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In utero exposure to DDT and incidence of diarrhea among boys from tropical Mexico

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Abstract

Background—A higher incidence rate (IR) of acute gastrointestinal (GI) infections associated with prenatal exposure to *p,p*'-DDE was suggested by the results in two studies. Given the high mortality rate due to childhood diarrhea in some countries with ongoing use of DDT, additional data on this association is relevant for those making decisions about vector-borne disease control.

Objective—To evaluate whether higher levels of prenatal exposure to *p,p*'-DDE and *p,p*'-DDT increase the risk of having diarrhea in a birth cohort of boys from tropical Mexico.

Methods—Our analysis was based on 747 boys whose exposure was measured in maternal serum collected at delivery (2002–2003). Mothers reported the number of diarrhea episodes of their children during in-person interviews. The median age of the children at their last interview was 21.4 months. Poisson regression models were fitted to estimate adjusted incidence rate ratios (aIRR) of diarrhea by levels of *p,p*'-DDE and *p,p*'-DDT.

Results—Overall, there were 1.7 episodes of diarrhea per child-year. Among those in the highest category of exposure (>9 μg DDE/g serum lipid), the aIRR for diarrhea was 1.14 (95% CI: 0.94, 1.30) compared to those in the lowest category of exposure (≤3 μg/g). Among boys living in the urban area, the corresponding aIRR was 1.39 (95% CI: 1.07–1.80). Among rural boys, no associations emerged.

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Conflict of interest:
None

IRB approval:
The study was approved by the Institutional Review Boards at the Instituto Nacional de Salud Pública in México and the National Institute of Environmental Health Sciences in the United States.

Conclusion—Although the results were consistent with a small positive association, the overall estimate was not precise. While urban boys in this study appeared to be more susceptible to DDE-associated diarrhea, a ready explanation for such increased susceptibility was not apparent.

Keywords

boys; diarrhea; *p,p*'-DDE; *p,p*'-DDT; prenatal exposure

1. INTRODUCTION

DDT is a pesticide extensively applied to crops in the past; its current use is restricted to vector-borne disease control in a few countries, mostly in Africa (Rogan and Chen 2005; Turusov et al. 2002; WHO 2010). Previous animal and human studies suggest that developmental exposure to *p,p*'-DDE (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane), the main and most persistent breakdown product of DDT, might cause various adverse outcomes including alterations of the immune system; nonetheless, these results are far from conclusive (ATSDR 2002; Eskenazi et al. 2009; Rogan and Chen 2005). Both prenatal and postnatal exposure to *p,p*'-DDE have been linked to immune responses in children, and disruptions of the immune system during development may induce long-lasting alterations in susceptibility to infectious diseases (Bilrha et al. 2003; Brooks et al. 2007; Daniel et al. 2002; Dietert et al. 2000; Glynn et al. 2008; Jusko et al. 2016; Karmaus et al. 2005; Nagayama et al. 2007; Vine et al. 2000; Vine et al. 2001).

A higher incidence rate of acute gastrointestinal (GI) infections (*i.e.*, dysentery, infectious diarrhea or vomiting, and gastric flu) in the first 12 months of life was reported in association with prenatal exposure to *p,p*'-DDE among Inuit villagers (Dallaire et al. 2004). Another study also reported a higher prevalence of gastroenteritis (including diarrhea and vomiting) with increasing levels of *p,p*'-DDE at ages 6 to 12 months among predominantly city dwellers in North Carolina (Rogan et al. 1987). Thus, the only two reports addressing this association were suggestive but not conclusive.

Diarrhea is the second leading cause of death due to infectious disease among children under 5 years of age worldwide (Black et al. 2010; Chopra et al. 2013). Diarrhea is a symptom of gastrointestinal (GI) infection, and common pathogens implicated in its etiology are viruses, bacteria, and parasites (Ahs et al. 2010; WHO 2009). Major risk factors for diarrhea include low socioeconomic status, suboptimal human waste disposal, and lack of adequate hygiene. Children from low to middle income countries are more susceptible to severe diarrhea resulting in dehydration and less likely to receive timely and adequate medical care, increasing the number of avoidable children's deaths in such countries (Ahs et al. 2010; Genser et al. 2006; WHO 2009). Cognitive deficit and physical growth shortfall have also been reported as long-term consequences of childhood diarrhea (Moore et al. 2001; Niehaus et al. 2002). Thus, identifying environmental exposures potentially associated with a higher risk of infections is useful for those making decisions about vector-borne disease control policy, especially in countries where deaths due to childhood infections are still a public health concern and the use of DDT is ongoing.

The main purpose of this study was to evaluate whether relatively high levels of prenatal exposure to *p,p*'-DDE and *p,p*'-DDT were associated with a higher risk of diarrhea in a birth cohort of boys under two years of age from Tapachula, Chiapas. In this area of Mexico, DDT was used for almost four decades; DDT was used on crops until 1991 and for malaria control until 1998 (Chanon et al. 2003; ISAT 2000).

2. MATERIAL AND METHODS

We analyzed data from a birth cohort of mothers and their singleton newborn sons from the State of Chiapas, Mexico; details of this cohort has been described elsewhere (Cupul-Uicab et al. 2008; Cupul-Uicab et al. 2010). In brief, 870 healthy newborn males born at term with normal birth weight and their mothers were enrolled through 2002 and 2003 at the time of delivery; the participation rate was 95% (Cupul-Uicab et al. 2010; Longnecker et al. 2007). Maternal serum samples were collected at the time of enrollment. From January 2004 to June 2005, mothers and their sons were visited at their homes to ascertain the duration of lactation and to obtain information on growth and health status of the children; the follow-up rate was 91% (Cupul-Uicab et al. 2008). As the initial study hypothesis was related to the potential androgen-blocking effects of DDT, only boys were enrolled (Longnecker et al. 2007). The study was approved by the Institutional Review Boards at the Instituto Nacional de Salud Pública in México and the National Institute of Environmental Health Sciences in the United States. All mothers gave written informed consent.

The following exclusion criteria were applied for the present analysis: unavailable information on the outcome of interest (n=10, because they answered an earlier version of the first follow-up questionnaire that did not include the section on child's health status) and those whose first follow-up visit took place after 30 months of age (n=32), because few visits occurred after that age. Therefore, our analysis was based on 747 boys. When the follow-up began the median age of these boys was 12.3 months (quartiles 7.7 and 16.1 months). Scheduling of the follow-up visits was determined by logistic considerations. Thus, they were visited between 1 and 6 times during the follow-up period (~17 months), with a median of 2 visits (quartiles 2 and 4); the median gap between each visit was 2.8 months (quartiles 1.8 and 4.1 months). When children were last seen, their median age was 21.4 months (quartiles 19.1 and 25.3 months).

2.1 DDE and DDT determination

Concentrations of *p,p*'-DDE and *p,p*'-DDT were determined in maternal serum samples collected within a day of delivery; samples at the beginning of the pregnancy, which might be the critical window of *in utero* exposure, were not available. Nonetheless, an earlier study showed a high correlation of *p,p*'-DDE levels from the first trimester with those from the third trimester or shortly after birth (Longnecker et al. 1999). Serum levels of *p,p*'-DDE and *p,p*'-DDT were quantified after solid phase extraction, using gas chromatography with mass spectrometry detection (Saady and Poklis 1990; Smith 1991). The limit of detection (LOD) was 0.2 μg/L and the recovery was 97% for both analytes. The between-assay coefficient of variation was 7% for *p,p*'-DDE (at 10 μg/L) and 6% for *p,p*'-DDT (at 2.5 μg/L). All samples had levels of *p,p*'-DDE that were above the LOD; 18 observations had levels of *p,p*'-DDT

below the LOD, for these we used the measured values reported by the laboratory in the analyses. Hence, no imputation of values below LOD was done. Total serum lipid was calculated based on triglycerides, phospholipids, free and total cholesterol measured using standard enzymatic methods (Patterson et al. 1991). Concentrations of *p,p*'-DDE and *p,p*'-DDT were expressed as micrograms per gram of lipid (μg/g).

2.2 Episodes of diarrhea

Information on children's diarrhea episodes were reported by the mothers during in-person interviews conducted by specially trained personnel during home visits. Diarrhea episodes were defined as any diarrhea for which the children had to drink oral electrolytes as a rehydration therapy. On the first visit, mothers answered the following questions: *Since your baby was born, has the baby had diarrhea (liquid or semiliquid evacuations)? Did you have to give [him] any oral electrolytes to drink ("Vida Suero Oral or Pedialite")? How many times did [he] have an episode like this?* In order to differentiate each episode, mothers were told that the baby had to stay completely healthy for more than 3 days between episodes. For subsequent visits, the queries were the same, except that the first question began with "*Since our last visit*". Only the cases with positive answers to the first two questions were considered as diarrhea episodes in our analysis. Information about the date of the beginning and ending of each episode was not collected.

2.3 Covariates

Maternal socio-demographic and household characteristics, reproductive history, and lifestyle were ascertained at enrollment; and children's health status, growth, and feeding practices were ascertained during follow-up visits. Poverty status was defined based on monthly per capita income in Mexican pesos according to national standards (Palacios-Escobar and Martinez-Romero 2004). In urban areas, the poorest category included mothers with an income below 672 Mexican pesos (*i.e.*, those who struggled to buy adequate food) and the "less poor": those with an income below 1,367 pesos (*i.e.*, those who lacked adequate income for other human needs). In rural areas, the corresponding cut points were 495 pesos for the poorest and 946 for the less poor (Cupul-Uicab et al. 2008). Urban (places with 2500+ inhabitants) and rural (places with <2500 inhabitants) residence was determined according to the National Institute of Statistics and Geography (INEGI), which is the Mexican government agency in charge of the national system of statistical and geographical information (INEGI 2001). Mothers reported their smoking habits during pregnancy and after birth; however, because very few of them smoked during pregnancy (n=9) we only report maternal smoking ever. Child's underweight status during follow-up was defined based on the 2000 growth chart for boys from the Centers for Disease Control and Prevention (CDC) as weight-for-age *z*-scores ≤ −2.00 (Kuczmarski et al. 2000). Age at last breastfeeding of any type ("any breastfeeding", in months and days) was defined as the child's last age he received any breast milk, regardless of the consumption of liquids or solid foods. Age at last exclusive breastfeeding (in months and days) was defined as the child's last age he was breastfed only and no other liquid or solid food had yet been introduced to his diet.

2.4 Statistical analysis

The association of prenatal exposure to *p,p*'-DDE and *p,p*'-DDT with incidence of diarrhea was assessed using Poisson regression with robust standard errors to adjust for the clustering of observations within children (90.5% of them had 2+ visits). All models included an offset for person-time to account for the differences in span of time that each child was followed. The person-time in months, for each child was calculated from the date of birth to the first visit and, for children who had two or more visits, from the time since the previous visit. We used the same cut points applied in prior analyses of these data for *p,p*'-DDE (≤3.00 μg/g [53.6%], 3.01–6.00 μg/g [22.2%], 6.01–9.00 μg/g [9.1%], >9.00 μg/g [15.1%]) and *p,p*'-DDT (≤0.25 μg/g [48.5%], 0.26–0.75 μg/g [26.2%], 0.76–1.99 μg/g [13.9%], ≥2.00 μg/g [11.4%]) in our main analysis. As previously described, these categories resulted in a large exposure difference between those in the high and low categories while maintaining adequate numbers for analysis (Cupul-Uicab et al. 2008; Longnecker et al. 2007). The incidence rate ratio (IRR) of diarrhea was also estimated per interquartile range (IQR) increase in exposure (μg/g).

Potential confounders were selected based on directed acyclic graphs (DAGs) (Greenland et al. 1999); the minimal sufficient set of variables for adjustment included maternal age at delivery, parity, urban or rural residence, and poverty status. Other variables not selected by the DAG that were predictors (*p*-value <0.20, model's Wald X^2 test) of the outcome in models that also included the exposure variable were assessed as potential confounders with the change in estimate method (*i.e.*, change in IRR ≥10%), starting with all variables in the models and deletion of one by one in a stepwise manner (Greenland 1989). Variables tested with such strategy were maternal education, trimester of first prenatal care (never, first, after first), type of delivery (vaginal and C-section), marital status (married, not married with partner, and single), house connected to a sewer system, child's exact age at measurements, gestational age at birth (≤37, 37.1–39.9, and ≥40.0 weeks), and child's underweight status during follow-up. None of these variables caused a change ≥10% in the IRR.

We explored potential interactions of *p,p*'-DDE and *p,p*'-DDT (μg/g) with the adjustment variables (*i.e.*, maternal age at delivery [years], parity [1, 2+], residence [urban, rural], and poverty status [poorest, less poor, not poor]) to assess homogeneity of the effects; cross-term products for these variables were added to the fully adjusted models to test such interactions (*i.e.*, *p,p*'-DDE μg/g × maternal age in years). Diarrheal disease is known to be related to low socioeconomic status and rural residence (Boschi-Pinto et al. 2006; Johansson et al. 2012), and exposure to DDT was higher among boys from rural settings in the present study. Thus, *a* priori potential effect modification by socioeconomic status and rural residence were of particular interest. Additional interactions of *p,p*'-DDE and *p,p*'-DDT with variables that are considered potential intermediates such as breastfeeding (months), gestational age at birth (weeks) and child's underweight status (no, yes) at first follow-up were tested in a similar fashion in fully adjusted models. An interaction was considered for further analysis when the *p*-values of the cross-term products were ≤0.20; for the interaction with poverty index this *p*-value came from the overall Chi square test of the cross-term products. There was evidence of potential interactions of *p,p*'-DDE and *p,p*'-DDT with residence and poverty index (*p*-values <0.17). Further stratification showed heterogeneity for residence only; thus, we also

presented our results stratified by residence. Linearity of *p,p*'-DDE and *p,p*'-DDT were verified in unadjusted and adjusted models. For all models, a 1 df (degrees of freedom) trend test was conducted by introducing ordinal variables of *p,p*'-DDE or *p,p*'-DDT coded with the median levels of each category.

In sensitivity analyses, because the number of follow-up visits varied among children, we assessed whether the number of follow-up visits predicted the outcome and whether there was a potential interaction of this variable with the exposures. Potential effect modification of *p,p*'-DDE and *p,p*'-DDT exposure by type of delivery (vaginal and C-section) was also assessed in sensitivity analyses, as children born by C-section might be at a higher risk of developing immune alterations as compared to those delivered vaginally (Neu and Rushing 2011). To test the robustness of our stratified results, the final models were additionally adjusted for a number of variables that showed statistically significant differences by residence (see below). All analyses were conducted using Stata (release 14.2; StataCorp, College Station, TX, USA).

3. RESULTS

Mothers in the present study were young (median age, 23.7 years), 90.6% were married or had a partner, 80.5% never smoked in their life, 89.7% were poor or very poor; 66.7% attended less than high school; 58.9% were multiparous and 60% were from urban dwellings; a 33.5% did not have prenatal care, and 27.0% lived in a house with a dirt floor. The median duration of breastfeeding was long (11 months), while for exclusive breastfeeding it was short (0.10 months) (Table 1). These mothers have higher levels of *p,p*'-DDE (median, 2.7 μg/g lipid) and *p,p*'-DDT (median, 0.27 μg/g lipid) than those reported for U.S. women in the 2003–2004 National Health and Nutrition Examination Survey (medians: *p,p*'-DDE, 0.21 μg/g lipid; *p,p*'-DDT, <0.0078 mg/g lipid) (CDC 2009); median of *p,p*'-DDE was approximately 13 times higher in the present study. Concentrations of *p,p*'-DDE and *p,p*'-DDT were higher among mothers who never smoked, from rural areas, and those who lived in a house with a dirt floor and without a sewer system; *p,p*'-DDE exposure increased with age and decreased with parity (Supplement Table 1).

Compared to mothers from urban areas, those from rural settings were slightly younger (median age: 23.2 vs. 24 years), a lower percentage had a high school education (22.8% vs. 40.4%) and smoked ever (14.0% vs. 23.2%); a higher percentage were enrolled at the Ministry' of Health hospital (which serves people without insurance) (60.5% vs. 44.2%), live in a house with no connection to a sewer system or septic tank (16.1% vs. 4.0%) and with flooring made of dirt (41.8% vs. 17.2%)). Likewise, compared to urban settings, children from rural areas were more likely to be underweight at the first follow-up visit (17.4% vs. 12.3%), to have regular well care visits (61.5% vs. 46.2%), and begin drinking milk other than breast milk after four months of age (48.8% vs. 36.6%), were less likely to drink bottled water (6.0% vs. 15.4%), and were breastfeed longer (12 months vs. 9.0 months) (Table 1).

Most of the children (82.2%) had at least one episode of diarrhea during the follow-up period, and the median number of episodes was 3 (quartiles 2 and 5). A slightly higher

percentage of children from rural areas had at least one episode of diarrhea (83.3%) compared to those from urban areas (81.5%). Overall, the incidence rate (IR) of diarrhea was 1.7 per child-year; the IR was slightly higher in children from rural (1.81 per child-year) compared to those from urban (1.62 per child-year) settings (unadjusted incidence rate difference, $p<0.01$) (Supplement Table 2).

Adjustment for potentially confounding factors had little effect on the results (Table 2). Children in the highest category (>9.00 μg/g) of *p,p*'-DDE compared to the lowest (≤3.00 μg/g) had a slightly higher IR of diarrhea (adjusted IRR [aIRR] = 1.14; 95% CI: 0.94, 1.39). Adjusted results after stratifying on urban-rural residence are shown in Table 3. Among children from urban settings, increasing levels of *p,p*'-DDE were associated with a higher IR of diarrhea (*p*-trend = 0.01), and we observed an aIRR of 1.39 (95% CI: 1.07, 1.80) for those in the highest category of *p,p*'-DDE compared with the lowest. The adjusted results for *p,p*'-DDT indicated a slightly weaker association (*p*-trend = 0.13), and the aIRR was 1.32 (95% CI: 0.95, 1.84) for children in the highest category (≥2.00 μg/g) relative to the lowest (≤0.25 μg/g). Higher levels of *p,p*'-DDE and *p,p*'-DDT were not associated with a higher IR of diarrhea among children from rural settings (Table 3). Our results were similar when we estimated the aIRR per IQR increase in *p,p*'-DDE levels and *p,p*'-DDT instead of using categories of exposure (data not shown).

Results from our sensitivity analyses showed that number of follow-up visits was a statistically significant predictor of the outcome in the overall models of *p,p*'-DDE (aIRR=1.05; 95% CI: 1.00, 1.10) and *p,p*'-DDT (aIRR=1.05; 95% CI: 1.01, 1.10). However, there was no evidence of interaction between *p,p*'-DDE or *p,p*'-DDT with number of visits (*p*-interaction ≥0.48). No evidence of effect modification by type of delivery (*p-interactions*: *p,p*'-DDE = 0.60 and *p,p*'-DDT = 0.93) was observed (data not shown). Results from tables 2 and 3 remained unchanged with additional adjustment for number of visits. Our results from table 3 did not materially change after additional adjustment for variables that showed statistically significant differences by residence (from Table 1, data not shown).

4. DISCUSSION

In this cohort of boys from a highly exposed area of Mexico, those in the highest category of prenatal exposure to *p,p*'-DDE had a slightly higher incidence rate of diarrhea as compared to the lowest category. We identified an interaction between prenatal exposure to *p,p*'-DDE and maternal urban or rural residence. Among boys from urban settings, increasing levels of prenatal exposure to *p,p*'-DDE were associated with a higher incidence rate of diarrhea; similarly, those in the highest exposure category of *p,p*'-DDT had the highest incidence rate of diarrhea. Among those from rural settings no association was apparent.

The association of prenatal exposure to *p,p*'-DDE with a higher incidence of diarrhea limited to boys from urban settings was unexpected and there was no simple, straightforward explanation. We expected a stronger association among boys from rural areas given that they were the most exposed and had less favorable living conditions as shown in Table 1. However, boys from rural areas had a median duration of any breastfeeding that was 3 months longer than those from urban areas. Breastfeeding decreases the risk of

gastrointestinal infections up to 40% in the first year of life (Kramer et al. 2001; Oddy 2001). Selective colonization of the gut microbiota due to breastfeeding seems to aid the development of the infant's immune system (Abrahamsson et al. 2015; Backhed et al. 2015; Ottman et al. 2012). But breastfeeding duration did not modify the association with DDE in the present study.

Exposure to other organochlorines (*i.e.*, dioxins, polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and aldrin) linked to adverse effects of the immune system in animal studies (WHO 2010) that were not measured in the present study, could potentially explain our findings if such exposures differed between urban and rural settings. Nevertheless, a recent study among children (6–12 years old) living in urban or rural settings around eight states of Mexico, reported non-detectable levels of PCBs and aldrin; moreover, children from rural Chiapas had detectable levels of HCB and higher levels of lindane compared to those from an urban area in a neighboring state, Veracruz (Trejo-Acevedo et al. 2009). Pyrethroids and carbamates had been used in the region for malaria control (DOF 2003, 2011; Ordóñez González et al. 2008) and agriculture (Ortíz et al. 2014); at least one study reported detectable levels of 3-phenoxibenzoic acid among children from a malarious community of Oaxaca (a state contiguous to Chiapas) two days after their household was sprayed with deltametrin (Yanez et al. 2002). Differential exposure to toxic compounds other than DDT by urban and rural settings cannot be ruled out with our data, this could possibly account for our null results among rural boys.

On the other hand, children living in urban settings tend to have higher rates of sensitization and allergies (Stemeseder et al. 2017), they also have a less diverse skin and gut microbiota than those living in rural settings (Lehtimaki et al. 2017; Mah et al. 2008). Living conditions, such as farms, that allow early contact with diverse microbial agents from the natural environment are thought to help the developing immune system of children (Ege et al. 2006; Rook and Brunet 2005; Rook et al. 2014).

Our results were consistent with the two earlier studies that reported an association between prenatal exposure to *p,p*'-DDE with a higher incidence rate of gastrointestinal infections. Among Inuit children from Canada (Dallaire et al. 2004), with the lowest quartile of *p,p*'-DDE as the reference, their aIRR was 1.35 (95% CI: 0.54, 3.42) for the highest quartile at six months of age, the corresponding aIRR at 12 months was 1.43 (95% CI: 0.87, 2.34). The lack of statistical significance was perhaps due to the small sample size (n=177) in tha:t study. The present analysis included a bigger sample size and the level of prenatal exposure was much higher; median levels of *p,p*'-DDE were higher (2.7 μg/g lipids) among mothers in Tapachula than the maximum value (2.27 μg/g lipids) reported among the Inuit mothers.

Similar to our findings, a higher frequency of gastroenteritis between 6 and 12 months of age with increasing levels of *p,p*'-DDE in breastmilk was reported in a birth cohort from North Carolina (only unadjusted descriptive results were presented) (Rogan et al. 1987). The percentage of children with gastroenteritis according to categories of *p,p*'-DDE (ppm milk fat) were: 13% (0–2 ppm), 9% (2–3 ppm), 18% (3–5 ppm), 20% (5–8 ppm) and 2%4 (8+ ppm). The odds of having gastroenteritis among children in highest category of *p,p*'-DDE

(8+ ppm) was 2.11 (95% CI: 0.81, 5.52) compared to the lowest (calculated from the raw data).

Unlike the two earlier studies, we did not have a broad definition of gastrointestinal infections as the outcome; our definition was limited to episodes of diarrhea that required the use of oral electrolytes as reported by the mother. Differential reports of the outcome by the mothers was unlikely because mothers and interviewers were blinded regarding the levels of DDT exposure, though random errors in the classification of the outcome cannot be ruled out, in which case the IRR could have been underestimated.

Studies based on animals and humans have reported mixed evidence regarding potential immunosuppressive effects of developmental exposure to *p,p*'-DDT and its metabolites (ATSDR 2002; Eskenazi et al. 2009; Rogan and Chen 2005). For example, postnatal exposure to *p,p*'-DDE (*i.e.*, child's serum or breastmilk levels) was associated with decreased specific BCG (*mycobacterium bovis* bacille Calmette–Guérin) vaccine antibody levels in infants (Jusko et al. 2016), but increased white blood cells (WBC), IgG, IgA, and IgE among school age children (Karmaus et al. 2005). Exposure to *p,p*'-DDE during adulthood was also associated with increased total WBC, lymphocytes, IgA in one study (Vine et al. 2001), and decreased IgG levels in another (Cooper et al. 2004). Although DDT-related immunologic alterations are not consistent across studies, such an effect could lead to important long lasting health effects. For example, immunoglobulins have the ability to neutralize pathogens and newborn babies can produce them after exposure to antigens; IgA plays an important role in protecting mucosal surfaces such as the intestine (Holt and Jones 2000; Ygberg and Nilsson 2012). Thus, it is possible that fetal exposure to chemicals during critical periods could adversely affect the development of the innate or adaptive immune system, perhaps altering its structure or function, potentially increasing the risk of infectious diseases later in life.

In the present study we estimated 1.7 episodes of diarrhea per child-year, which is slightly lower compared to the incidence of diarrhea for children under five from low and middle income countries (3.2 episodes per child-year), in the Americas (3.2 to 5.25 episodes per child-year) and the African regions (6.2 to 10.4 episodes per child-year), as well as from children 12 months and younger from Mexico (2.9 episodes per child-year) (Fischer Walker et al. 2012; Kosek et al. 2003). Our observed incidence was similar to that observed in the developed world (1 to 2 episodes per child-year) (Thapar and Sanderson 2004). This underestimation of incidence rate of diarrhea could be explained by our definition (required use of electrolytes). But it could also be explained in part by the long duration of lactation in our study population (median, 11 months); the median duration of lactation in Mexico is 9 months (Gonzalez-Cossio et al. 2003). Breastfeeding protects against life-threatening dehydration due to diarrhea and is known to support children's hydrating status throughout diarrheal episodes (Huffman and Combest 1990; Huttly et al. 1997). Another limitation of our study is that we did not collect enough information to identify the cause of each diarrhea episode.

As mentioned earlier, children delivered by C-section might develop immune alterations (Neu and Rushing 2011); a slightly higher risk of intestinal bacterial infections before 5

years of age was reported in a large birth cohort (Bager et al. 2010). Nonetheless, in the present study type of delivery was not selected as a confounder with the change in estimate method previously described and did not modify the association of *p,p*'-DDE and *p,p*'-DDT exposure with the incidence of diarrhea.

As noted earlier, the present cohort included only healthy newborn males as the initial study hypothesis was related to the potential androgen-blocking effects of DDT (Longnecker et al. 2007). The effect of excluding females on our estimated IRR is unknown, however, in the previous studies no differential effects of prenatal exposure to *p,p*'-DDE with GI infections based on sex were reported (Dallaire et al. 2004; Rogan et al. 1987).

5. CONCLUSION

Our results based on a highly exposed population of boys from Mexico support the earlier findings of a higher risk of GI infections with prenatal exposure to *p,p*'-DDE. Overall, boys with high prenatal exposure to *p,p*'-DDE had a slightly higher incidence rate of diarrhea. Among boys from urban settings, increasing levels of prenatal exposure to *p,p*'-DDE were more clearly associated with a higher incidence rate of diarrhea. While urban boys in this study appeared to be more susceptible to DDE-associated diarrhea than rural boys, a ready explanation for such increased susceptibility was not apparent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

***p,p'*-DDE**	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene
***p,p'*-DDT**	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
IQR	interquartile range
IRR	incidence rate ratio

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Highlights

- A higher risk of GI infections with DDE exposure was consistent with previous studies
- Prenatal exposure to DDE increased the rate of diarrhea in boys from urban settings
- Among boys from rural settings no association was apparent
- A ready explanation for such increased susceptibility was not apparent

Table 1

Characteristics of the mothers and their sons at enrollment or during follow-up. Tapachula, Chiapas, Mexico 2002–2005

Characteristics	n	Overall	Urban residence	Rural residence	
		Median (IQR) or % n = 747	Median (IQR) or % n = 448	Median (IQR) or % n = 299	*p-value*[*]
Mothers					
Age	747	23.7 (7.2)	24.0 (7.0)	23.2 (7.2)	0.05
Education (year)					<0.01
0 – 6	262	35.1	27.9	45.8	
7 – 9	236	31.6	31.7	31.4	
10 – 12	173	23.2	26.6	18.1	
> 12	76	10.2	13.8	4.7	
Parity					0.39
1	307	41.1	40.6	41.8	
2	261	34.9	33.7	36.8	
3+	179	24.0	25.7	21.4	
Trimester of first prenatal visit					0.82
Never	250	33.5	32.6	34.8	
1st	330	44.2	44.9	43.1	
After first	167	22.4	22.5	22.1	
Type of delivery					0.98
Caesarean	277	37.1	37.1	37.1	
Vaginal	470	62.9	62.9	62.9	
Marital status					0.75
Married	357	47.8	46.7	49.5	
Not married with partner	320	42.8	43.8	41.5	
Single	70	9.4	9.6	9.0	
Hospital of recruitment					<0.01
Social Security	368	49.3	55.8	39.5	
Ministry of Health	379	50.7	44.2	60.5	

Characteristics	n	Overall Median (IQR) or % n = 747	Urban residence Median (IQR) or % n = 448	Rural residence Median (IQR) or % n = 299	*p-value**
Mom smoked ever					<0.01
Yes	146	19.5	23.2	14.0	
No	601	80.5	76.8	86.0	
Poverty index					0.34
Poorest	531	71.1	69.4	73.6	
Less poor	139	18.6	20.3	16.1	
Not poor	77	10.3	10.3	10.4	
Residence area					
Urban	448	60.0	100.0	0.0	
Rural	299	40.0	0.0	100.0	
Type of sanitation system					<0.01
Sewer system	555	74.3	88.6	52.8	
Septic tank	126	16.9	7.4	31.1	
Latrine	44	5.9	2.9	10.4	
Other	22	2.9	1.1	5.7	
House's type of floor					<0.01
Dirt/Soil	202	27.0	17.2	41.8	
Concrete	487	65.2	73.0	53.5	
Mosaic/other	58	7.8	9.8	4.7	
Number of inhabitants in the household	747	5.0 (2.0)	5.0 (2.0)	5.0 (2.0)	0.99
p,p'-DDT (μg/g)	747	0.27 (0.67)	0.19 (0.29)	0.66 (1.48)	<0.01
p,p'-DDE (μg/g)	747	2.70 (4.50)	2.21 (2.90)	4.27 (6.95)	<0.01
Sons					
Birth season					0.63
Winter	142	19.0	17.9	20.7	
Spring	212	28.4	27.7	29.4	
Summer	216	28.9	30.1	27.1	
Autumn	177	23.7	24.3	22.7	
Gestational age at birth (wk)					0.45

Characteristics	n	Overall Median (IQR) or % n = 747	Urban residence Median (IQR) or % n = 448	Rural residence Median (IQR) or % n = 299	*p-value**
≤ 37	25	3.3	2.9	4.0	
37.1 – 39.9	277	37.1	38.6	34.8	
≥ 40	445	59.6	58.5	61.2	
					0.05
WfA < −2.00 SD					
No	640	85.7	87.7	82.6	
Yes	107	14.3	12.3	17.4	
					<0.01
Child attended well care					
No	356	47.7	53.8	38.5	
Yes	391	52.3	46.2	61.5	
					<0.01
Type of drinking water					
Bottle	87	11.6	15.4	6.0	
Boiled	647	86.6	83.3	91.6	
Raw	13	1.7	1.3	2.3	
					<0.01
Child's age at introduction of other milk (mo)					
≤ 1	255	34.1	35.5	32.1	
1.1 – 4.0	182	24.4	27.9	19.1	
> 4.0	310	41.5	36.6	48.8	
					1.00
Ever exclusively breastfed					
No	120	16.1	16.1	16.1	
Yes	627	83.9	83.9	83.9	
Any breastfeeding (mo)	747	11.0 (12.9)	9.0 (12.0)	12.0 (12.0)	<0.01
Exclusive breastfeeding (mo)	747	0.10 (0.97)	0.13 (0.97)	0.10 (0.97)	0.15

IQR, interqartile range; WfA, weight-for-age

* *P-values* from Pearson's Chi2 and Fisher's exact test comparing participants from urban versus rural areas

Table 2

IRR of diarrhea according to prenatal exposure to *p,p'*-DDE and *p,p'*-DDT among 747 boys from Tapachula, Chiapas, Mexico 2002–2005

Organochlorine (μg/g)	Unadjusted IRR	Adjusted[a] IRR
p,p'-DDE		
≤ 3.00	1.00	1.00
3.01 – 6.00	1.07 (0.89, 1.28)	1.08 (0.90, 1.30)
6.01 – 9.00	1.05 (0.82, 1.35)	1.04 (0.80, 1.35)
> 9.00	1.16 (0.96, 1.40)	1.14 (0.94, 1.39)
P-trend[*]	*0.13*	*0.23*
p,p'-DDT		
≤ 0.25	1.00	1.00
0.26 – 0.75	1.01 (0.85, 1.20)	1.01 (0.85, 1.20)
0.76 – 1.99	0.94 (0.78, 1.13)	0.87 (0.71, 1.06)
≥ 2.00	1.08 (0.86, 1.35)	0.99 (0.78, 1.25)
P-trend[*]	*0.59*	*0.85*

IRR, incidence rate ratio

[a] Adjusted for mother's age at delivery, parity, area of residence, and poverty index

[*] Trend tests from models ran with *p,p'*-DDE or *p,p'*-DDT as ordinal variables coded with median levels of each category

Table 3

Adjusted[a] IRR of diarrhea according to prenatal exposure to *p,p'*-DDE and *p,p'*-DDT among boys from Tapachula, Chiapas, Mexico 2002–2005. Stratified on residence area

	Urban (n=448)	Rural (n=299)	
Organochlorine (μg/g)	**IRR**	**IRR**	***p-interaction***
p,p'-DDE			0.04
≤ 3.00	1.00	1.00	
3.01 – 6.00	1.12 (0.86, 1.46)	1.01 (0.77, 1.31)	
6.01 – 9.00	1.26 (0.83, 1.91)	0.85 (0.62, 1.16)	
> 9.00	1.39 (1.07, 1.80)	0.97 (0.74, 1.26)	
P-trend[*]	*0.01*	*0.73*	
p,p'-DDT			0.08
≤ 0.25	1.00	1.00	
0.26 – 0.75	0.99 (0.78, 1.25)	1.01 (0.78, 1.33)	
0.76 – 1.99	0.95 (0.72, 1.26)	0.81 (0.62, 1.06)	
≥ 2.00	1.32 (0.95, 1.84)	0.85 (0.63, 1.16)	
P-trend[*]	*0.13*	*0.27*	

IRR, incidence rate ratio

[a]Adjusted for mother's age at delivery, parity, and poverty index

[*]Trend tests from models ran with *p,p'*-DDE or *p,p'*-DDT as ordinal variables coded with median levels of each category