for gestational age. Premature newborns showed higher concentrations of HCB, p,p'-DDE, β-HCH, and total PCB at birth than those born at term, being only significantly different for p,p'-DDE. When the above analyses were repeated after excluding prematures, HCB cord serum levels were also associated with a decrease in crown-heel length, either in a continuous [for each doubling of a dose there was a decrease of −0.53 (0.24) cm] or in a categorical way [coefficients (SE) = −0.68 (0.54) and −1.10 (0.55) for the second and third tertile, respectively]. Moreover, those infants born with a small length for gestational age had higher concentrations of HCB in cord serum than those with an adequate length for gestational age [geometric mean = 1.87

Table 2. *Coefficients (SE) from multivariate models of birth weight and crown-heel length*

	Birth weight (g)	Crown-heel length (cm)
Reference†	3220.6 (59.9)	50.08 (0.51)
HCB 0.9–1.48 ng/mL	−66.28 (109.1)	−0.70 (0.53)
HCB > 1.48 ng/mL	29.09 (114.9)	−1.08 (0.53)*
Sex, boys	122.1 (93.5)	0.87 (0.42)*
Cigarettes/day	−21.7 (9.6)*	−0.10 (0.04)*
Alcohol intake, yes	—	−1.28 (0.59)*
Gestational age (wk)	206.9 (30.2)*	0.78 (0.13)*
Maternal age (y)	15.9 (9.4)	—
Maternal body mass index (kg/m^2)	24.7 (13.3)	0.12 (0.06)*
Gestational diabetes, yes	—	1.97 (0.70)*
Parents' education,	—	
Low in one of them		−0.27 (0.49)
Low in both of them		−0.93 (0.52)

Each column is a different multivariate model (birth weight and crown-heel length). The covariates included in regression models were levels of HCB in cord serum (three categories: in tertiles), sex (two categories), smoking in pregnancy (continuous), alcohol intake during pregnancy (two categories), gestational age (continuous), maternal age (continuous), maternal body mass index (continuous), gestational diabetes (two categories), and parent's education (three categories).

† Newborns with a HCB level < 0.09 ng/mL in cord serum, female, nonexposed to alcohol or tobacco during gestation, born at 39.7 wk, whose mothers were 30.7 y old and had a body mass index of 23.7 kg/m^2, did not have a gestational diabetes and neither the paternal nor the maternal education were low (up to primary school). Values for each variable (β) are change (per unit increase if continuous or category change if categorical) in the reference value; *i.e.* a newborn who differed to the reference in the HCB levels (1.26 ng/mL) and the sex (male) would have a predicted birth weight of 3220.6−66.28 + 122.1 g and a crown-heel length of 50.08−0.70 + 0.87 cm.

* $p < 0.05$.

— Variables not included due to $p > 0.20$.

Table 3. *Effects (coefficient and SE) of other organochlorine compounds in cord serum on birth weight and crown-heel length*

	Birth Weight (g)†	Crown-heel Length (cm)‡
HCB (ng/mL)#	19.8 (50.9)	−0.48 (0.23)*
p,p′-DDE (ng/mL)#	−16.8 (37.8)	−0.20 (0.15)
β-HCH (ng/mL)#	17.5 (17.6)	−0.02 (0.08)
All PCB (ng/mL)#	−5.6 (36.1)	−0.12 (0.16)
Quantifiable levels of PCB138§	−41.4 (97.6)	−0.46 (0.46)
Quantifiable levels of PCB180§	118.7 (104.2)	−0.49 (0.52)
Quantifiable levels of PCB153§	−50.1 (107.9)	−0.52 (0.48)
Detectable levels of PCB118¶	−18.2 (86.4)	−0.20 (0.40)

Each coefficient derives from a different multivariate model. # Each unit change represents a doubling of the concentration in ng/mL of each organochlorine compound in cord serum. § Levels of each specific PCB > 0.05 ng/mL in cord serum. ¶ Levels of PCB118 > 0.01 ng/mL in cord serum.

* $p < 0.05$.

† Adjusted for sex, gestational age, number of cigarettes per day during gestation, maternal body mass index, and maternal age.

‡ Adjusted for sex, gestational age, number of cigarettes per day and alcohol intake during gestation, maternal body mass index, parents' education and gestational diabetes.

Table 4. *Geometric mean of organochlorine compounds in cord serum according to prematurity and small weight and length for gestational age*

	Prematurity (≤37 wk)		Small weight for gestational age†		Small length for gestational age‡	
	Yes $n = 4$	No $n = 66$	Yes $n = 7$	No $n = 63$	Yes $n = 12$	No $n = 58$
HCB (ng/mL)	1.94	1.10**	1.10	1.10	1.64	1.00*
p,p′-DDE (ng/mL)	2.40	0.80*	0.47	0.77	0.88	0.65
β-HCH (ng/mL)	0.55	0.26	0.15	0.32	0.54	0.23
All PCB (ng/mL)	0.70	0.34**	0.28	0.37	0.51	0.32

Each coefficient derives from a different multivariate model.

* $p < 0.05$; ** $p < 0.10$.

† Adjusted for sex and number of cigarettes per day.

‡ Adjusted for sex, number of cigarettes per day during gestation, and maternal body mass index.

ng/mL *versus* gm = 0.96 ng/mL ($p = 0.01$)]. Findings for maternal HCB (instead of cord serum HCB) were similar.

DISCUSSION

The main finding of the present study was that higher HCB levels in maternal and in cord serum were negatively associated with crown-heel length but not with birth weight. This association was not explained by prematurity or gestational age, although premature newborns had higher levels of HCB. These associations were independent of the role of maternal smoking during pregnancy. Most of the studies of prenatal organochlorine exposure in relation to size at birth have only assessed the effects on birth weight.

In utero exposure to PCBs was related to an increased risk of having an infant with low birth weight in a number of studies (5, 11–14), but not in studies conducted in North Carolina (15) and in Finland (16). Only one study reported an association between prenatal exposure to HCB and lower birth weights of female infants (17) and a recent large study reported a strong relation between p,p'-DDE and lower birth weight (18). In our study, no effects on birth weight could be observed for any of the organochlorine compounds but levels of p,p'-DDE were much lower than in the previous study and levels of PCB in the cord serum of our newborns were lower than those reported recently in other sites (19).

Among the studies reporting effects of prenatal exposure to PCB on birth length (5, 14), only the study in Taiwan, with higher levels of PCB and other compounds, found a statistically significant association with birth length. We similarly found no association between PCB and length but we observed a significant association with HCB. It is important to note that skeletal growth, as reflected by body length, may have considerable prognostic value for subsequent growth and development of the newborn. Morris *et al.* (20) found a strong direct association between birth length on both the psychomotor development and attained length at 12 mo.

Premature newborns had higher levels of organochlorines, particularly of p,p'-DDE. Previous results in the literature are quite controversial. Longnecker *et al.* (18) have recently found that maternal serum concentration of p,p'-DDE was associated with increased odds of premature births, but in another study, with lower levels of p,p'-DDE in women, a lack of an association between p,p'-DDE and preterm delivery was found (21). Two human population-based studies had previously found an association with PCB levels (12, 22). The role of organochlorines in prematurity could be of a different nature than the mechanisms underlying the linear growth delay, and should be reassessed in larger populations.

A recent study in Germany has shown that HCB and PCB levels at birth were higher in smokers' newborns than in nonsmokers' newborns (23). We did not find that levels of HCB (or other organochlorine compounds) were higher in smokers' children, and we observed that the association between HCB and length was independent of the smoking effect.

The present study was able to find significant results despite its small size. This might be explained by the strength of the associations. This cohort is representative of the infants born in an HCB-polluted area. Participation rates in this study were high and selection bias is unlikely. These data suggest that HCB reduces intrauterine physical linear growth.

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