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Association of prenatal pesticide exposures with adverse pregnancy outcomes and stunting in rural Bangladesh

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Abstract

Background: Pesticide exposure during pregnancy is thought to adversely affect fetal growth, which in turn may impact child growth, but results have been inconsistent across studies and few have explored these effects in developing countries.

Objectives: To quantify urinary concentrations of pesticide biomarkers in early pregnancy (<16 weeks' gestation), and to estimate the association of these concentrations with preterm birth, low birth weight, small for gestational age, and stunting at ~1 and 2 years of age.

Methods: Eight pesticide biomarkers were quantified in urine collected from 289 pregnant women (aged 18 to 40 years) participating in a birth cohort study in Bangladesh. Anthropometry measurements were conducted on the index child at birth and approximately 1 and 2 years of age. A directed acyclic graph was used to identify minimal sufficient adjustment sets. Log-binomial regression was used to estimate the relative risk (RR) with 95% confidence intervals (CI).

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Declaration of interests

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Results: 3,5,6-trichloro-2-pyridinol (TCPY), a metabolite of chlorpyrifos and chlorpyrifos methyl, and 4-nitrophenol, a metabolite of parathion and methyl parathion, were detected in nearly all women with geometric mean (95% CI) values of 3.17 (2.82–3.56) and 18.66 (17.03–20.46) μg/g creatinine, respectively. 3-phenoxybenzoic acid (3-PBA), a non-specific metabolite of several pyrethroids, and 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY), a diazinon metabolite, were detected in 19.8% and 16.1% of women, respectively. The remaining four pesticide biomarkers were detected in <10% of women. Women in the highest quartile of 4-nitrophenol were more than 3 times more likely to deliver preterm than women in the lowest quartile: unadjusted RR (95% CI), 3.57 (1.65, 7.73). Women in the highest quartile of 4-nitrophenol were also at increased risk of having a child born small for gestational age: RR (95% CI) adjusted for household income, maternal education, and maternal total energy and meat intake, 3.81 (1.10, 13.21). Women with detectable concentrations of IMPY were at increased risk of having a child born with low birth weight compared to women with non-detectable concentrations: adjusted RR (95% CI), 2.13 (1.12, 4.08). We observed no association between any of the pesticide biomarkers and stunting at 1 or 2 years of age.

Discussion: Exposure to the insecticides parathion and diazinon during early pregnancy may increase the risk of adverse birth outcomes.

Keywords

Biomonitoring; Children's health; Birth outcomes; Herbicides; Organophosphate pesticides

INTRODUCTION

Two-thirds of adults living in low-income countries are employed in the agricultural sector compared to less than 2% in the United States (International Labour Organization 2017). The unsafe handling, storage, and disposal of pesticides is widespread in low-resource settings where illiteracy rates are high, yet few research studies have evaluated the potential health implications of these practices. Exposure to pesticides has been linked to adverse pregnancy outcomes and impaired child growth in several epidemiological studies (Berkowitz et al. 2003; Berkowitz et al. 2004; Chevrier et al. 2011; Kartini et al. 2019; Levario-Carrillo et al. 2004; Naksen et al. 2015; Paudel et al. 2012; Perera et al. 2003; Whyatt et al. 2004; Wohlfahrt-Veje et al. 2011; Xiang et al. 2000), though not all (Eskenazi et al. 2004).

Experimental studies in laboratory animals have also demonstrated adverse effects of exposure to pesticides, particularly organophosphate insecticides, on birth weight and postnatal growth (Chanda and Pope 1996; Muto et al. 1992; Spyker and Avery 1977). Potential mechanisms include an influence on placental nutrient transport (Spyker and Avery 1977) and altered activity of the adenylyl cyclase signaling cascade resulting in disrupted cell development (Song et al. 1997). However, not all studies have observed such effects: one study of rats fed dimethoate or methyl parathion (both organophosphate insecticides) found no impact on birth weight (Institoris et al. 1995) and another study of mice fed dimethoate observed no impact on birth weight but significantly lower growth rates (Budreau and Singh 1973).

The incidence of low birth weight in Bangladesh is among the highest in the world (Arifeen et al. 2000), and 41% of children under 5 years old are stunted (e.g. low length/height-for-age) (Sarma et al. 2017). Intrauterine growth restriction is the leading risk factor for low birth weight and stunting in developing countries (Danaei et al. 2016), thus, innovative interventions and programs targeting in utero exposures are needed to address this significant global disease burden.

To our knowledge, no study has explored the association of exposure to pesticides in utero and child growth in Bangladesh. We conducted a study using stored samples from a prospective birth cohort in rural Bangladesh in order to: (1) quantify urinary concentrations of select pesticide biomarkers in early pregnancy (<16 weeks' gestation) and (2) estimate the association of these pesticide biomarkers with preterm birth, low birth weight, small for gestational age, and stunting at ~1 and 2 years of age.

METHODS

Sample population

Stored maternal urine samples came from a prospective birth cohort study conducted in rural Bangladesh (Kile et al. 2014). The aim of the parent study was to evaluate the effects of chronic low-level arsenic exposure on reproductive health outcomes, thus, the two study areas were selected based on British Geological Survey data that indicated the groundwater was moderately contaminated with arsenic (Kinniburgh and Smedley 2001). Between 2008 and 2011, 1613 pregnant women were enrolled in the cohort. Inclusion criteria were as follows: ≥18 years of age, singleton pregnancy <16 weeks' gestation confirmed by ultrasound, used a well as their primary drinking water source, planned to live at their current residence for the duration of pregnancy, and planned to deliver at a local rural health clinic or at home with a trained midwife. Between 2010 and 2013, children born to mothers in the original study were invited to participate in follow-up studies at approximately 1 and 2 years of age. Stored baseline (<16 weeks' gestation) maternal urine samples were selected for this sub-study on pesticide biomarkers if they met the following inclusion criteria: sufficient volume (at least 1.5 mL) and non-missing data on urinary creatinine, birth outcomes, maternal age, infant sex, maternal education, and household drinking water source. A total of 289 mother-child pairs met these criteria and were included in this study. Compared to the 1613 mother-child pairs enrolled, these 289 mother-child pairs were more likely to have a household income >4,000 tk ($p=0.01$), less likely to have a husband engaged in agricultural work ($p=0.003$), and had lower concentrations of arsenic in their drinking water ($p<0.0001$) (Supplemental Table 1). We did not observe any significant differences between this sub-sample and the larger study sample with respect to maternal age, gravidity, maternal education, maternal body mass index (BMI) at enrollment, or secondhand smoke exposure.

The study was approved by the Human Research Committees at the Harvard T.H. Chan School of Public Health (protocol number IRB17-1036) and Dhaka Community Hospital, and informed consent was obtained from all participants. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects' research.

Exposures

A summary of biomarker assessments including the laboratory that conducted the measurements and years as well as the specific method used is provided in Supplemental Table 2. Baseline maternal urine samples were collected by trained healthcare workers in a clinical setting and immediately stored at −20°C. Frozen urine samples were shipped on dry ice to Taipei Medical University in Taiwan and stored at −80°C. Creatinine was measured by Taipei Medical University using a colorimetric assay on a Roche Modular P800 instrument (Roche Inc., Mannheim, Germany) between 2009 and 2012.

Remaining frozen urine samples were shipped on dry ice from Taipei Medical University to the Harvard T.H. Chan School of Public Health where samples were stored at −80°C. Frozen samples were pulled from storage and shipped to the CDC on dry ice in two batches (December 2017 and August 2018) for analysis of urinary pesticide biomarkers. The CDC used standard methodologies described in Supplementary Materials, pp. 3-6, and previously published (Davis et al. 2013). We chose to measure specific pesticide biomarkers instead of dialkyl phosphates (DAPs) given concerns regarding the degradation of DAPs relative to specific pesticide biomarkers under field conditions common in many developing countries (Wylie et al. 2017). Specific pesticide biomarkers, on the other hand, appear to be more stable over time and temperature gradients (Hoppin et al. 2006; Wylie et al. 2017). The eight pesticide biomarkers included: the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D); four organophosphate insecticide metabolites, 3,5,6-trichloro-2-pyridinol (TCPY, a metabolite of chlorpyrifos and chlorpyrifos methyl), 4-nitrophenol (a metabolite of parathion and methyl parathion), malathion dicarboxylic acid (MDA, a malathion metabolite), and 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY, a diazinon metabolite); and three metabolites of pyrethroid insecticides, 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA, a metabolite of cyfluthrin and flumethrin), 3-phenoxybenzoic acid (3-PBA, a non-specific metabolite of several pyrethroids including cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, phenothrin permethrin, tralomethrin, and esfenvalerate), and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (trans-DCCA, a metabolite of permethrin, cypermethrin, and cyfluthrin) (Davis et al. 2013). The limit of detection (LOD) was 0.3 μg/L for 2,4-D, 0.1 μg/L for TCPY, 1.0 μg/L for MDA, 0.6 μg/L for trans-DCCA, and 0.2 μg/L for all other biomarkers. Observations with a concentration below the LOD were assigned a value equal to LOD divided by the square root of two (Hornung and Reed 1990).

We used creatinine-adjusted urinary concentrations (μg/g creatinine) for all analyses. Pesticide biomarkers detected in fewer than 10% of samples were excluded from subsequent analyses. Concentrations of pesticide biomarkers detected in 10-60% of samples were treated as binary variables (detectable versus non-detectable). Concentrations of pesticide biomarkers detected in at least 60% of samples were categorized into quartiles according to the distribution of creatinine-adjusted urinary concentrations. We also calculated geometric means (95% confidence intervals [CI]) for pesticides biomarkers detected in at least 60% of samples.

Outcomes

Gestational age was determined by ultrasound at the time of enrollment. Birth weight was measured to the nearest 10 g on a calibrated pediatric scale. Infant length and weight were measured using standardized procedures by trained study staff (Cheikh Ismail et al. 2013a). Preterm birth was defined as delivery at <37 weeks' gestation. Low birth weight was defined as birth weight <2500 g. Small for gestational age was defined as weight-for-gestational age percentile <10 based on the INTERGROWTH-21st standards (Cheikh Ismail et al. 2013b). Stunting was defined as length-for-age z-score of more than 2 SD below the median using the WHO growth references (de Onis 2006). These standards aim to provide a single international standard of physiological growth for all children from birth to 5 years of age.

Covariates

Women completed three clinical visits during the study: baseline (<16 weeks' gestation), approximately 28 weeks' gestation, and ≤1 month post-delivery. At the baseline clinical visit, a whole blood sample was collected and serum was isolated by centrifugation and stored at −20°C immediately after processing. Serum samples were shipped on dry ice to the Harvard T.H. School of Public Health where samples underwent one freeze-thaw cycle in order to create aliquots prior to storage at −80°C. Frozen serum samples were transported on dry ice to the University of Massachusetts Amherst where they were stored at −80 °C until biomarker assessment. Maternal hemoglobin was measured using the sodium lauryl sulphate (SLS) method on a Sysmex XS-800i instrument (Sysmex Europe GmbH, Norderstedt, Germany) between 2010 and 2012. The SLS method has previously been shown to have excellent correlation with the cyanmethemoglobin method ($r^2 = 0.996$) (Karsan et al. 1993). The justification for including maternal hemoglobin was that it is an important indicator of maternal nutritional status and low hemoglobin levels in the first trimester of pregnancy have been associated with increased risk of preterm birth, low birth weight, and small for gestational age (Jung et al. 2019).

After their first clinical visit, during which weight and height were measured, trained health care providers visited participants in their homes once per month to weigh participants using standardized protocols; these weights were used to calculate total gestational weight gain. Enrollment BMI was calculated as measured weight divided by height-squared, and categorized according to the World Health Organization guidelines as underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5 to <25.0 kg/m^2), or overweight (BMI ≥25.0 kg/m^2) (World Health Organization 2003).

During the second clinical visit at approximately 28 weeks' gestation, a validated semiquantitative food frequency questionnaire was administered (Lin et al. 2017). In addition, total arsenic was measured in nitric acid-preserved maternal drinking water samples using inductively coupled plasma mass spectrometry by Environmental Laboratory Services, North Syracuse, NY, USA (Kile et al. 2014).

Statistical analysis

Summary statistics were used to summarize the socio-demographic and clinical characteristics of women at baseline, and birth outcomes and infant growth. Characteristics

included maternal age, gravidity, maternal education, monthly household income, whether or not the husband was engaged in agricultural work, maternal enrollment BMI status, self-reported secondhand smoke exposure, maternal hemoglobin level, total gestational weight gain, and infant sex. No participants were missing data for most of these characteristics except for one participant missing data for secondhand smoke exposure and 43 participants missing data for maternal dietary intake (Supplemental Table 3). Bivariate associations between these characteristics and the pesticide biomarkers were evaluated using non-parametric Kruskal-Wallis tests for continuous characteristics and Chi-square tests for categorical characteristics. The assumption of cell sizes >5 for all Chi-square tests was met. Shapiro-Wilk tests (Shapiro and Wilk 1965) and visual inspections of histograms were used to evaluate normality of continuous variables.

We estimated relative risks for the four binary outcomes (low birth weight, preterm birth, stunting at follow-up 1, and stunting at follow-up 2) using log-binomial regression (Spiegelman and Hertzmark 2005). Effects were adjusted for a minimal sufficient adjustment set of confounders, identified using a directed acyclic graph [DAG] (Supplemental Figure 1) and DAGitty software v2.3 (Textor et al. 2011; VanderWeele et al. 2008). Based on this DAG, no adjustment was necessary to estimate the total effect of maternal pesticide exposure on preterm birth (gestational age). For low birth weight, small for gestational age, and stunting, the minimal sufficient adjustment set included household income, maternal education, maternal dietary intake, and infection. We did not have information on infection and therefore the final adjustment set for these outcomes included household income (≤4,000 tk versus >4,000 tk), maternal education (no formal schooling or primary school versus secondary school and higher), maternal total energy intake (log-transformed kcal/day), and maternal meat intake (log-transformed g/day). Maternal total energy intake and meat intake, which were non-normally distributed, were log-transformed in all models.

We conducted several sensitivity analyses including: (1) specifying the pesticides biomarkers detected in at least 60% of samples continuously in models as (a) μg/g creatinine and (b) μg/L; and (2) specifying the outcomes as continuous (gestational age at birth, birth weight, weight-for-gestational age z-score, length-for-age z-score at 1 year of age, and length-for-age z-score at 2 years of age) using linear regression. All statistical analyses were performed using SAS software v9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

The mean ±SD gestational age was 11.6 ±2.9 weeks at the time of urine collection for pesticide analysis. Women were, on average, 23.1 years old (ranging from 18 to 40 years), and all women were married and reported “housewife” as their occupation. Only 22.8% of women reported that their husband was engaged in agricultural work as his primary occupation (Table 1). There were 66 cases of preterm birth (22.8%), 39 cases of low birth weight (13.5%), 35 cases of small for gestational age (12.1%), 197 cases of stunting at 1 year of age (68.2%), and 141 cases of stunting at 2 years of age (48.8%). Babies born preterm were more likely to be low birth weight ($p<0.0001$, Supplemental Table 4), and babies born with a low birth weight were more likely to be small for gestational age

(p<0.0001). Infants that were stunted at 1 year of age were more likely to be stunted at 2 years of age (p=0.05). We did not observe a significant association between preterm birth, low birth weight, or small for gestational age and stunting at either 1 or 2 years of age (all p>0.05).

TCPY and 4-nitrophenol were detected in nearly all women (97.9% and 100%, respectively) with GM (95% CI) values of 3.17 (2.82–3.56) μg/g creatinine and 18.66 (17.03–20.46) μg/g creatinine, respectively (Table 2). 3-PBA and IMPY were detected in 19.8% and 16.1% of women, whereas 2,4-D, MDA, and trans-DCCA were detected in less than 10% of women. We did not detect 4-F-3-PBA in any women. With respect to relationships between pesticide biomarkers (Supplemental Table 5), women in quartiles 3 and 4 of 4-nitrophenol were more likely to have detectable levels of IMPY (p=0.01) and 3-PBA (p=0.02) than women in quartiles 1 and 2 of 4-nitrophenol. Women with detectable concentrations of IMPY were more likely to have detectable concentrations of 3-PBA (p=0.005). Women in quartiles 2, 3 and 4 of TCPY were more likely to have detectable concentrations of 3-PBA than women in quartile 1 of TCPY (p=0.007).

Compared to women in the lowest quartile of TCPY, women in the highest quartile were younger (p=0.02), more likely to have a household income >4,000 tk (p=0.004), had lower fruit intakes (p=0.0002), and tended to have higher dal intakes (p=0.08) (Supplemental Table 6). Women in quartile 3 of TCPY tended to be more likely to have a husband engaged in agricultural work compared to women in the lowest quartile (p=0.05). Compared to women in the lowest quartile of 4-nitrophenol, women in the highest quartile were less likely to have a household income >4,000 tk (p=0.04) and had lower hemoglobin levels (p<0.0001), lower total gestational weight gain (p=0.004), and lower total energy intakes (p=0.04) (Supplemental Table 7). Women with detectable concentrations of IMPY tended to be older (p=0.06), more likely to have given birth to a female infant (p=0.05), and had lower hemoglobin levels (p=0.05) compared to those with non-detectable concentrations of IMPY (Supplemental Table 8). Women with detectable concentrations of 3-PBA were less likely to have a household income >4,000 tk (p=0.04), more likely to have a husband engaged in agriculture (p=0.01), more likely to be overweight (p=0.04), and had lower hemoglobin levels (p=0.01) and lower total gestational weight gain (p=0.01) compared to women with non-detectable concentrations of 3-PBA (Supplemental Table 9). The outcomes of small for gestational age and stunting at follow up 1 and 2 were not associated with any of the dietary intake variables (all p>0.05, data not shown). However, we did observed that women with lower energy and meat intake at approximately 28 weeks' gestation were more likely to deliver preterm and to have a low birth weight baby (all p<0.05, data not shown).

Women in the highest quartile of 4-nitrophenol were more than 3 times more likely to deliver preterm than women in the lowest quartile [unadjusted RR (95% CI), 3.57 (1.65, 7.73)] and nearly 4 times more likely to have a child small for gestational age [unadjusted RR (95% CI), 4.00 (1.18, 13.58); adjusted RR (95% CI), 3.81 (1.10, 13.21)] (Table 3). In unadjusted models (Supplemental Table 10), women in quartile 3 of 4-nitrophenol were at increased risk of having a child born with low birth weight compared to women in the lowest quartile: unadjusted RR (95% CI), 2.50 (1.03, 6.08), but the association was attenuated in adjusted models: 1.70 (0.69, 4.22). Women with detectable concentrations of IMPY were at

increased risk of having a child born with low birth weight compared to women with non-detectable concentrations: adjusted RR (95% CI), 2.13 (1.12, 4.08). Women with detectable concentrations of 3-PBA, on the other hand, were less likely to have a child small for gestational age compared to women with non-detectable concentrations: adjusted RR (95% CI), 0.13 (0.02, 0.95). There was no association between any of the pesticide biomarkers and stunting at 1 or 2 years of age.

In sensitivity analyses, 4-nitrophenol specified continuously in μg/g creatinine was associated with preterm birth [unadjusted OR (95% CI), 1.44 (1.17, 1.78)]. Without creatinine adjustment (e.g. specified continuously in μg/L), this association was attenuated [unadjusted OR (95% CI), 1.17 (0.99, 1.37)] (Supplemental Table 11). When gestational age was modeled continuously as an outcome, women in the highest 4-nitrophenol quartile had a gestational age, unadjusted parameter estimate (standard error), 1.0 (0.3) weeks shorter than women in the lowest quartile (p=0.0009) (Supplemental Table 12), and the association with continuous gestational age remained significant when 4-nitrophenol was modeled continuously as μg/g creatinine (p=0.0002) or μg/L (p<0.0001) (Supplemental Table 13). Women in quartile 3 of 4-nitrophenol had babies that were, unadjusted parameter estimate (standard error), 170 (67) g smaller than women in the lowest quartile of 4-nitrophenol (p=0.01), and women with detectable concentrations of IMPY tended to have babies, unadjusted parameter estimate (standard error), 126 (65) g smaller than women with non-detectable concentrations of IMPY (p=0.05) (Supplemental Table 12). In general, women with higher levels of 4-nitrophenol had children with higher length-for-gestational age z-scores, and lower weight-for-age z-scores and weight-for-length z-scores at follow-up 1 compared to women in the lowest quartile (Supplemental Tables 12 **and** 13).

DISCUSSION

Only two of the eight biomarkers evaluated were detected in at least 60% of samples from pregnant women in Bangladesh participating in this study: TCPY, a metabolite of chlorpyrifos and chlorpyrifos methyl, and 4-nitrophenol, a metabolite of parathion and methyl parathion. For these two biomarkers, however, levels were substantially higher than those reported among non-pregnant women in the United States (Centers for Disease Control and Prevention 2019): GM (95% CI) concentrations 3.17 (2.82–3.56) μg/g creatinine in Bangladesh versus 0.855 (0.765 – 0.954) μg/g creatinine in the United States for TCPY, and 18.66 (17.03–20.46) versus 0.480 (0.430 – 0.536) μg/g creatinine, in Bangladesh and the United States, respectively, for 4-nitrophenol.

With respect to associations with birth outcomes and child growth, higher concentrations of 4-nitrophenol in early pregnancy (<16 weeks' gestation) were associated with an increased risk of preterm delivery and small for gestational age, and higher concentrations of IMPY were associated with increased risk of low birth weight. We found that women with lower energy and meat intake at approximately 28 weeks' gestation were more likely to deliver preterm and to have a low birth weight baby, but adjustment for these risk factors did not change the conclusions of the study, suggesting that there may be independent effects of certain pesticides on these adverse birth outcomes. We did not observe an association between any of the pesticide biomarkers evaluated – or maternal dietary intake, preterm

birth, low birth weight, or small for gestational age – and stunting at either 1 or 2 years of age in this sample.

Studies on pesticide use and biomonitoring of pesticide exposures in Bangladesh are limited. To the best of our knowledge, this is the first study to evaluate non-persistent pesticide biomarkers in urine samples in Bangladesh, finding concentrations of two organophosphate insecticide metabolites – TCPY and 4-nitrophenol – to be the most frequently detected whereas the one herbicide metabolite measured (2,4-D) and two of the three pyrethroid insecticide metabolites measured (4-F-3-PBA and trans-DCCA) were detected in very few women. The most recent analysis of trends in pesticide use in Bangladesh reported a 1341% increase from 3135 metric tons of active ingredient in 1977 to 45,172 metric tons in 2009 with insecticides making up the vast majority (>90%) of pesticides applied, and herbicides and fungicides making up a much smaller proportion (Rahman 2013). With regards to the specific type of insecticide, several farm-level surveys in Bangladesh support that organophosphates are the most commonly used (Bhattacharjee et al. 2013; Dey 2010; Islam et al. 2016; Rahman 2003; Shammi et al. 2018). Thus, the biomonitoring results from this study are consistent with national trend data and farm-level surveys in Bangladesh.

Some previous studies evaluating the effects of pesticide exposures on birth outcomes have used residence near agricultural land, parental occupation, or other proxies of pesticide exposure (Chiu et al. 2018; Ling et al. 2018; Sanjose et al. 1991; Schreinemachers 2003; Schwartz et al. 1986; Shirangi et al. 2011), and were conducted in the United States or Europe. In our study, which used a more objective exposure measurement – pesticide biomarkers – in a low-income country, women in the highest quartile of 4-nitrophenol were more than 3 times more likely to deliver preterm than women in the lowest quartile. This result is consistent with several recent studies in the United States, which have reported increases in risk of preterm birth among pregnant women with higher pesticide exposures (Ling et al. 2018; Winchester et al. 2016), though the magnitude of the effect in those studies was much smaller than what we reported in this sample from Bangladesh. In contrast, a large (n=1,777 mothers), prospective study conducted from 1999-2002 (Project Viva) recently estimated maternal intake of pesticide residues from fruits and vegetables based on food frequency data and data on residues from the U.S. Department of Agriculture, found no association between estimated pesticide exposure and preterm birth: adjusted odds ratio (95% CI) for the highest versus lowest quintile of high-pesticide fruit and vegetable intake, 1.01 (0.49, 2.10) for first trimester and 0.60 (0.27, 1.34) for second trimester (Chiu et al. 2018). These differences could be the result of many factors including differences in timing of exposure during pregnancy, sources of exposure (e.g. diet versus occupational versus residential), level and type of exposure including differences in the cocktail of pesticides commonly used in a given setting, and within-person variability in urinary pesticide biomarkers, among other factors.

We also found that women with higher concentrations of 4-nitrophenol during pregnancy were at increased risk of having a child born small for gestational age, and women with a detectable concentration of IMPY were at increased risk of having a child born with low birth weight compared to women with non-detectable concentrations. This is consistent with a study of 52 pregnant women in Thailand that found higher maternal organophosphate

concentrations during pregnancy (mean gestational age of 12 weeks) were significantly associated with lower birth weight (Naksen et al. 2015). However, several studies in the United States (Ling et al. 2018), including a recent pooled analysis of four longitudinal birth cohort studies with measurements of non-specific organophosphate metabolites in maternal urine (CHAMACOS, HOME, Columbia, and Mount Sinai) (Harley et al. 2016), have found no association between biomarkers of pesticide exposures and low birth weight.

We did not find an association between pesticide biomarker concentration in early pregnancy and child stunting at 1 or 2 years of age. We hypothesized that maternal pesticide exposure would increase the risk of child stunting through its effects on low birth weight or small for gestational age, which are well-established risk factors for stunting (Danaei et al. 2016), especially in the first year of life (Krishna et al. 2016). However, in this sample, we did not find an association between low birth weight or small for gestational age and stunting, which may in turn explain why we did not observe an association between maternal pesticide exposure and stunting. Three previous studies have explored the relationship between pesticide exposure and stunting in children. Consistent with our finding of a null association, a study of 72 primary school children in an area of Ecuador with intensive floriculture, found no difference in height-for-age z-score between children classified as exposed to pesticides prenatally from their mother's work and those who were classified as unexposed: mean (SD) height-for-age z-score of −1.84 (0.83) among exposed and −1.59 (0.99) among controls, p=0.26 (Grandjean et al. 2006). In contrast, two case-control studies, one in Nepal and one in Indonesia, found significant associations between self-reported pesticide exposure and stunting. The study in Nepal, conducted among 118 stunted children (6-59 months) and 236 controls, found a substantially increased odds of having been exposed to pesticides (self-report) among stunted children compared to controls: unadjusted odds ratio (95% CI), 3.51 (1.33, 9.23) (Paudel et al. 2012). The other case-control study, in Indonesia, conducted among 48 stunted children (8-12 years) and 112 controls, reported an odds ratio (95% CI) of 3.90 (1.15, 13.26) after adjustment for insulin-like growth factor 1 (IGF-1) (Kartini et al. 2019). Additional research is needed to understand the potential effects of pesticide exposures on impaired child growth in these agrarian settings where relatively high rates of stunting persist.

An important strength of this study is the prospective design, which strengthens the interpretation of the findings. However, this study was limited by a small sample size of 289 pregnant women, which may have limited our power to detect associations, particularly for biomarkers with low detection frequencies. Another major limitation is that we estimated the associations of each pesticide biomarker individually, but women are exposed to multiple pesticides and indeed we found significant correlations between certain pesticide biomarkers in this sample. Future research in larger samples is needed to determine potential interactive versus independent effects of these pesticide biomarkers. The specific pesticide biomarkers evaluated were part of a standard CDC panel used for US biomonitoring and therefore we found several biomarkers were not detected in most participants from this Bangladeshi sample. As a result, we had to specify several pesticides as binary variables (<LOD versus ≥ LOD) to avoid left censoring bias and therefore lost information on the specific values of those above the LOD. Building off of this study and information on pesticides sold by registered dealers, future studies should explore additional biomarkers in this population. We

did not measure polymorphisms of paraoxonase 1 (PON1), an enzyme involved in the detoxification of organophosphate insecticides, which has been shown to be an important effect modifier of the association between pesticides and birth outcomes in a handful of studies (Harley et al. 2011; Harley et al. 2016; Naksen et al. 2015). Future research should consider exploring these polymorphisms in the Bangladeshi population. Additional limitations include potential selection bias given that women in this sub-sample had higher household incomes and were less likely to have a husband engaged in agricultural work, which may have biased the associations towards the null; lack of data on important confounders, particularly maternal infections during pregnancy; and the use of creatinine to adjust for urine dilution given that it may be affected by meat intake, gestational age, and glomerular filtration, among other variables.

In addition, we did not collect information on potential routes of exposure to pesticides aside from whether or not the women and their husbands were engaged in agricultural work as an occupation. None of the women were engaged in agricultural work as an occupation and only 23% of their husbands were, suggesting that exposures were likely via non-occupational routes. Malaria is not endemic to either district covered by this study (Pabna and Munshiganj Districts) (Haque et al. 2009). While Pabna District is the second-highest district in Bangladesh in terms of visceral leishmaniasis cases, all reported cases were from two upazilas (sub-districts), neither of which was included in our study (Chowdhury et al. 2014). Chlorpyrifos has been recommended for spraying potential sand fly breeding sites (Chowdhury et al. 2017), but according to both the 2008 and 2011 WHO Malaria Report Country Profiles for Bangladesh, and the Demographic and Health Surveys, less than 5% of households report that their dwelling was sprayed with a residual insecticide in the last 12 months (USAID Unknown; World Health Organization 2008, 2011). We therefore suspect that the primary route of exposure among these women was diet, especially considering the high insecticide application rates in agriculture mentioned previously (Rahman 2013). Moreover, several studies have found particularly high levels of chlorpyrifos and parathion on vegetable samples from Bangladesh (Hossain et al. 2015; Islam et al. 2019), and another study reported that diazinon was the most commonly detected residue (Alam et al. 2015). More research on routes of exposure is needed to understand the high levels of TCPY, a metabolite of chlorpyrifos and chlorpyrifos methyl; 4-nitrophenol, a metabolite of parathion and methyl parathion; and IMPY, a metabolite of diazinon, in this population.

We relied on a single measurement of maternal biomarkers of these non-persistent pesticides to assess exposure, which was hypothesized to impact the developing fetus. The latter point is supported by previous studies among pregnant women in New York City that reported a high correlation between concentrations of chlorpyrifos in maternal and cord plasma ($r=0.76$, $p<0.001$) (Whyatt et al. 2004) and concentrations of TCPY in maternal urine and meconium ($r=0.31$, $p<0.01$) (Whyatt et al. 2009), which is consistent with animal studies demonstrating that chlorpyrifos and its metabolite TCPY are distributed to maternal and fetal tissues including the placenta (Abdel-Rahman et al. 2002; Magnarelli and Guiñazú 2012). With respect to the non-persistent nature of these pesticide exposures, a widely cited pharmacokinetics study in humans found that TCPY was eliminated in urine with a half-life of 27 hours and 70% of an oral dose of chlorpyrifos was eliminated as urinary TCPY within 4 days (Nolan et al. 1984). Related to this rapid metabolism and excretion rate, recent

studies of pregnant women in six European countries (Casas et al. 2018) and the United States (Barkoski et al. 2018) have shown that urinary concentrations of organophosphate insecticide metabolites are highly variable within individuals. However, older US studies and studies outside the United States and Europe have shown better reliability, potentially because in sample populations with a continuous source of exposure, within-subject variability may be lower. For example, a study of second-morning void samples collected during each trimester of pregnancy among 21 pregnant women in Mexico City reported an intraclass correlation coefficient (ICC) of 0.41 for TCPY, reflecting a fair level of reliability (Fortenberry et al. 2014), and that value was similar to the ICC of 0.43 for TCPY reported for repeated samples in the third trimester among 253 pregnant women in New York City (Whyatt et al. 2009). Similarly, a recent study in Japan of 62 pregnant women found that the ICC for spot urine concentrations of DAP metabolites was generally greater than 0.4, indicating moderate reliability, and that categorization – as was done in our study – improved the accuracy of exposure: less than 10% of spot urine samples were classified in the wrong quartile (Hioki et al. 2019). While the exact reliability of TCPY and the other non-persistent pesticide biomarkers in our study is not known, it should be acknowledged that these biomarkers represent a major improvement over self-reported, questionnaire-based data on pesticide exposures (Barr 2008). Future studies should consider this limitation when developing urine collection procedures and, ideally, aim to collect multiple samples over the course of a day on multiple days over the course of a week, and analyze a composite of those samples to better classify participants' usual exposure. However, in the context of countries with high average daily temperatures and limited refrigeration capacity, the logistical challenges of participants storing multiple samples or field teams picking up multiple samples from participants' homes are significant barriers to overcome.

To the best of our knowledge, this is the first study to quantify concentrations of a wide range of pesticide biomarkers among pregnant women in Bangladesh, and to evaluate their association with birth outcomes and child growth. Results suggest that exposure to the common insecticides during early pregnancy may increase the risk of adverse birth outcomes. In 2015, the Gates Foundation announced a new approach to nutrition, emphasizing improvements to the food system (Bill & Melinda Gates Foundation 2015). However, here and elsewhere where "improvements to the food system" are mentioned in the context of improving maternal and child health, the impact of pesticides is overlooked. Results of this study suggest that future work in this area would be strengthened by broadening the discussion to include these important exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

Abdel-Rahman AA, Blumenthal GM, Abou-Donia SA, Ali FA, Abdel-Monem AE, Abou-Donia MB. 2002 Pharmacokinetic profile and placental transfer of a single intravenous injection of chlorpyrifos in pregnant rats. Arch Toxicol 76:452–459. [PubMed: 12185413]

Alam M, Chowdhury M, Hossain MS, Mijanur Rahman M, Rahman MA, Gan SH, et al. 2015 Detection of residual levels and associated health risk of seven pesticides in fresh eggplant and tomato samples from Narayanganj district, Bangladesh. Journal of Chemistry: 243574.

Arifeen SE, Black RE, Caulfield LE, Antelman G, Baqui AH, Nahar Q, et al. 2000 Infant growth patterns in the slums of Dhaka in relation to birth weight, intrauterine growth retardation, and prematurity. Am J Clin Nutr 72:1010–1017. [PubMed: 11010945]

Barkoski J, Bennett D, Tancredi D, Barr DB, Elms W, Hertz-Picciotto I. 2018 Variability of urinary pesticide metabolite concentrations during pregnancy in the marbles study. Environ Res 165:400–409. [PubMed: 29860212]

Barr DB. 2008 Biomonitoring of exposure to pesticides. Journal of Chemical Health and Safety 15:20–29.

Berkowitz GS, Obel J, Deych E, Lapinski R, Godbold J, Liu Z, et al. 2003 Exposure to indoor pesticides during pregnancy in a multiethnic, urban cohort. Environ Health Perspect 111:79–84. [PubMed: 12515682]

Berkowitz GS, Wetmur JG, Birman-Deych E, Obel J, Lapinski RH, Godbold JH, et al. 2004 In utero pesticide exposure, maternal paraoxonase activity, and head circumference. Environ Health Perspect 112:388. [PubMed: 14998758]

Bhattacharjee S, Chowdhury M, Fakhruddin A, Alam M. 2013 Impacts of pesticide exposure on paddy farmers' health. Jahangirnagar University Environmental Bulletin 2:18–25.

Bill & Melinda Gates Foundation. 2015 The Bill & Melinda Gates Foundation announces new $776 million investment in nutrition to tackle child mortality and help all women and children survive and thrive. Seattle, WA: Bill & Melinda Gates Foundation.

Budreau CH, Singh RP. 1973 Effect of fenthion and dimethoate on reproduction in the mouse. Toxicol Appl Pharmacol 26:29–38. [PubMed: 4748136]

Casas M, Basagana X, Sakhi AK, Haug LS, Philippat C, Granum B, et al. 2018 Variability of urinary concentrations of non-persistent chemicals in pregnant women and school-aged children. Environ Int 121:561–573. [PubMed: 30300814]

Centers for Disease Control and Prevention. 2019 Fourth national report on human exposure to environmental chemicals Updated tables, 1 2019, Vol. 2 Atlanta, GA: Centers for Disease Control and Prevention.

Chanda SM, Pope CN. 1996 Neurochemical and neurobehavioral effects of repeated gestational exposure to chlorpyrifos in maternal and developing rats. Pharmacol Biochem Behav 53:771–776. [PubMed: 8801577]

Cheikh Ismail L, Knight H, Bhutta Z, Chumlea W. 2013 International fetal and newborn growth consortium for the 21st century. Anthropometric protocols for the construction of new international fetal and newborn growth standards: The intergrowth-21st project. BJOG 120:42–47. [PubMed: 23841804]

Cheikh Ismail L, Knight H, Bhutta Z, Chumlea W, Fetal ftI, Century NGCfts. 2013b Anthropometric protocols for the construction of new international fetal and newborn growth standards: The intergrowth-21st project. BJOG 120:42–47. [PubMed: 23841804]

Chevrier C, Limon G, Monfort C, Rouget F, Garlantezec R, Petit C, et al. 2011 Urinary biomarkers of prenatal atrazine exposure and adverse birth outcomes in the Pelagie birth cohort. Environ Health Perspect 119:1034–1041. [PubMed: 21367690]

Chiu Y-H, Williams PL, Gillman MW, Hauser R, Rifas-Shiman SL, Bellavia A, et al. 2018 Maternal intake of pesticide residues from fruits and vegetables in relation to fetal growth. Environ Int 119:421–428. [PubMed: 30029096]

Chowdhury R, Mondal D, Chowdhury V, Faria S, Alvar J, Nabi SG, et al. 2014 How far are we from visceral leishmaniasis elimination in Bangladesh? An assessment of epidemiological surveillance data. PLoS Negl Trop Dis 8:e3020. [PubMed: 25144317]

Chowdhury R, Faria S, Huda MM, Chowdhury V, Maheswary NP, Mondal D, et al. 2017 Control of phlebotomus argentipes (diptera: Psychodidae) sand fly in Bangladesh: A cluster randomized controlled trial. PLoS Negl Trop Dis 11:e0005890. [PubMed: 28873425]

Danaei G, Andrews KG, Sudfeld CR, Fink G, McCoy DC, Peet E, et al. 2016 Risk factors for childhood stunting in 137 developing countries: A comparative risk assessment analysis at global, regional, and country levels. PLoS Med 13:e1002164. [PubMed: 27802277]

Davis MD, Wade EL, Restrepo PR, Roman-Esteva W, Bravo R, Kuklenyik P, et al. 2013 Semi-automated solid phase extraction method for the mass spectrometric quantification of 12 specific metabolites of organophosphorus pesticides, synthetic pyrethroids, and select herbicides in human urine. Journal of Chromatography B 929:18–26.

de Onis M. 2006 Who child growth standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age:WHO.

Dey NC. 2010 Use of pesticides in vegetable farms and its impact on health of farmers and environment. Environ Sci Technol(II):134–140.

Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, et al. 2004 Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect:1116–1124. [PubMed: 15238287]

Fortenberry GZ, Meeker JD, Sanchez BN, Barr DB, Panuwet P, Bellinger D, et al. 2014 Urinary 3,5,6-trichloro-2-pyridinol (tcpy) in pregnant women from mexico city: Distribution, temporal variability, and relationship with child attention and hyperactivity. Int J Hyg Environ Health 217:405–412. [PubMed: 24001412]

Grandjean P, Harari R, Barr DB, Debes F. 2006 Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in Ecuadorian school children. Pediatrics 117:e546–556. [PubMed: 16510633]

Haque U, Ahmed SM, Hossain S, Huda M, Hossain A, Alam MS, et al. 2009 Malaria prevalence in endemic districts of Bangladesh. PLoS One 4:e6737. [PubMed: 19707580]

Harley KG, Chevrier J, Schall RA, Sjödin A, Bradman A, Eskenazi B. 2011 Association of prenatal exposure to polybrominated diphenyl ethers and infant birth weight. Am J Epidemiol 174:885–892. [PubMed: 21878423]

Harley KG, Engel SM, Vedar MG, Eskenazi B, Whyatt RM, Lanphear BP, et al. 2016 Prenatal exposure to organophosphorous pesticides and fetal growth: Pooled results from four longitudinal birth cohort studies. Environ Health Perspect 124:1084–1092. [PubMed: 26685281]

Hioki K, Ito Y, Oya N, Nakayama SF, Isobe T, Ebara T, et al. 2019 Intra-individual variations of organophosphate pesticide metabolite concentrations in repeatedly collected urine samples from pregnant women in japan. Environ Health Prev Med 24:7. [PubMed: 30654738]

Hoppin JA, Ulmer R, Calafat AM, Barr DB, Baker SV, Meltzer HM, et al. 2006 Impact of urine preservation methods and duration of storage on measured levels of environmental contaminants. J Expo Sci Environ Epidemiol 16:39–48. [PubMed: 16007114]

Hornung RW, Reed LD. 1990 Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg 5:46–51.

Hossain M, Fakhruddin A, Alamgir Zaman Chowdhury M, Rahman M, Khorshed Alam M. 2015 Health risk assessment of selected pesticide residues in locally produced vegetables of Bangladesh. International Food Research Journal 22.

Institoris L, Siroki O, Desi I. 1995 Immunotoxicity study of repeated small doses of dimethoate and methylparathion administered to rats over three generations. Hum Exp Toxicol 14:879–883. [PubMed: 8588948]

International Labour Organization. 2017 ILOSTAT database In: Employment in agriculture (% of total employment) (modeled ILO estimate). Geneva: International Labour Organization.

Islam MA, Ullah A, Habib M, Chowdhury MTI, Khan MSI, Kaium A, et al. 2019 Determination of major organophosphate pesticide residues in cabbage collected from different markets of Dhaka. Asia Pacific Environmental and Occupational Health Journal 5.

Islam MS, Alam MS, Uddin MN, Zabir A, Islam MS, Haque KA, et al. 2016 Farm level pesticides use in patuakhali and comilla region of bangladesh and associated health risk. Journal of Health and Environmental Research 2:20–26.

Jung J, Rahman MM, Rahman MS, Swe KT, Islam MR, Rahman MO, et al. 2019 Effects of hemoglobin levels during pregnancy on adverse maternal and infant outcomes: A systematic review and meta-analysis. Ann N Y Acad Sci.

Karsan A, Maclaren I, Conn D, Wadsworth L. 1993 An evaluation of hemoglobin determination using sodium lauryl sulfate. Am J Clin Pathol 100:123–126. [PubMed: 8356944]

Kartini A, Subagio HW, Hadisaputro S, Kartasurya MI, Suhartono S, Budiyono B. 2019 Pesticide exposure and stunting among children in agricultural areas. Int J Occup Environ Med 10:17–29. [PubMed: 30685774]

Kile ML, Rodrigues EG, Mazumdar M, Dobson CB, Diao N, Golam M, et al. 2014 A prospective cohort study of the association between drinking water arsenic exposure and self-reported maternal health symptoms during pregnancy in bangladesh. Environmental Health 13:1. [PubMed: 24405644]

Kinniburgh D, Smedley P. 2001 Arsenic contamination of groundwater in Bangladesh. Keyworth: British Geological Survey.

Krishna A, Fink G, Berkman LF, Subramanian SV. 2016 Short- and long-run associations between birth weight and children's height. Econ Hum Biol 21:156–166. [PubMed: 26922363]

Levario-Carrillo M, Amato D, Ostrosky-Wegman P, Gonzalez-Horta C, Corona Y, Sanin LH. 2004 Relation between pesticide exposure and intrauterine growth retardation. Chemosphere 55:1421–1427. [PubMed: 15081785]

Lin PD, Bromage S, Mostofa MG, Allen J, Oken E, Kile ML, et al. 2017 Validation of a dish-based semiquantitative food questionnaire in rural Bangladesh. Nutrients 9.

Ling C, Liew Z, von Ehrenstein OS, Heck JE, Park AS, Cui X, et al. 2018 Prenatal exposure to ambient pesticides and preterm birth and term low birthweight in agricultural regions of California. Toxics 6:E41. [PubMed: 30037110]

Magnarelli G, Guiñazú N. 2012 Placental toxicology of pesticides. INTECH Open Access Publisher. Available from: https://www.intechopen.com/books/recent-advances-in-research-on-the-human-placenta/placental-toxicology-of-pesticides [accessed 10 August 2019]

Muto MA, Lobelle F Jr., Bidanset JH, Wurpel JN. 1992 Embryotoxicity and neurotoxicity in rats associated with prenatal exposure to dursban. Vet Hum Toxicol 34:498–501. [PubMed: 1283795]

Naksen W, Prapamontol T, Mangklabruks A, Chantara S, Thavornyutikarn P, Srinual N, et al. 2015 Associations of maternal organophosphate pesticide exposure and pon1 activity with birth outcomes in sawasdee birth cohort, thailand. Environ Res 142:288–296. [PubMed: 26186137]

Nolan RJ, Rick DL, Freshour NL, Saunders JH. 1984 Chlorpyrifos: Pharmacokinetics in human volunteers. Toxicol Appl Pharmacol 73:8–15. [PubMed: 6200956]

Paudel R, Pradhan B, Wagle RR, Pahari DP, Onta SR. 2012 Risk factors for stunting among children: A community based case control study in nepal. Kathmandu Univ Med J (KUMJ) 10:18–24.

Perera FP, Rauh V, Tsai WY, Kinney P, Camann D, Barr D, et al. 2003 Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. Environ Health Perspect 111:201–205. [PubMed: 12573906]

Rahman S. 2003 Farm-level pesticide use in bangladesh: Determinants and awareness. Agriculture, Ecosystems and Environment 95:241–252.

Rahman S. 2013 Pesticide consumption and productivity and the potential of ipm in bangladesh. Sci Total Environ 445-446:48–56. [PubMed: 23314122]

Sanjose S, Roman E, Beral V. 1991 Low birthweight and preterm delivery, scotland, 1981-84: Effect of parents' occupation. Lancet 338:428–431. [PubMed: 1678094]

Sarma H, Khan JR, Asaduzzaman M, Uddin F, Tarannum S, Hasan MM, et al. 2017 Factors influencing the prevalence of stunting among children aged below five years in Bangladesh. Food Nutr Bull 38:291–301. [PubMed: 28758423]

Schreinemachers DM. 2003 Birth malformations and other adverse perinatal outcomes in four us wheat-producing states. Environ Health Perspect 111:1259. [PubMed: 12842783]

Schwartz DA, Newsum LA, Heifetz RM. 1986 Parental occupation and birth outcome in an agricultural community. Scand J Work Environ Health:51–54. [PubMed: 3485819]

Shammi M, Sultana A, Hasan N, Rahman MM, Islam MS, Bodrud-Doza M, et al. 2018 Pesticide exposures towards health and environmental hazard in Bangladesh: A case study on farmers' perception. Journal of the Saudi Society of Agricultural Sciences.

Shapiro SS, Wilk MB. 1965 An analysis of variance test for normality (complete samples). Biometrika 52:591–611.

Shirangi A, Nieuwenhuijsen M, Vienneau D, Holman CD. 2011 Living near agricultural pesticide applications and the risk of adverse reproductive outcomes: A review of the literature. Paediatr Perinat Epidemiol 25:172–191. [PubMed: 21281330]

Song X, Seidler FJ, Saleh JL, Zhang J, Padilla S, Slotkin TA. 1997 Cellular mechanisms for developmental toxicity of chlorpyrifos: Targeting the adenylyl cyclase signaling cascade. Toxicol Appl Pharmacol 145:158–174. [PubMed: 9221834]

Spiegelman D, Hertzmark E. 2005 Easy SAS calculations for risk or prevalence ratios and differences. Am J Epidemiol 162:199–200. [PubMed: 15987728]

Spyker JM, Avery DL. 1977 Neurobehavioral effects of prenatal exposure to the organophosphate diazinon in mice. J Toxicol Environ Health 3:989–1002. [PubMed: 599594]

Textor J, Hardt J, Knuppel S. 2011 Dagitty: A graphical tool for analyzing causal diagrams. Epidemiology 22:745.

USAID. Unknown. Demographic and health surveys. Malaria corner: Bangladesh Available: https://dhsprogram.com/topics/malaria-Corner/Bangladesh.cfm [accessed 29 July 2019].

VanderWeele TJ, Hernán MA, Robins JM. 2008 Causal directed acyclic graphs and the direction of unmeasured confounding bias. Epidemiology 19:720. [PubMed: 18633331]

Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R, et al. 2004 Prenatal insecticide exposures and birth weight and length among an urban minority cohort. Environ Health Perspect: 1125–1132. [PubMed: 15238288]

Whyatt RM, Garfinkel R, Hoepner LA, Andrews H, Holmes D, Williams MK, et al. 2009 A biomarker validation study of prenatal chlorpyrifos exposure within an inner-city cohort during pregnancy. Environ Health Perspect 117:559–567. [PubMed: 19440494]

Winchester P, Proctor C, Ying J. 2016 County-level pesticide use and risk of shortened gestation and preterm birth. Acta Paediatr 105:e107–115. [PubMed: 26613363]

Wohlfahrt-Veje C, Main KM, Schmidt IM, Boas M, Jensen TK, Grandjean P, et al. 2011 Lower birth weight and increased body fat at school age in children prenatally exposed to modern pesticides: A prospective study. Environmental Health 10:79. [PubMed: 21933378]

World Health Organization and Food and Agriculture Organization. 2003 Diet, Nutrition and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation. Geneva:World Health Organization.

World Health Organization. 2008 World malaria report. Geneva: World Health Organization.

World Health Organization. 2011 World malaria report Geneva: World Health Organization.

Wylie BJ, Ae-Ngibise KA, Boamah EA, Mujtaba M, Messerlian C, Hauser R, et al. 2017 Urinary concentrations of insecticide and herbicide metabolites among pregnant women in rural ghana: A pilot study. Int J Environ Res Public Health 14:354.

Xiang H, Nuckols JR, Stallones L. 2000 A geographic information assessment of birth weight and crop production patterns around mother's residence. Environ Res 82:160–167. [PubMed: 10662530]

Highlights:

- Among first studies to evaluate pesticide exposures in pregnant women in Bangladesh
- Levels of urinary metabolites of chlorpyrifos and parathion were notably high
- Higher exposure to parathion was associated with increased risk of preterm birth
- Exposure to parathion was also associated with increased risk of small for gestational age
- Exposure to diazinon was associated with increased risk of low birth weight

Table 1.

Characteristics at enrollment (≤16 weeks' gestation) and infant outcomes among pregnant women in rural Bangladesh (n=289).

	% (n) or mean ±SD
Maternal age (years)	23.1 ±4.3
Gravidity	
0	41.2 (119)
1	31.8 (92)
≥2	27.0 (78)
Maternal education	
No formal schooling or primary school	47.4 (137)
Secondary school and higher	52.6 (152)
Monthly household income	
≤4,000 tk (~ $48)	33.9 (98)
>4,000 tk (~ $48)	66.1 (191)
Husband engaged in agricultural work (% yes)	22.8 (66)
Body mass index at enrollment (<16 weeks' gestation)	
Underweight (<18.5 kg/m^2)	23.2 (67)
Normal weight (18.5 to <25.0 kg/m^2)	67.8 (196)
Overweight (≥25.0 kg/m^2)	9.0 (26)
Secondhand smoke exposure (% yes)	38.5 (111)
Concentration of arsenic in participant's drinking water (μg/L)	21.1 ±58.3
Maternal hemoglobin level (g/dL)	11.3 ±1.4
Maternal dietary intake at approximately 28 weeks' gestation	
Total energy intake (kcal/day)	3589 ±1115
Carbohydrate intake (% calories)	63.6 ±5.0
Fat intake (% calories)	16.9 ±3.4
Protein intake (% calories)	17.1 ±2.3
Grain intake (g/day)	1782 ±354
Dal intake (g/day)	100 ±79
Vegetable intake (g/day)	190 ±119
Fruit intake (g/day)	130 ±84
Meat intake (g/day)	724 ±304
Dairy intake (g/day)	241 ±139
Birth outcomes	
Total gestational weight gain (kg)	7.9 ±3.0
Gestational age at birth (weeks)	37.7 ±1.8
Preterm birth (% yes) [a]	22.8 (66)
Infant sex	
Male	52.6 (152)
Female	47.4 (137)

	% (n) or mean ±SD
Low birth weight (% yes) [b]	13.5 (39)
Small for gestational age [c]	12.1 (35)
Follow-up 1 outcomes	
Infant age (years)	1.1 ±0.1
Stunted (% yes) [d]	68.2 (197)
Follow-up 2 outcomes	
Infant age (years)	2.2 ±0.2
Stunted (% yes) [d]	48.8 (141)

[a] Preterm birth was defined as delivery at <37 weeks' gestation.

[b] Low birth weight was defined as birth weight <2.5 kg.

[c] Small for gestational age was defined as weight-for-gestational age percentile <10 based on the INTERGROWTH-21st standards.

[d] Stunting was defined as length-for-age z-score <−2 SD based on the WHO Growth Reference Charts.

Table 2.

Concentrations of pesticide biomarkers among pregnant women in rural Bangladesh (n=289)

Pesticide biomarker	>LOD, n (%)[a]	Geometric Mean (95% CI), µg/g creatinine [b]	U.S. Population, Non-pregnant Females, Geometric Mean (95% CI), µg/g creatinine[c]
2,4-D	5.6 (16)	-	0.334 (0.302 – 0.369)
TCPY	97.9 (283)	3.17 (2.82–3.56)	0.855 (0.765 – 0.954)
4-nitrophenol	100 (288)	18.66 (17.03–20.46)	0.480 (0.430 – 0.536)
MDA	2.8 (8)	-	Not calculated
IMPY	16.1 (46)	-	Not calculated
4-F-3-PBA	0 (0)	-	Not calculated
3-PBA	19.8 (57)	-	0.505 (0.453 – 0.564)
trans-DCCA	6.2 (18)	-	Not calculated

Abbreviations: confidence interval (CI); 2,4-dichlorophenoxyacetic acid (2,4-D); 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA); 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY); limit of detection (LOD); malathion dicarboxylic acid (MDA); 3-phenoxybenzoic acid (3-PBA); 3,5,6-trichloro-2-pyridinol (TCPY); trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (trans-DCCA).

[a] LOD was 0.3 µg/L for 2,4-D, 0.1 µg/L for TCPY, 1.0 µg/L for MDA, 0.6 µg/L for trans-DCCA, and 0.2 µg/L for all other biomarkers.

[b] Not reported for pesticide biomarkers detected in fewer than 40% of participants.

[c] Values are for non-pregnant females (all ages), U.S. population from the National Health and Nutrition Examination Survey, 2009-2010. Centers for Disease Control and Prevention. 2019. Fourth national report on human exposure to environmental chemicals. Updated tables, January 2019. Atlanta, GA: Centers for Disease Control and Prevention. Available at: www.cdc.gov/exposurereport.

Table 3.

Association between pesticide concentrations and infant outcomes among pregnant women in rural Bangladesh (n=289).

	Concentration Range	Preterm Birth [a]	Low Birth Weight [b]	Small for Gestational Age [b]	Stunting at Follow-up 1 [b]	Stunting at Follow-up 2 [b]
TCPY	Quartile 1 (n=73) 0.37 to 1.79 μg/g creatinine	Ref	Ref	Ref	Ref	Ref
	Quartile 2 (n=71) 1.79 to 2.77 μg/g creatinine	1.17 (0.63, 2.15)	2.15 (0.82, 5.66)	0.97 (0.42, 2.28)	0.96 (0.75, 1.23)	0.97 (0.65, 1.43)
	Quartile 3 (n=72) 2.81 to 4.94 μg/g creatinine	0.95 (0.49, 1.82)	0.88 (0.27, 2.86)	0.66 (0.26, 1.67)	1.06 (0.85, 1.32)	1.13 (0.79, 1.62)
	Quartile 4 (n=73) 4.97 to 795.37 μg/g creatinine	1.33 (0.74, 2.39)	1.76 (0.63, 4.97)	0.38 (0.12, 1.19)	0.99 (0.77, 1.27)	1.16 (0.80, 1.68)
4-nitrophenol	Quartile 1 (n=72) 1.06 to 11.12 μg/g creatinine	Ref	Ref	Ref	Ref	Ref
	Quartile 2 (n=72) 11.18 to 18.11 μg/g creatinine	1.29 (0.51, 3.27)	0.40 (0.11, 1.52)	2.93 (0.82, 10.40)	0.86 (0.65, 1.14)	0.84 (0.61, 1.16)
	Quartile 3 (n=72) 18.27 to 31.75 μg/g creatinine	**3.57 (1.65, 7.73)**	1.70 (0.69, 4.22)	2.61 (0.71, 9.61)	1.07 (0.83, 1.36)	0.74 (0.51, 1.07)
	Quartile 4 (n=72) 31.90 to 293.00 μg/g creatinine	**3.57 (1.65, 7.73)**	1.87 (0.74, 4.72)	**3.81 (1.10, 13.21)**	1.10 (0.87, 1.39)	0.85 (0.61, 1.19)
IMPY	< LOD (n=240)	Ref	Ref	Ref	Ref	Ref
	≥ LOD (n=46) 0.14 to 7.62 μg/g creatinine	1.16 (0.68, 1.99)	**2.13 (1.12, 4.08)**	1.53 (0.70, 3.35)	1.02 (0.82, 1.27)	1.04 (0.73, 1.49)
3-PBA	< LOD (n=231)	Ref	Ref	Ref	Ref	Ref
	≥ LOD (n=57) 0.17 to 7.57 μg/g creatinine	0.99 (0.58, 1.69)	0.78 (0.32, 1.94)	**0.13 (0.02, 0.95)**	1.01 (0.83, 1.24)	0.95 (0.69, 1.32)

Values are relative risk (95% confidence interval) from log-binomial regression. Bolded values significant at p<0.05.

Abbreviations: 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY); limit of detection (LOD); 3-phenoxybenzoic acid (3-PBA); 3,5,6-trichloro-2-pyridinol (TCPY). LOD was 0.1 μg/L for TCPY, and 0.2 μg/L for 4-nitrophenol, IMPY, and 3-PBA.

[a]Directed acyclic graph analysis indicated no adjustment was needed to estimate the total effect of maternal pesticide exposure on preterm birth and therefore final model was unadjusted.

[b] Adjusted for household income (≤4,000 tk versus >4,000 tk), maternal education (no formal schooling or primary school versus secondary school and higher), maternal total energy intake (log-transformed kcal/day), and maternal meat intake (log-transformed g/day).