**ABSTRACT**

**Importance:** Prenatal exposure to BTEX (benzene, toluene, ethylbenzene and the isomers of xylene)” has been associated with numerous adverse health outcomes in animal studies; however, there have been limited studies of the associations between prenatal exposure to BTEX and measurable outcomes in human newborns.

**Objective:** To examine whether prenatal exposure to BTEX compounds is associated with changes in anthropometric measurements in newborns.

**Design:** Cross-sectional study.

**Settings:** Two major hospitals in Israel, between January 2016 and May 2020.

**Participants:** In total, 883 mother and newborn pairs who lived in urban areas and were admitted to the delivery room of either hospital were recruited.

**Exposure:** BTEX metabolites as measured in maternal urine.

**Main Outcomes and Measures:** The main outcomes were newborn birthweight, length, and head circumference, measured by neonatologists in delivery rooms. Urine samples were collected from all mothers on the day of delivery, for further BTEX and creatinine analysis. Self-reported environmental exposures, lifestyle, and sociodemographic characteristics were captured during the recruitment interview by a study coordinator. Obstetrics and medical history were obtained from medical records.

**Results:** A total of 883 mothers (mean [standard deviation (SD)] age 32.4 [4.6] years) and their healthy, term-born infants (462 [52%] male; mean [SD] infant gestational age, 39.5 [1.3] weeks) were included in the analysis. Traces of toluene, xylene, and ethylbenzene metabolites were detected in all samples, with mean [SD] concentrations of benzylmercapturic acid (23.8 [51.5] μg/g creatinine); methylhippuric acid (292.5 [527.2] μg/g creatinine); and phenylglyoxylic acid (555.2 [737.7] μg/g creatinine), respectively. Following covariate adjustments, an increase in log-transformed and interquartile-scaled ethylbenzene was found to be associated with a decrease in the birthweight of males (unstandardized β= -40.856 g; 95% confidence interval (CI) -73.073 to -8.639; P=.013). Further exclusion of newborns considered small and large for gestational age (n = 72 [8.2%]; n =84 [9.5%], respectively) resulted in an association between an increase in log-transformed and interquartile-scaled ethylbenzene to the head circumference of males (unstandardized β= -.07 cm; 95%CI:-.146 to -.005; P=.035).

**Conclusions and Relevance:** These results suggest that prenatal exposure to ethylbenzene may be associated with a decrease in male birthweight and head circumference. Expanding current knowledge concerning prenatal BTEX exposure and its possible association with fetal development is crucial and should be encouraged by health systems around the world.

**INTRODUCTION**

BTEX (benzene, toluene, ethylbenzene, and isomers of xylene) are volatile compounds present in the air of typical urban environments1. They are emitted by numerous products, including paints, solvents, automobile exhausts, and tobacco smoke2–4. Human exposure to BTEX compounds has been associated with short-term effects, including nausea, headaches, skin irritation, and asthma exacerbation5–8, and long-term effects, including various cancers and respiratory diseases9,10. BTEX compounds enter the body via skin and airways11,12. Studies among pregnant women suggest these compounds can cross the placenta13 and accumulate in the placenta and fetal tissues, explaining BTEX embryotoxicity seen in animal studies14,15. Accumulation of these compounds in fetal and maternal tissues may be associated with adverse health outcomes among newborns of exposed mothers.

Health outcomes easily monitored immediately following childbirth include the anthropometric measurements (e.g., birth weight, birth length, and head circumference). While abnormal anthropometric measurements alone can be associated with morbidity in early childhood and adulthood16,17, they may result from a series of intrauterine events18 that could instigate other future complications. Hence, it is crucial to investigate associations between various prenatal exposures with these measurable and sensitive birth outcomes. Assessment of prenatal exposure during pregnancy is challenging, involving analysis of maternal blood and urine samples. Traces of exposure found in such specimens are assumed to correlate with their levels in cord blood and a newborn’s circulation14. Epidemiological studies have examined associations between individual BTEX compounds in maternal urine samples and various adverse health outcomes in newborns, including smaller birth size and head circumference19, neurological impairments20, and pulmonary changes21.

Most human studies have focused on exposure to a single BTEX compound, rather than multiple9,20,22. Where the effects of several compounds were investigated23,24, possible interactions between compounds and their combined effects were not considered. The concentrations of BTEX compounds detected in specimens varied; some studies10,25 detected relatively high levels of volatile compounds among participants while others struggled to include compounds in their analysis due to missing data and low concentrations23. These inconsistent results emphasize the need for studies with larger sample sizes and greater validity and reliability.

We examined associations between prenatal exposure to various BTEX compounds, as measured in maternal urine, with anthropometric measurements of newborns; this included individual compounds and modeling possible interactions between compounds.

**METHODS**

***STUDY DESIGN AND POPULATION***

Between January 2016 and May 2020, pregnant women and their newborns were recruited at delivery rooms of two hospitals in Israel: Rambam Medical Center (~5500 births/year) and Shamir Medical Center (~8000 births/year). Inclusion criteria were being a Hebrew speaker, aged ≥18 years, and pregnant with a singleton. Exclusion criteria included high-risk pregnancy, preterm birth (gestational age <37 weeks), and congenital malformations. Each woman provided written informed consent to participate and completed a questionnaire, including sociodemographic characteristics, smoking status, health, and pregnancy and obstetric history. In total, 883 mother–newborn pairs were recruited (458 and 425 from Rambam and Shamir, respectively). Each pair was anonymized and assigned a unique identifier. Maternal urine samples were collected from participants on the day of delivery. Neonatologists took newborns’ anthropometric measurements. All procedures were approved by the hospitals’ Institutional Review Boards.

***MEASUREMENTS***

***URINARY BTEX AND CREATININE***

Upon admission, each participant provided a urine sample that was immediately stored at −80°C prior to analysis at a reference laboratory. BTEX analysis was performed26 for benzene metabolites (trans-trans-muconic acid [ttMA], S-phenylmercapturic acid [SPMA]), toluene metabolite (benzylmercapturic acid [BMA]), ethylbenzene metabolite (phenylglyoxylic acid [PGA]), and xylene metabolites (dimethylphenyl acid [DPMA] and methylhippuric acid [MHA]). Samples were diluted in various concentrations of fumaric acid (1%) in a 1:1 ratio, and the presence of these compounds in urine samples was analyzed using high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS\MS)27,28.

Urine creatinine was determined using a colorimetric method and used to standardize BTEX concentrations detected in urine samples.

***NEWBORNS’ HEALTH MEASUREMENTS***

All infants received a standard examination; birth weight, length, and head circumference were recorded. Each measurement was repeated three times; all results were documented in their medical records.

***LIFESTYLE AND MEDICAL RECORDS***

Using data from the questionnaires and medical registries, we adjusted our final models for potential confounders, including maternal age (continuous, in years), newborn gender, previous parities (nulliparous vs. multiparous), smoking exposure during pregnancy (yes vs. no), socioeconomic status (SES) (standardized score), and geographic area. The maternal standardized SES scores were individually calculated using a geographical information system (GIS) to match maternally reported zip codes and the annually reported geographical distribution of SES29.

As gestational age could function as a mediator affecting the pathway between exposure and outcome30, leading to over- or under-estimation of the true effect31, it was excluded from further analysis.

Information about smoking and the degree of exposure to environmental tobacco smoke during pregnancy were self-reported. Women were considered smoke-exposed if they reported being an active smoker or being exposed to environmental tobacco smoke for ≥1 hour per week during at least half their pregnancy.

***STATISTICAL ANALYSIS***

Distributional plots and descriptive statistics were examined for all variables. Mean values and standard deviations (SDs) were used to describe continuous variables; two-sided t-tests were used to compare differences between groups. Median values, interquartile ranges (IQRs), and Mann–Whitney U tests were used to describe and compare maternal urinary BTEX concentrations. We calculated z-scores for the anthropometric measurements, standardized to the mean and SD of the study population. All BTEX concentrations were divided by creatinine level, log-transformed, and standardized for the IQR to achieve a common scale and account for the positive skewness detected. ttMA, SPMA, and DPMA were detected in <5% of urine samples and thus excluded from the final models.

Multivariate linear models were used to examine associations between independent variables and anthropometric z-scaled measurements. Single-exposure models were run separately for males and females and adjusted for maternal age, previous parities, SES, and geographic area. The standardized birthweight of newborns was an independent variable in the models to examine the association of exposure to BTEX with birth length and head circumference. For further analysis, both double and triple possible interactions of BTEX variables were included in the models. The numbers of term newborns considered small or large for their gestational age32 (SGA and LGA, respectively) were relatively low (72 and 84, respectively), so we could not analyze each group separately; therefore, we repeated the linear models for newborns considered an appropriate size for gestational age (AGA). Results are presented as mean differences in SD of anthropometric measurements (with 95% confidence intervals) per IQR change in the log-transformed urine BTEX compound concentrations. Sensitivity analyses conducted included linear models run again to examine associations between non-scaled anthropometric measurements and independent variables; and several logistic models, with birth weight and head circumference coded as dichotomous variables (higher/lower than the median) and an examination of their associations with the independent variables.

For further analyses, statistical significance was two-sided and set at p < .05. All statistical processes were performed in R (version 4.1.1; R Foundation for Statistical Computing), using the packages data.table, ggplot2, dply, and lubridat.

**RESULTS**

Among 883 mother–newborn pairs recruited (Table 1), mean maternal age (SD) was 32.4 (4.6) years, and mean (SD) gestational age at delivery was 39.50 (1.31) weeks; 462 newborns (52%) were male; anthropometric measurements are shown in Table 2.

BMA, PGA, and MHA were detected in all urine samples (Figure 1). ttMA was detected in just 4.2% of samples, while neither SPMA nor DPMA were detected in any samples; these compounds were excluded from further analyses. The only BTEX compounds found to be significantly correlated were BMA and MHA, with a Spearman correlation coefficient of .09 (P = .011), and PGA and MHA, with a correlation coefficient of .07 (P = .036).

After adjusting the z-scaled anthropometric measurements for background characteristics (Figure 2), males’ birthweight was the only measure significantly associated with BTEX compound concentrations; an increase in the log-transformed IQR of PGA was associated with a decrease of .09 SD in birthweight (95%CI: -.173 to - .020; P = .013). No other significant associations were detected between any BTEX metabolite concentrations with anthropometric measurements in the single-exposure models. Examination of the triple-interaction model suggested no association between BTEX compound interactions and anthropometric measurements; however, the double-exposure (BMA:PGA) interaction model suggested significant associations between this combination and male birthweight (βBMA:PGA= -0.089 SD; P = 0.046; 95%CI: -0.177 to -0.002).

When only newborns considered AGA were included (n = 727; Figure 3), a slight change was observed in the beta coefficient of males’ birthweight as a function of PGA (β= -.08; 95%CI: -.148 to -013; P =.019), while a negative association was revealed between the increasing IQR of log-transformed PGA and males’ head circumference (β= -.062; 95%CI: -.120 to -.004; P =.036).

Possible associations of interactions between BTEX compounds and anthropometric measurements were tested again for AGA newborns only, however none were significant.

For the sensitivity analysis, we re-ran single-exposure linear models using the non-scaled anthropometric measurements as dependent variables. In this analysis, male birthweight was the only measurement significantly associated with increased IQR of the log-transformed PGA concentration (β= -40.856 g; 95%CI: -73.073 to -8.639; P =.013).

Exclusion of SGA and LGA newborns resulted in similar findings to those obtained from the models conducted previously for the scaled anthropometric measures. PGA remained significantly and negatively associated with the non-scaled male birthweight (β= -33.866 g; 95%CI: -62.122 to -5.603; P =.019) and head circumference (β= -.07 cm; 95%CI: -.146 to -.005; P =.035). For further analysis, the weight, head circumference, and length were coded as dichotomous variables, either higher or lower than the medians for males (Mewt: 3385 g; Mehc: 35 cm; Melength: 50 cm) and females (Mewt: 3225 g; Mehc: 34.5 cm; Melength: 49.4 cm). The logistic models suggested a negative association between PGA and male birthweight (odds ratio (OR) = .814; 95%CI: .687-.964; P = .017). No other significant associations were found between BTEX compounds and anthropometric measurements of either gender. Similar results were obtained from the logistic model for weight when SGA and LGA newborns were excluded (OR = .838; 95%CI: .702-.997; P = .049).

**DISCUSSION**

Previous studies have examined associations between BTEX exposures and adverse health outcomes among the general population and among pregnant women. We recruited a large sample of mother–newborn pairs and investigated the presence of BTEX metabolites in maternal urine and its possible associations with anthropometric measurements of newborns. We assumed these measurements were strongly associated with intrauterine growth and useful for evaluating the future morbidity of newborns. SPMA and DPMA were not detected in any urine samples, while ttMA was detected in <5% of samples; however, BMA, PGA, and MHA were detected in all samples. BMA levels we detected were lower (Table 3) compared with previous results among workers in factories33 and gasoline stations34, but higher than levels reported among Canadian pregnant women25. MHA levels were similar to those measured in the urine of the general population in Korea35, although with a relatively wide range. PGA levels we detected in maternal urine were higher than those detected among the Korean general population35, but lower than those found among workers in Korean factories36.

BTEX compounds can be absorbed by the human body via several routes, before being metabolized and eliminated by various processes37–40; while some are unique, most elimination processes are common for all. Most studies in humans examined associations between benzene exposure and health outcomes 19,22,41–43; however, it is reasonable to assume that other compounds, including ethylbenzene44, could affect similar metabolic pathways. The rates of metabolization and degradation of some compounds differ among males and females22, suggesting sex-related differences in metabolite clearance that could potentially be associated with different health outcomes between the sexes. Interestingly, the associations we found between prenatal exposure to BTEX compounds and anthropometric measurements were significant only among male newborns. In all models run for males, increasing levels of PGA were significantly associated with lower birthweight; these findings were supported both by the sub-analysis of the AGA group and by the sensitivity analysis.

These results support those of a previous study19; although that study examined a possible association between prenatal exposure to benzene and birthweight, they did report a stronger negative association among males than females. Our finding of a negative association between PGA levels and newborns’ head circumference at birth agreed with their findings regarding benzene exposure. This association between pollutant exposure and head circumference among male newborns was reported previously45. The question of sex-differences in the prevalence of adverse health outcomes among newborns in general following exposure to BTEX, and the higher prevalence among males in particular, has been explored in numerous *in vivo* studies. Prenatal exposure to benzene is associated with a higher risk of hematotoxicity46, metabolic imbalances47, and neuroinflammation48 among male compared with female rodents. *In vivo* experiments suggest BTEX compounds can cross the placenta to the fetus13,49,50; again, different sex-based effects were observed47, with males apparently more vulnerable to toxic effects and oxidative stress caused by exposure to these compounds51. However, the precise mechanisms involved in BTEX toxicity due to prenatal exposure remain unknown.

One hypothesis suggests BTEX compounds interfere with the pathways or action of endogenous hormones52,53, with a more prominent effect among males than females. As birthweight does not appear to be a sensitive and conclusive marker for exposure to endocrine disrupting chemicals (EDCs)54, this hypothesis should be examined further, with studies of more sensitive biomarkers to EDC exposure (e.g., anogenital distance55) performed.

As newborn weight is largely associated with intrauterine fetal metabolism56,57, and fetal metabolic rates are suggested to differ between the sexes58, it is reasonable to assume that differences in the rates of degradation and elimination of BTEX compounds between males and females could be associated with differences in concentrations of these compounds in the fetal circulation between the sexes. A study of human adults59 found that elimination of benzene metabolites was slower in women than men, likely due to the higher percentage of body fat tissue in the former and its distribution. However, the question of whether the proportion of fat tissue is higher in newborn females than males remains controversial: some studies reported significant differences60,61, others did not62. However, if female fetuses do have a higher proportion of fat, it is likely that the highly lipophilic BTEX compounds are rapidly distributed throughout their tissues, decreasing the concentration of these compounds in plasma while prolonging the duration of exposure. Conversely, a lower proportion of fat tissue in male fetuses could result in higher plasma concentrations, exposing male fetuses to higher doses for a shorter time. Relatively high levels of BTEX compounds could directly interfere with cellular mechanisms, e.g., affecting electron transfer pathways63, increasing apoptosis and mitosis15, blocking membrane transporters64, and binding to DNA and proteins65. The disruption of multiple mechanisms could indirectly increase oxidative DNA and cellular damage66, affecting fetal metabolism and homeostasis48, leading to altered fetal anthropometric measurements67.

Fetus–placenta interactions are crucial for fetal development and weight determination68–70; any disturbance of these interactions could affect fetal growth. Placental oxidative stress71 and inflammatory reactions72, for example, can impact fetal development, leading to preterm birth and reduced birthweight73. Studies have shown associations between exposure to air pollution during pregnancy with placental oxidative stress, DNA damage, an aging phenotype, and inflammation74,75. BTEX compounds as a group have been associated with an increase in mid-pregnancy inflammation markers24; other studies suggested associations between prenatal exposure and placental damage, but focused on specific BTEX metabolites76,77. Although the mechanisms mediating placental damage associated with BTEX exposure remain poorly understood, they may increase the likelihood of preterm birth24,78 and morbidity of exposed newborns76. One study79 suggested that female fetuses exhibited higher total antioxidant status and lower plasma membrane hydroperoxides in the umbilical cord artery, together with higher catalase, glutathione peroxidase, and superoxide dismutase activity, compared with males, and therefore cope with oxidative stress better than male fetuses. Higher resilience to oxidative stress in females has been linked to their highly active estrogen metabolism80, which in turn promotes the activation of glutathione. It is reasonable to assume that prenatal exposure to ethylbenzene could increase placental and fetal oxidative stress, exposing male fetuses to greater risk of developmental impairments. This proposed mechanism could explain the reduced weight and head circumference among male newborns in our study; however, *in vivo* studies should be conducted to further investigate this, along with epidemiological studies analyzing stress biomarkers in cord blood of both males and females.

***LIMITATIONS***

Our study had some limitations. As we included only full-term newborns, associations between prenatal maternal exposure to BTEX and preterm deliveries could not be examined. BTEX metabolite levels observed in our study varied, enabling us to examine the possible effect of daily exposures but also limiting the validity of our findings due to the high variances. Although BTEX metabolites could be measured in urine and were adjusted according to maternal hydration conditions, their half-lives varied. Thus, our findings cannot reflect any association between duration or prenatal timing of exposure with any of the anthropometric measurements.

***CONCLUSION***

We examined associations between BTEX metabolites in maternal urine at delivery with anthropometric measurements of their newborns, assuming the presence of BTEX traces in urine can shed light on prenatal exposure. Using a large sample of mother–child pairs, we detected metabolites of xylene, toluene, and ethylbenzene in all samples. Overall, our findings showed a significant association between increased levels of PGA and reduced birthweight, but only among male newborns. Some models suggested a negative association between PGA levels and head circumference among males. Their unique metabolism and vulnerability mean that fetuses require a relatively stable intrauterine environment. Exposure to oxidative stress agents and disruptors of DNA and hormones, as well as placental inflammation, can affect fetal homeostasis and development, leading to adverse health effects. The fetal biological and cellular mechanisms affected by prenatal exposure to BTEX compounds are incompletely understood; therefore, the associations of these compounds with the development and health of newborns should be further examined in both pathobiological and epidemiological studies. We recommend health systems around the world monitor BTEX exposures carefully to expand our current knowledge concerning their possible associations with children’s health.