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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 18–40 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).

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.

1. 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

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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.

2. Methods

2.1. 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 > 4000 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.

2.2. 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 $0.3\ \mu\text{g/L}$ for 2,4-D, $0.1\ \mu\text{g/L}$ for TCPY, $1.0\ \mu\text{g/L}$ for MDA, $0.6\ \mu\text{g/L}$ for trans-DCCA, and $0.2\ \mu\text{g/L}$ 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 ($\mu\text{g/g}$ 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.

2.3. 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 World Health Organization (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.

2.4. Covariates

Women completed three clinical visits during the study: baseline (< 16 weeks' gestation), approximately 28 weeks' gestation, and \leq 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 WHO guidelines as underweight (BMI < 18.5 kg/m²), normal weight (BMI 18.5 to < 25.0 kg/m²), or overweight (BMI \geq 25.0 kg/m²) (World Health Organization, 2003).

During the second clinical visit at approximately 28 weeks' gestation, a validated semi-quantitative 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).

2.5. 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 five binary outcomes (low birth weight, preterm birth, small for gestational age, 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 Fig. 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 (\leq 4000 tk versus > 4000 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) $\mu\text{g/g}$ creatinine and (b) $\mu\text{g/L}$; 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).

3. Results

The mean \pm SD gestational age was 11.6 \pm 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) $\mu\text{g/g}$ creatinine and 18.66 (17.03–20.46) $\mu\text{g/g}$ 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 ($p = 0.007$).

Table 1
Characteristics at enrollment (≤ 16 weeks' gestation) and infant outcomes among pregnant women in rural Bangladesh (n = 289).

	% (n) or mean \pm SD
Maternal age (years)	23.1 \pm 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	
≤ 4000 tk (~ \$48)	33.9 (98)
> 4000 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 ($\mu\text{g/L}$)	21.1 \pm 58.3
Maternal hemoglobin level (g/dL)	11.3 \pm 1.4
<i>Maternal dietary intake at approximately 28 weeks' gestation</i>	
Total energy intake (kcal/day)	3589 \pm 1115
Carbohydrate intake (% calories)	63.6 \pm 5.0
Fat intake (% calories)	16.9 \pm 3.4
Protein intake (% calories)	17.1 \pm 2.3
Grain intake (g/day)	1782 \pm 354
Dal intake (g/day)	100 \pm 79
Vegetable intake (g/day)	190 \pm 119
Fruit intake (g/day)	130 \pm 84
Meat intake (g/day)	724 \pm 304
Dairy intake (g/day)	241 \pm 139
<i>Birth outcomes</i>	
Total gestational weight gain (kg)	7.9 \pm 3.0
Gestational age at birth (weeks)	37.7 \pm 1.8
Preterm birth (% yes) ^a	22.8 (66)
Infant sex	
Male	52.6 (152)
Female	47.4 (137)
Low birth weight (% yes) ^b	13.5 (39)
Small for gestational age ^c	12.1 (35)
<i>Follow-up 1 outcomes</i>	
Infant age (years)	1.1 \pm 0.1
Stunted (% yes) ^d	68.2 (197)
<i>Follow-up 2 outcomes</i>	
Infant age (years)	2.2 \pm 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.

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 > 4000 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 > 4000 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 > 4000 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 observe 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 $\mu\text{g/g}$ creatinine was associated with preterm birth [unadjusted odds ratio (OR) (95% CI), 1.44 (1.17, 1.78)]. Without creatinine adjustment (e.g. specified continuously in $\mu\text{g/L}$), 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 $\mu\text{g/g}$ creatinine ($p = 0.0002$) or $\mu\text{g/L}$ ($p < 0.0001$) (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).

4. 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

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
4-nitrophenol	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 (≤ 4000 tk versus > 4000 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).

(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; Shammii 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 ($n = 1777$ mothers), prospective study conducted from 1999 to 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 OR (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 OR (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 OR (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 ($< \text{LOD}$ versus $\geq \text{LOD}$) 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 Guíñazú, 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 h 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 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 and 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.

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6. Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC) or the National Institute of Environmental Health Sciences. Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2019.105243>.

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