Prenatal exposure to 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene (p,p'-DDE) in relation to child growth

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Objective To examine the relation between prenatal 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (p,p'-DDE) exposure (a metabolite of the insecticide DDT) and child growth during the first 7 years of life.

Design Prospective cohort study.

Participants 1712 children born between 1959 and 1966 with measured p,p'-DDE concentrations in their mother’s serum samples from pregnancy.

Setting Multicenter US Collaborative Perinatal Project (CPP).

Results The highest prenatal concentrations of p,p'-DDE (>60 µg/l), as compared with the lowest (<15 µg/l), were associated with decreased height at age 1 year [adjusted coefficient (SE) = −0.72 cm (0.37), n = 1540], 4 years [−1.14 cm (0.56), n = 1289], and 7 years [−2.19 (0.46), n = 1371]. Among subjects in lower categories of exposure no association was observed.

Conclusions The findings suggest that high prenatal exposure to p,p'-DDE decreases height in children. Impaired growth may be a general indicator of toxicity and suggests that specific organ systems (e.g. endocrine) could be affected.

Keywords p,p'-DDE, growth, children, cohort

The insecticide DDT [2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane] was banned or restricted in most of the industrialized countries in the 1970s, but it is still used in some countries for disease-vector control. The debate about the urgency of eliminating or extending its use remains open.1,2

Reintroduction of DDT in South Africa has resulted in fewer cases of malaria3 and other countries are considering its use.1 The toxic effects in humans of DDT and its degradates, however, have not been adequately characterized.4,5 Both DDT and its primary degrade, p,p'-DDE [1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene, hereafter DDE] are resistant to degradation and bioaccumulate in the food chain.

Data from some studies suggest that perinatal exposure to certain organochlorine compounds may affect body size in children, though the human data are scarce and inconsistent. Reduced height among female but not male children was associated with higher childhood DDE concentrations in one study conducted in Germany.6 Another report found that higher in utero exposure to DDE was associated with greater heights around the time of puberty in boys but not girls.7 A recent study with 304 males born in Philadelphia found no association between anthropometric measurements during their adolescence and prenatal exposure to DDE.8 In adult rodents, a decrease in the body weight gain has been reported after acute or chronic administration of DDE in the diet.9

We measured concentrations of DDE in stored serum samples from pregnant women from the US Collaborative Perinatal Project (CPP). Mothers were enrolled in the early 1960s, when DDT use in the US was at its peak.10 A previous study based on CPP subjects found that DDE concentrations in maternal pregnancy serum were associated with an increased risk of
small-for-gestational-age birth,\textsuperscript{11} consistent with an effect on fetal growth. Information on child’s height was available up to the age of 7 years in the CPP. We examined the association between prenatal DDE exposure and child height among boys and girls in a setting with relatively high levels of exposure to this contaminant.

Methods

Study participants

The CPP was a prospective study of neurological disorders and other conditions in US children.\textsuperscript{12} Pregnant women were recruited between 1959 and 1965 in 12 US study centres (11 university hospital clinics and one group of private practices) from the North-east [Boston, Buffalo, New York (2 centres), Philadelphia, and Providence], South (Baltimore, New Orleans, Richmond, and Memphis), and Midwest–West (Minneapolis and Portland). Researchers enrolled 42 000 women, who gave birth to \~55 000 babies. Study data were collected at each prenatal visit, at delivery, and when the child’s age was 24 h, 4 months, 8 months, and 1, 3, 4, 7, and 8 years. Samples of the mothers’ non-fasting blood were taken about every 8 weeks before delivery. Serum samples were stored in glass at \text{–}20^{\circ}\text{C} and no thaw was recorded.

Singleton live-born children were eligible for the present study if a 3 ml maternal serum sample taken in the third trimester was available. From the 43 628 eligible children, 1200 were selected at random and 993 were selected according to the outcomes of their tests of neonatal tone, neonatal reflexes, Bayley Scale of Infant Development at 8 months, intelligence quotient on the Wechsler Intelligence Scale for Children at age 7 years, and audiogram results at age 8 years.\textsuperscript{11} The samples were obtained independently and the pregnancies selected for more than one type of sample were included only once in this analysis.

Assessment of exposure

Serum concentrations of \textit{p,p’}-DDE, \textit{p,p’}-DDT, and several PCB (polychlorinated biphenyl) congeners were measured at the US Centers for Disease Control and Prevention between 1997 and 1999 after solid-phase extraction followed by dual-column gas chromatographic separation with electron capture detection.\textsuperscript{13} The proportion of DDE in the samples recovered by extraction averaged 70\% (range, 57–85\%). The results shown are not adjusted for recovery.\textsuperscript{14} Serum cholesterol and triglycerides were measured with standard enzymatic methods.

Outcome measures

Children’s weight and height were specifically measured for the CPP. Examination manuals were the same across centres and specially trained study personnel conducted the measurements. Measurements of height and weight between the ages of 1, 4, and 7 years were so sparse that we were unable to fit a smoothed model of growth trajectory. Thus, we examined DDE effects at 1 year (10–14 months), 4 years (46–52 months), and 7 years (82–90 months). Subjects with observations outside the given band were considered as not having an observation at that follow-up examination. We excluded children who were missing all three height measurements (164 exclusions), maternal serum DDE concentration (159 exclusions), or covariates (148 exclusions). Of the remainder, not all children had heights at all three ages. Our final analyses included 1540 children at age 1, 1289 at age 4, and 1371 at age 7.

Statistical analysis

We examined DDE concentrations in relation to height at ages 1, 4, and 7 years. DDE was treated both as a continuous and as a categorical variable. To divide individuals into categories based on their DDE concentrations, a set of four equally spaced cut points was used that contained at least 50 children per category. Study centre (12 categories), age at height measurement (in months), gender, race (white, African American, and other), socioeconomic index (SEI) (three categories), maternal smoking status during the end of pregnancy (0, 1–10, and >10 daily cigarettes), maternal age, and concentrations of triglycerides and cholesterol were considered a priori to be confounding variables and were included in the baseline multivariable linear regression models. SEI was the mean of three percentile scores for education, occupation, and family income. In models in which DDE was represented as a categorical variable, we used only the coefficient for the highest category of DDE to evaluate confounding by additional factors. If adjustment for an additional variable altered the DDE coefficient by 10\% or more in the categorical or continuous model at each age, we retained the variable in the final set of covariates, which was the same for all the ages. Maternal height, parity, and pre-pregnancy body mass index (BMI) were additional variables that met the criteria for confounding.

We repeated all the analyses adjusting for birth size in order to determine whether associations seen were solely attributable to continued effects of lowered birth weight or birth length. To determine whether the results were sensitive to the outcome-dependent sampling scheme, we also repeated all analyses on the random sample only. To study the growth estimates we fit models where the outcome was the increase in height at age 7 taking into account the measurements at previous ages (birth, 1 and 4) and the increase in height at age 4.

Finally, we performed analyses that included data for all three ages simultaneously while accounting for the correlations of height at different ages. We fit a mixed model with an unstructured covariance matrix, including in the model for the mean all the same terms as in the previous analyses plus the interaction of those terms with age at measurement.

Results

Boys and girls were present in essentially equal proportions and most of the children were either African American or white. The median age of the mothers was 23 years. Most mothers were born in urban areas of the US and were non-smokers (Table 1). The median concentration of DDE was 24.4 $\mu$g/l. There were no differences in DDE concentrations between the children with height measurements and those without. Children with height measurements at ages 1 and 4 were essentially equal to those with missing values according to all the factors shown in Table 1. At age 7, children with missing values were more likely to be of other ethnic origin rather than white or African American and to belong to the New York Medical College study area. No differences were found for concentrations of DDE.
Table 1 Characteristics of mothers and children in the entire population and in the two extreme categories of exposure

<table>
<thead>
<tr>
<th>Maternal variables</th>
<th>Median prepregnancy BMI, kg/m²</th>
<th>Median height, cm</th>
<th>Median weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 1712)</td>
<td>(n = 157–165)</td>
<td>(n = 2863–3515)</td>
</tr>
<tr>
<td></td>
<td>(20.0–24.7)</td>
<td>(163)</td>
<td>(3203)</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>56</td>
<td>22.4</td>
<td>3289</td>
</tr>
<tr>
<td>Smoker 0–10 cig/day</td>
<td>32</td>
<td>22.4</td>
<td>3289</td>
</tr>
<tr>
<td>Smoker &gt;10 cig/day</td>
<td>11</td>
<td>22.4</td>
<td>3289</td>
</tr>
<tr>
<td>Place of birth (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US urban</td>
<td>68</td>
<td>23.9</td>
<td>2948–3629</td>
</tr>
<tr>
<td>US rural</td>
<td>23</td>
<td>23.9</td>
<td>2948–3629</td>
</tr>
<tr>
<td>US non-urban</td>
<td>6</td>
<td>163</td>
<td>163</td>
</tr>
<tr>
<td>US non-rural</td>
<td>3</td>
<td>163</td>
<td>163</td>
</tr>
<tr>
<td>Study region (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South (Baltimore,</td>
<td>30</td>
<td>18.4</td>
<td>163</td>
</tr>
<tr>
<td>New Orleans,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richmond, Memphis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midwest–West (</td>
<td>12</td>
<td>18.4</td>
<td>163</td>
</tr>
<tr>
<td>Minneapolis, Portland)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median triglycerides, mg/dl</td>
<td>195 (155–247)</td>
<td>183 (146–226)</td>
<td>194 (161–242)</td>
</tr>
<tr>
<td>Median total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cholesterol, mg/dl</td>
<td>233 (197–275)</td>
<td>220 (186–258)</td>
<td>244 (201–288)</td>
</tr>
</tbody>
</table>

Table 1 continued

<table>
<thead>
<tr>
<th>Maternal variables</th>
<th>Median PCBs, µg/l</th>
<th>Median total cholesterol, mg/dl</th>
<th>Median triglycerides, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 300)</td>
<td>(n = 201–288)</td>
<td>(n = 163–242)</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
<td>2.0</td>
<td>220</td>
</tr>
</tbody>
</table>

Values are percentages or medians (and interquartile ranges).

a Children with height measurements at ages 1, 4, or 7 with complete data on covariates.

Children in the highest category of in utero DDE exposure (≥60 µg/l) were more likely to be African American, had a lower birth weight, and had a shorter birth length; their mothers had a lower SEI and were more likely to have been born in rural areas. Higher exposure occurred in the South. Among these children in the highest DDE category, concentrations of total cholesterol, triglycerides, and PCBs in the mother’s serum were higher (Table 1).

The results of the final adjusted linear regression model for the relative height are shown in Figure 1. At all assessed ages, there was a reduction in height in the highest concentration group compared with the lowest concentration group, the reduction being statistically significant at ages 4 and 7. The DDE effect on height increased with age in both absolute and percentage terms. Although no dose response was observed within the other categories of DDE exposure, DDE as a continuous variable was also associated with a decreased height at age 1 year [coefficient (SE) = −0.008 cm (0.004)] and at age 7 years [coefficient (SE) = −0.02 cm (0.007)].

In a separate height analysis, we added birth length as a covariate to the final model. We found that greater birth length was associated with increased height at later ages [coefficient (SE) = 0.44 cm (0.03) at age 1, 0.38 cm (0.04) at age 4, and 0.44 cm (0.05) at age 7], but the parameter estimates for the highest DDE category were not appreciably different from those shown in the figure.

Crude mean height means at age 7 by DDE exposure and race are shown in Table 2. Children in the highest DDE category had a shorter mean height than children in the other categories. African American children had a greater height in all categories of exposure than whites. In the highest category of DDE the population was mostly African American. Thus, important confounding of the crude results by race was anticipated.

The crude and adjusted results for height at age 7 are shown in Table 3. A reduction in height was observed for the highest DDE category in the crude, partially adjusted, and fully adjusted models, being larger and statistically significant in both adjusted models. As expected, the change between the crude and the partially adjusted estimates was due mainly to confounding by race. When the multivariable models were stratified by race (Table 3), we found that the association between DDE and height was clear only in African Americans (P for interaction = 0.05). Further stratification by both gender and race suggested that among African American females the effect of high exposure to DDE on height was greater.
Figure 1 Adjusted regression results [β (SE)] for height in centimetres according to DDE category by age. (Relative height is the ratio of adjusted mean height among those in a given exposure group to those in the referent exposure group. Reference group: <15 µg/L. The reference category is firstborn white females from the Boston centre whose mothers were 24.3 years-old, non-smokers, had a low SEI, a prepregnancy BMI of 22.8 kg/m², a height of 1.61 m, 239 mg/dl of total cholesterol, and 212 mg/dl of triglycerides.)

Table 2 Crude mean height in centimetres at the 7 years in relation to in utero exposure to p,p'−DDE and race

Table 3 Crude and adjusted regression results for height in centimetres at the 7 years in relation to in utero exposure to p,p'−DDE

A decrease in growth of 2.4 and 2.6%, respectively, compared with the lowest, were associated with decreased height at any age. However, further adjustment for PCBs in the fully adjusted DDE models slightly increased the effect of DDE on height in the highest category of exposure [coefficient (SE) for highest category compared with lowest = −1.97 cm (0.66)] at age 1, −1.24 cm (0.57) at age 4, and −2.43 (0.69) at age 7). The DDE/DDT ratio was not associated with decreased height at any age.

When we restricted the fully adjusted, wet weight analyses to only children who were selected randomly, the effects of the highest DDE category on height remained statistically significant [coefficient (SE) for highest category compared with the lowest = −1.81 (0.90) at age 7 years].

Finally, when DDE was expressed on a lipid basis (µg DDE/g lipid) in a fully adjusted model without cholesterol and triglycerides, the association with decreased height was still evident [coefficient (SE) for highest category (>8 µg/g) compared with the lowest (<2 µg/g) = −1.33 cm (0.66) at age 7 years].
Discussion

Our results suggest that in utero exposure to high concentrations of $p,p'$-DDE ($>$60 µg/l) decreases height in children. Among subjects in lower categories of exposure no association was observed, suggesting a non-linear dose-response pattern. When we stratified by race, the observed association was clear only among African Americans, who comprised the majority of those most highly exposed.

In a previous study, serum DDE concentrations at 8 years of age were examined in relation to height among 175 boys and 125 girls from birth to the age of 10 years; in girls, but not in boys, higher exposures were associated with shorter height. In another study of 278 adolescent boys and 316 girls, prenatal, but not lactational, DDE exposure was associated with a higher height and BMI of the boys. The subjects of the present study, who were born during the peak of DDT use in the US, had higher exposures than the subjects in the above-mentioned studies. In a related recent study with CPP subjects, 304 males born in Philadelphia were studied during their adolescence. Although the degree of exposure was the same as in the present study, associations between prenatal exposure to DDT compounds and male anthropometric measurements were not seen. Although the findings in studies of DDE and height have not been reported, they overlap little in terms of age at exposure assessment, level of exposure, and age at outcome ascertainment. Furthermore, the present study, due to the size and exposure level, had greater statistical power than the earlier studies.

Impaired growth may be a general indicator of toxicity and suggests specific organ systems (e.g. endocrine) could be affected. Although DDE is a hormonally active agent, a plausible biological mechanism for an effect on growth is not readily apparent. Animal experiments with large doses of DDE showed decreased weight among adults; however, effects on height or length of in utero exposure have not been reported.

In a previous publication with the same group of children, an association between DDE and decreased birth weight in both boys and girls was described. Adjustment by birth measurements did not materially affect the results of the present study, suggesting that the apparent effect of prenatal DDE exposure on growth continues post-natally. Post-natal exposure to DDE could not be assessed in this study. Breastfeeding, a source of DDE post-natally, was not a confounder or effect modifier in this study, but our sample included very few breast-fed children. Although some studies have reported effects of PCBs on child's growth, we did not find any association between prenatal exposure to PCBs and height.

At age 7 years, children in the highest DDE group were 2 cm shorter than those in the lowest category. Several studies have reported that shorter height can be a marker of deficits in late-life cognitive function, increased cardiovascular risk, and reduced workplace success and income.

About half the participants in the present study were from a random sample of the CPP population; the remainder were selected because of their outcomes on certain neurological examinations. However, when we restricted the analyses to only children who were selected randomly, the association between DDE and decreased height remained. There were no differences in DDE concentrations between the children with height measurements and those lost to follow-up.

Few data exist on DDE stability in serum during the frozen storage. However, in pooled breast milk samples from Swedish mothers, the DDE concentrations assayed after 15 years, and then 25 years of storage, showed no decline.

In summary, our findings suggest that high exposure to DDE in utero decreases height throughout childhood. Because the average serum DDE concentrations in developed countries are now substantially <15 µg/l, the likelihood of replicating these findings there is low, though susceptibility among a subgroup may exist. However, in the tropical countries where DDT is still used for malaria control, blood concentrations of DDE among women of childbearing age who live in houses sprayed with DDT could exceed the range observed in the present study, and the consequent effects on health in offspring might be important.

Acknowledgements

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**KEY MESSAGES**

- Data from studies suggest that perinatal exposure to organochlorine compounds may affect body size in children.
- Impaired growth may be a general indicator of toxicity and suggest specific organ systems (e.g. endocrine) could be affected.
- Our results suggest that perinatal exposure to high concentrations of $p,p'$-DDE decreases height throughout childhood. The observed association is clear among African Americans, who comprise the majority of the most highly exposed.

References

Commentary: Halogenated organic compounds and child’s growth: a growing public health problem

Wilfried Karmaus

Halogenated organic compounds (HOCs) such as polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethylene (DDE) may interfere with normal hormonal function and, thereby, affect growth and maturation. Thus, these toxicants were termed ‘endocrine disruptors’. Two outcomes, easily observed and frequently linked to HOCs, are birth size and post-natal height and growth. Both outcomes can indicate adverse intrauterine and post-natal development and are associated with several adult diseases. A number of studies have reported that DDE and PCBs are associated with reduced birth sizes and with children’s height and growth (Table 1).

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