Exposure to Hexachlorobenzene during Pregnancy and Children’s Social Behavior at 4 Years Age

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BACKGROUND: Hexachlorobenzene (HCB) is an organochlorine chemical that has been used in agriculture and industrial processes. Behavioral impairment after HCB exposure has been described in animal models, but little information is available in humans.

OBJECTIVES: Our goal was to study the association of prenatal exposure to HCB with the social behavior of preschool children.

METHODS: Two birth cohorts in Ribera d’Ebre and Menorca (Spain) were set up between 1997 and 1999 (n = 475). The California Preschool Social Competence Scale and the Attention-Deficit Hyperactivity Disorder (ADHD) were scored by each 4-year-old child’s teacher. Organochlorine compounds were measured in cord serum. Children’s diet and parental sociodemographic information were obtained through questionnaire.

RESULTS: Children with concentrations of HCB > 1.5 ng/mL at birth had a statistically significant increased risk of having poor Social Competence [relative risk (RR) = 4.04; 95% confidence interval (CI), 1.76–9.58] and ADHD (RR = 2.71; 95% CI, 1.05–6.96) scores. No association was found between HCB and the cognitive and psychomotor performance of these children.

CONCLUSIONS: Prenatal exposure to current concentrations of HCB in Spain is associated with a decrease in the behavioral competence at preschool ages. These results should be considered when evaluating the potential neurotoxicologic effects of HCB.

KEY WORDS: behavior, children, cord serum, HCB, hexachlorobenzene, hyperactivity, prenatal exposure, social competence. Environ Health Perspect 115:447–450 (2007). doi:10.1289/ehp.9314 available via http://dx.doi.org/ [Online 6 November 2006]
to calculate the intertest reliability. We applied the absolute kappa coefficient weighted with the “prerecorded” weights, taking into account the potential missing. An acceptable kappa coefficient of agreement would be > 0.40. The test–retest reliability by kappa formula was satisfactory for the CPSCS (mean = 0.59) and the ADHD checklist (mean = 0.71). The teachers did not know the degree of exposure to OCs. The cognitive skills of the children were also evaluated with the McCarthy Scales for Infant Development as described in previous studies (Ribas-Fitó et al. 2006).

We used a gas chromatograph with electron capture detection (Hewlett Packard 6890N GC-ECD; Hewlett Packard, Avondale, PA) to quantify HCB and other OCs (Carrizo et al. 2006). Quantification was performed using external standards, with the polychlorinated biphenyl (PCB)-142 injection standard used to correct for volume. Recovery of 1,2,4,5-tetrabromobenzene and PCB-209 (75–115%) was used to correct results. Limit of detection was 0.02 ng/mL. A value of 0.01 ng/mL was given for the nonquantifiable concentrations. Serum samples were stored at –40°C until analysis. All the analyses were carried out in the Department of Environmental Chemistry (IQAB-CSIC) in Barcelona, Spain.

Information on socioeconomic background, maternal diseases and obstetric history, parity, sex, fetal exposure to alcohol (at least two glasses per week during the entire pregnancy) and cigarette smoking (at least one cigarette per day during the last trimester), type and duration of breast-feeding, education, and social class was obtained through questionnaires administered in person after delivery and at 48 months. We used the UK Registrar General’s 1990 classification to group the cohort by social class according to maternal and paternal occupation, coded using the International Standard Classification of Occupations (ISCO-88) (Warwick Institute for Employment Research 2006). Duration of breast-feeding was categorized into four groups: < 2, 2–16, 16–28, and > 28 weeks. Information on maternal diet was obtained through a food–frequency questionnaire administered in the third trimester of gestation.

Statistical analysis. The CPSCS scores were skewed to the left and were categorized into two subgroups according to a scoring of less or more than 80 points (which represents the social competency female and male mean raw score for a 4-year-old child according to the scale). The criterion for ADHD was the presence of either six or more symptoms of inattention or six or more symptoms of hyperactivity–impulsivity. Both components (inattention and hyperactivity) were also studied separately. Cord serum HCB concentrations were categorized into four categories (< 0.5, 0.5–0.99, 1.00–1.49, and > 1.50 ng/mL). We used a natural logarithmic transformed variable when HCB was treated as a continuous variable. We used multivariate models to adjust for the significant covariates reported from the literature, such as maternal and paternal social class and education, mother’s parity and marital status, child’s sex, age, and school season during test administration, cohort, and psychologist. Those variables that altered

### Table 1. Distribution (median and 25th and 75th percentiles) of HCB in cord serum (ng/mL) by cohort.

| Variable                        | Cohort          | No. | Minimum | 25th | Median | 75th | Maximum |
|---------------------------------|-----------------|-----|---------|------|--------|------|---------|
| **Maternal variables**          |                 |     |         |      |        |      |         |
| Maternal age (weeks)            | Total           | 475 | 30.5    | 32.0 | 33.4   | 34.1 | < 0.001 |
|                                 | Ribera d’Ebre   | 70  | 30.5    | 32.0 | 33.4   | 34.1 | < 0.001 |
|                                 | Menorca         | 405 | 30.5    | 32.0 | 33.4   | 34.1 | < 0.001 |
| **Paternal variables**          |                 |     |         |      |        |      |         |
| Paternal social class (%):       |                 |     |         |      |        |      |         |
| Professional                    |                 |     |         |      |        |      |         |
|                                 |                 |     |         |      |        |      |         |
| Skilled                         |                 |     |         |      |        |      |         |
|                                 |                 |     |         |      |        |      |         |
| Partially skilled               |                 |     |         |      |        |      |         |
|                                 |                 |     |         |      |        |      |         |
| Unemployed                      |                 |     |         |      |        |      |         |
|                                 |                 |     |         |      |        |      |         |
| Social class (%)                |                 |     |         |      |        |      |         |
|                                 |                 |     |         |      |        |      |         |
| Professional                    |                 |     |         |      |        |      |         |
|                                 |                 |     |         |      |        |      |         |
| Partially skilled               |                 |     |         |      |        |      |         |
| **Maternal variables**          |                 |     |         |      |        |      |         |
| Maternal age (years)            | Total           | 475 | 30.5    | 32.0 | 33.4   | 34.1 | < 0.001 |
|                                 | Ribera d’Ebre   | 70  | 30.5    | 32.0 | 33.4   | 34.1 | < 0.001 |
|                                 | Menorca         | 405 | 30.5    | 32.0 | 33.4   | 34.1 | < 0.001 |
| **Child variables**             |                 |     |         |      |        |      |         |
| Gender (years)                  | Total           | 475 | 30.5    | 32.0 | 33.4   | 34.1 | < 0.001 |
|                                 | Ribera d’Ebre   | 70  | 30.5    | 32.0 | 33.4   | 34.1 | < 0.001 |
|                                 | Menorca         | 405 | 30.5    | 32.0 | 33.4   | 34.1 | < 0.001 |
| **Social class (%)**            |                 |     |         |      |        |      |         |
|                                 |                 |     |         |      |        |      |         |
| Professional                    |                 |     |         |      |        |      |         |
|                                 |                 |     |         |      |        |      |         |
| Partially skilled               |                 |     |         |      |        |      |         |

### Table 2. Distribution of child, paternal, and maternal variables according to concentrations of HCB in cord serum (n = 405).

| Variable                        | HCB category (ng/mL) |   |   |   |   |   |   |
|---------------------------------|----------------------|---|---|---|---|---|---|
| Child variables                 |                      |   |   |   |   |   |   |
| Female sex (%)                  | 53                   | 51| 50| 41| NS|   |   |
| Population (%)                  | 53                   | 51| 50| 41| NS|   |   |
| Maternal age (years)            |                      |   |   |   |   |   |   |
| Age (years)                     | 30.5                 | 32.0| 33.4| 34.1| < 0.001| |
| Years of education (%)          |                      |   |   |   |   |   |   |
| > 16                            |                      |   |   |   |   |   |   |
| 12–15                          |                      |   |   |   |   |   |   |
| 8–11                           |                      |   |   |   |   |   |   |
| < 8                            |                      |   |   |   |   |   |   |
| Social class (%)                |                      |   |   |   |   |   |   |
| Professional                    |                      |   |   |   |   |   |   |
| Partially skilled               |                      |   |   |   |   |   |   |
| Maternal age (years)            |                      |   |   |   |   |   |   |
| Age (years)                     | 27.2                 | 29.1| 30.4| 30.8| < 0.001| |
| Weight (kg)                     | 56.3                 | 59.5| 60.6| 63.4| < 0.001| |
| Years of education (%)          |                      |   |   |   |   |   |   |
| > 16                            |                      |   |   |   |   |   |   |
| 12–15                          |                      |   |   |   |   |   |   |
| 8–11                           |                      |   |   |   |   |   |   |
| < 8                            |                      |   |   |   |   |   |   |
| Social class (%)                |                      |   |   |   |   |   |   |
| Professional                    |                      |   |   |   |   |   |   |
| Partially skilled               |                      |   |   |   |   |   |   |
| Maternal age (years)            |                      |   |   |   |   |   |   |
| Age (years)                     | 11.1                 | 11.0| 12.1| 11.9| NS|   |   |
| Breast-feeding, yes (%)         | 84                   | 83| 79| 78| NS|   |   |
| Birth weight (g)                | 3516.4               | 3259.3| 3125.0| 3290.5| NS|   |   |
| Birth weight (kg)               | 0.64                 | 0.86| 0.90| 0.90| 0.032| |
| Birth weight (mg)               | 0.10                 | 0.18| 0.17| 0.18| < 0.001| |
| Birth weight (g)                | 0.93                 | 1.58| 1.97| 2.44| < 0.001| |

NS, not statistically significant.
the HCB coefficient by ≥ 10% in the categorical or continuous models remained in the model. All the models were repeated for each specific cohort. All statistical analyses were conducted with the Stata 8.0 statistical software (StataCorp, College Station, TX, USA).

Results

HCB was detected and quantified in all cord serum samples from the two cohorts. Concentrations of HCB were, as expected, higher in Ribera d’Ebre, but the maximum values were higher in Menorca (Table 1). Children from the Ribera d’Ebre cohort were more likely to be an only child and to have been breast-fed for shorter periods. The mothers of this cohort were less educated and were more likely to be of lower social class and to drink and smoke during pregnancy than those of the Menorca cohort (n = 18). The crude behavioral evaluation was equal in both cohorts (the frequency of a low Social Competence scoring was 19% in Ribera d’Ebre and 23% in Menorca; the frequency of ADHD was 15% in the categorical Menorca cohort). The crude and adjusted RR of having ADHD symptoms at 4 years of age in relation to in utero exposure to HCB [coefficient (95% CI)] is shown in Table 2. Children with HCB concentrations in cord serum > 1.5 ng/mL had a 4-fold increase of having a poor Social Competence score and a 2.7-fold increase of having ADHD symptoms. No association was found between HCB and the cognitive and psychomotor performance of these children.

The evidence of neurotoxicity in humans exposed to HCB was provided by studies of people in southeast Turkey who consumed contaminated bread in the late 1950s. Neurologic symptoms included loss of appetite, tremors, convulsions, and weakness (Peters et al. 1982). Follow-up studies found that neurologic symptoms persisted in adults who had been exposed as children. During the grain poisoning epidemic, there was an extremely high (95%) rate of mortality in infants < 2 years of age who had been breast-fed by mothers who had ingested the contaminated bread; these children exhibited convulsions, tremors, and progressive weakness before death (Peters et al. 1966). A recent study described a significant association between cord blood HCB and one of the blocks of the Neurobehavioral Evaluation System Continuous Performance Test, which evaluates sustained attention, at 8 years of age (Stewart et al. 2005), but further adjustment for covariates has found the association nonsignificant. The authors also found an adjusted association between prenatal PCB exposure and an impaired response inhibition at 9.5 years of age, confirming what they had described at 4.5 years of age (Stewart et al. 2003). We did not find any association between PCBs or p,p’-DDE and the assessed behavioral patterns.

Table 3 shows the crude and adjusted associations between HCB and the Social Competence scores. All the categories of HCB exposure were associated with an increase in the risk of having a poorer Social Competence score, but only those children with concentrations of HCB > 1.5 ng/mL at birth had a statistically significant increased risk (relative risk (RR) = 4.04; 95% confidence interval (CI), 1.76–9.58). The crude and adjusted risks of having an ADHD criteria are shown in Table 3. Children with ADHD criteria had a score of 76.18 ± 1.34. The difference was statistically significant with a p-value < 0.001.

Table 4. Crude and adjusted RR of having ADHD symptoms at 4 years of age in relation to in utero exposure to HCB [coefficient (95% CI)].

| Exposure | Unadjusted | Adjusted | Adjusted for other OCsb | Menorca cohortb |
|----------|------------|----------|-------------------------|-----------------|
| HCB category |            |          |                         |                 |
| Referencec | 1          | 1        | 1                       | 1               |
| 0.5–0.99 ng/mL | 1.16 (0.62–2.18) | 1.40 (0.69–2.87) | 1.77 (0.83–3.79) | 1.84 (0.82–4.11) |
| 1–1.49 ng/mL | 1.04 (0.48–2.29) | 1.47 (0.59–3.62) | 1.83 (0.72–4.69) | 1.51 (0.52–4.35) |
| ≥ 1.5 ng/mL | 2.88 (1.39–5.97)* | 4.04 (1.76–9.58)* | 5.63 (2.13–14.88)* | 6.18 (2.06–18.50)* |

*Adjusted for age, cohort, sex, maternal education, paternal education, tobacco and alcohol exposure, maternal age in years, and type and duration of breast-feeding (see “Population and Methods”). Adjusted for same variables above and PCBs, p,p’-DDE and p,p’-DDT. Reference group: < 0.5 ng/mL. Natural log-transformed HCB concentration.

p < 0.05.

Table 5. Crude and adjusted RR of having ADHD symptoms at 4 years of age in relation to in utero exposure to HCB [coefficient (95% CI)].

| Exposure | Unadjusted | Adjusted | Adjusted for other OCsb | Menorca cohortb |
|----------|------------|----------|-------------------------|-----------------|
| HCB category |            |          |                         |                 |
| Referencec | 1          | 1        | 1                       | 1               |
| 0.5–0.99 ng/mL | 1.19 (0.58–2.42) | 1.23 (0.54–2.78) | 1.47 (0.63–3.46) | 1.38 (0.57–3.32) |
| 1–1.49 ng/mL | 1.73 (0.77–3.91) | 2.28 (0.88–5.96) | 2.74 (1.01–7.45)* | 2.17 (0.73–6.49) |
| ≥ 1.5 ng/mL | 2.05 (0.90–4.67)** | 2.71 (1.05–6.96)* | 3.43 (1.24–9.51)* | 3.11 (1.01–9.55)* |

*Adjusted for age, cohort, sex, maternal education, paternal education, tobacco and alcohol exposure, maternal age in years, and type and duration of breast-feeding (see “Population and Methods”). Adjusted for same variables above and PCBs, p,p’-DDE and p,p’-DDT. Reference group: < 0.5 ng/mL. Natural logarithmic transformed HCB concentration.

p < 0.05; **p < 0.10.
Taylor (1992), and changes in operant behavior in adult rats (Lillenthal et al. 1996), but no significant effects on learning or motor activity have been detected.

The mechanisms by which HCB may cause behavioral impairments is not known. It has been suggested that HCB interferes with myelination during development (Goldey and Taylor 1992). Alterations in regional brain concentrations of serotonin, dopamine, and norepinephrine have also been described by Bleavins et al. (1984), and a recent study has found that exposure to HCB can produce oxidative stress and that the brain is a sensitive target organ of HCB toxicity (Song et al. 2006). No signs of histopathologic changes in the brain, spinal cord, motor and sensory nerves, and skeletal muscles have been found (Campbell 1963; Kuiper-Goodman et al. 1977).

It is difficult to elucidate why the HCB effects were apparent only on the behavioral and not the cognitive functions. A study investigating the potential effects of consuming fish from the Great Lakes was unable to correlate HCB levels in umbilical blood or breast milk from infants with intelligence test results (Darvill et al. 2000). In a previous study with children from the Ribera d'Ebre cohort, we reported no association between prenatal exposure to HCB and the mental and psychomotor functions of the children at 13 months of age (Ribas-Fito et al. 2003), although no behavioral test batteries were performed. Most research on OCs and neurodevelopment has relied on broad measures of global cognitive functioning. These tests have not been designed to measure specific neuropsychological processes, such as attention and social behavior. In the present study, we also did not find any association between HCB and the cognitive skills in the two cohorts at 4 years of age (Ribas-Fito et al. 2006). The association was significant only when the behavioral domains such as the Social Competence and ADHD were studied. ADHD is a neuropsychiatric disorder characterized by pervasive inattention and/or hyperactivity–impulsivity and resulting in significant functional impairment. The Centers for Disease Control and Prevention estimate that 4.4 million youth 4–17 years of age have been diagnosed with ADHD by a health care professional, and as of 2003, 2.5 million youth 4–17 years of age are currently receiving medication treatment for the disorder (Lesene et al. 2000).

A potential limitation of the present study is the nonresponse rate (20.6%). Subjects were not included (n = 98) because no information could be obtained from the schools. The nonresponse rate is unlikely to be related to HCB levels or the Social Competence scores of the children. One of the cohorts in the present study was studied in an area where the main pollutant, deriving from an electrochemical factory in the vicinity, and the population living in this area has the highest levels of HCB ever reported for nonoccupational exposure (Sala et al. 2001). The Menorca cohort represents a general population with little industrial activity. Geographic differences between Menorca and Ribera d'Ebre were eliminated by analyzing the data by cohort. The results using the Menorca cohort alone showed no differences compared with the two-cohorts analyses, and the results from the Ribera d'Ebre cohort alone were in the same direction but not statistically significant. This lack of significance could be explained by the small size of the cohort. Because Menorca has no local pollution sources, the results encountered here could be representative of other areas where several years have elapsed since HCB use was banned. Residual confounding was minimized by the adjustment for the potential confounders.

Overall, prenatal exposure to current concentrations of HCB is associated with a decrease in the Social Competence scores and an increase of the ADHD symptomatology at preschool ages. These results suggest that some infants may be at risk for developing neurotoxicity from HCB due to relatively high concentrations of HCB detected in cord serum and breast milk from women in certain parts of the world.

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