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Associations of prenatal urinary melamine, melamine analogues, and aromatic amines with gestational duration and fetal growth in the ECHO Cohort

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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.

Disclaimer

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.109227.

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Abstract

Melamine, its analogues, and aromatic amines (AAs) were commonly detected in a previous study of pregnant women in the Environmental influences on Child Health Outcomes (ECHO) Cohort. While these chemicals have identified toxicities, little is known about their influences on fetal development. We measured these chemicals in gestational urine samples in 3 ECHO cohort sites to assess associations with birth outcomes (n = 1,231). We estimated beta coefficients and 95% confidence intervals (CIs) using adjusted linear mixed models with continuous dilution-standardized concentrations ($\log_2$ transformed and scaled by interquartile range, IQR) or binary indicators for detection. As secondary analyses, we repeated analyses using categorical outcomes. Forty-one of 45 analytes were detected in at least one sample, with > 95 % detection of melamine, cyanuric acid, ammelide, and aniline. Higher melamine concentration was associated with longer gestational age ($\hat{\beta}$ per IQR increase of $\log_2$-transformed: 0.082 [95 % CI: −0.012, 0.177]; 2nd vs 1st tertile: 0.173 [−0.048, 0.394]; 3rd vs 1st tertile: 0.186 [−0.035, 0.407]). Similarly in secondary analyses using categorical outcomes, an IQR increase in $\log_2$(melamine) was associated with 1.22 [0.99, 1.50] higher odds of post-term (>40 & ≤42 weeks) as compared to full-term (≥38 & ≤40 weeks). Several AAs were associated with birthweight and gestational length, with the direction of associations varying by AA. Some stronger associations were observed in females. Our findings suggest melamine and its analogs and AAs may influence gestational length and birthweight.

Keywords

Melamine; Cyanuric acid; Aromatic amines; Gestational age; Birthweight; Preterm birth; Low birthweight; Large for gestational age; Small for gestational age

1. Introduction

Melamine and aromatic amines (AAs) are high-production-volume chemicals in the U.S. (USEPA 2021) that are frequently detected in pregnant people (Choi et al. 2022). Exposure to melamine and AAs is ubiquitous as a result of contact with contaminated water,

foodstuffs, dust, and air (Palmiotto et al. 2001; Tkaczyk et al. 2020; Turesky et al. 2005; Zhu and Kannan 2018; 2019b) and the variety of products that contain these chemicals, including melamine in plastics, textiles, flame retardants, and disinfectant (NLM 2021a; b; Takazawa et al. 2020; Zhu and Kannan 2020) as well as AAs in dyes and pigments, rubber, pesticides, tobacco smoke, and pharmaceuticals (Edebali et al. 2024; IARC 2010). Widespread exposure to melamine and AAs raises health concerns due to their established toxicity in humans. Melamine and several AAs are group 1 (carcinogenic to humans), 2A (probably carcinogenic to humans), or 2B (possibly carcinogenic to humans) carcinogens as classified by the International Agency for Research on Cancer (IARC, 2024). Such carcinogenicity has been reported with exposure starting as early as pregnancy, for example, maternal pregnancy exposure to hair dyes was associated with greater odds of leukemia in children under ages 2 years (Couto et al. 2013). In utero exposures to chemicals are thought to be more toxicologically potent than in adulthood (Rice and Ward 1982). Additionally, for melamine, evidence shows harmful effects of early-life exposure on kidney such as stone formation and abnormal levels of biomarkers for kidney injury and inflammation (Gossner et al. 2009; He et al. 2009; Lam et al. 2009; Sathyanarayana et al. 2019; Shen et al. 2011; Tsai et al. 2022; Yang et al. 2013), but evidence is more limited for other health endpoints. Given data on exposure and the potential for developmental toxicity, melamine and AAs were classified as understudied priority chemicals for biomonitoring and health evaluation in the National Institutes of Health's Environmental influences on Child Health Outcomes (ECHO) Cohort (Pellizzari et al. 2019). In a pilot study, we confirmed that pregnant women in the U.S. have detectable levels of melamine, 2 melamine analogs, and 14 AAs (Choi et al. 2022).

Gestational exposure to melamine and AAs has been linked with adverse reproductive and developmental effects in animal studies (Jones 1979), with both decreased (Kim et al. 2011; Kim et al. 2023; Stine et al. 2014) and increased (An and Zhang 2016; Chu et al. 2017; Tayem et al. 2019) fetal weight reported with pregnancy administration of melamine. While epidemiologic studies that directly measured melamine and AAs in human biospecimen are sparse, studies have assessed exposure to potential sources of these chemicals, such as dyes, textiles, tobacco smoke, and traffic-related air pollution. Such previous epidemiologic studies suggest links with preterm birth (PTB) and reduced fetal growth as indicated with small for gestational age (SGA) and low birthweight (LBW). For example, occupations with high levels of exposure to certain AAs (e.g., hairdressers (Casas et al. 2015; Henrotin et al. 2015; Kersemaekers et al. 1997; Seidler et al. 1999) and textile workers (Meyer et al. 2008)), as well as sources of AA exposure in the general population (e.g. hair dye use before pregnancy (Jiang et al. 2018) and road traffic, specifically particulate matter with diameter < 2.5 μm from non-exhaust sources that may include AAs from tire wear (Smith et al. 2017)) have been associated with an increased risk of adverse birth outcomes. For melamine, detectable levels in breast milk were associated with lower birthweight (Yalcin et al. 2020). Given such indications of the harm of melamine and AAs, there is a need to evaluate prenatal exposure using improved measurements to quantify relationships between individual melamine, melamine analogs, and AAs and birth outcomes.

To characterize potential developmental impacts of gestational exposure to melamine, melamine analogs, and AAs, we measured these chemicals in urine samples from over 1,000

pregnant women to describe population exposure and to assess associations with measures of gestational duration and birthweight.

2. Materials and methods

2.1. Study population

This study used data from the ECHO Cohort, a nationwide cohort assembled with the aim of improving knowledge of early environmental factors affecting children's health (Knapp et al. 2023). We included participants with existing measurements or stored maternal gestational urine samples for analysis of melamine, melamine analogs, and AAs from 3 ECHO sites: Chemicals in Our Bodies (CiOB) (San Francisco Bay Area, CA) (Eick et al. 2021; Eick et al. 2020; Morello-Frosch et al. 2016; Wang et al. 2018), Illinois Kids Development Study (IKIDS) (Illinois) (Eick et al. 2021), and New York University Children's Health and Environment Study (NYU CHES) (New York City, NY) (Trasande et al. 2020) (Supplementary Table 1). We selected sites based on logistical and cost considerations, availability of mid-pregnancy urine samples, and geographic/sociodemographic representation. Each site selected samples from participants with sufficient stored urine volume according to their own criteria, prioritizing mid-pregnancy urine samples from pregnant participants whose children were consented for ECHO and had child health outcomes data available. Of 1,577 births with maternal urinary assay data, there were 1,252 births with information on gestational age (GA) at birth, children's birthweight, and urinary dilution (i. e., specific gravity or creatinine). Missing data for GA, birthweight, or sex was due to information not being provided to ECHO (e.g., the child participant was not consented). We further excluded multiple gestations (n = 20). Because our approach for calculating birthweight for GA z-score requires a GA ≤ 42 weeks (Aris et al. 2019), we excluded 1 birth with a GA > 42 weeks, resulting in the final analytic sample of 1,231 births (Supplementary Fig. 1).

The study protocols were reviewed and approved by the ECHO Institutional Review Boards (IRBs) (local or central). All participants provided written informed consent. The IRB at Johns Hopkins Bloomberg School of Public Health approved the involvement of the ECHO Data Analysis Center (DAC).

2.2. Measurement of melamine, melamine analogs, and aromatic amines in urine

Urine samples were shipped on dry ice to the Wadsworth Center Human Health Exposure Analysis Resource (HHEAR) laboratory for analysis. Laboratory methods for the analysis of melamine, 3 melamine analogs (cyanuric acid, ammelide, and ammeline), 41 AAs, and cotinine in urine samples using high-performance liquid chromatography-tandem mass spectrometry have been described previously (Chinthakindi and Kannan 2021; Choi et al. 2022; Zhu and Kannan 2019a). Replicates of HHEAR Quality Control (QC) Pools A and B (Kannan et al. 2021) were processed with each batch of samples; coefficients of variation (CVs) were ≤ 20 % for all analytes in both pools, with the exception of melamine in HHEAR QC Pool A (CV = 49 %). However, the HHEAR QC Pool A CV was reduced to 7.9 % after the removal of an outlier. Further details of the analytical methods have been

described elsewhere (Chinthakindi and Kannan 2021; Choi et al. 2022; Zhu and Kannan 2019a).

2.3. Birth outcomes

We used GA at birth and birthweight data ascertained via maternal or child medical record abstraction, parent report, or site-provided data (e.g. staff-reported information obtained at hospital visit). Our primary outcomes of interest were continuously modeled GA at birth and sex-and GA-specific birthweight (BW-GA) Z-scores. We calculated sex- and GA-specific birthweight (BW-GA) Z-scores based on the 2017 U.S. birthweight reference, which is a nationally representative reference of birthweight and obstetric estimates of GA (Aris et al. 2019). As secondary outcomes, we categorized GA (i.e., preterm [<37 weeks], early-term [≥37 & < 38 weeks], full-term [≥38 & ≤40 weeks, referent category], post-term [>40 & ≤42 weeks]) and BW-GA Z-scores (i.e., for GA and SGA [<10th percentile], LGA [>90th percentile], and appropriate for gestational age [AGA; 10–90th percentile; referent category]).

2.4. Covariates

Covariate information was collected at each ECHO site and harmonized by the ECHO DAC. We used a directed acyclic graph to identify potential confounders, mediators, and precision variables for consideration in analytic models (Supplementary Fig. 2). We considered the following variables as potential adjustment variables: site, maternal self-reported race/ethnicity, maternal age at delivery, maternal education, maternal parity, season of urine sample collection, calendar year of urine sample collection, urine cotinine concentration, and child's sex assigned at birth.

2.5. Statistical approach

2.5.1. Descriptive statistics—We generated descriptive statistics of all analytes, outcomes, and covariates by site. For participants with multiple samples collected across different gestational weeks, we used the measurement at the gestational week closest to the cohort median so that analyte concentrations would represent mid-pregnancy for all participants. For analytes with values below the limit of detection (LOD; Table 2), we used machine-read values when reported by the laboratory or replaced with the value with the LOD/$\sqrt{2}$ otherwise (Hornung and Reed 1990). Next, we standardized each analyte for urinary dilution. Briefly, we multiplied each analyte by the ratio of the observed dilution value to the median dilution value in their ECHO site, which yields dilution-standardized values that are in the original units and can be directly combined regardless of whether specific gravity or creatinine is used (Kuiper et al. 2021). We used specific gravity except for 2 % of the samples, which were assayed for our previous pilot study and had data on creatinine (Choi et al. 2022). As a measure of combined exposure to multiple analytes, we calculated the molar sum for each chemical class (ΣMelamine and ΣAA). We divided the concentration of each analyte by its molar weight and then summed the concentrations; analytes with a detection frequency (DF) < 20 % were not included in the sums. We calculated Spearman correlation coefficients of dilution-adjusted concentrations among analytes with ≥ 20 % DF.

2.5.2. Estimating associations with birth outcomes—For analytes with a DF ≥ 80 %, we examined linear and non-linear dose–response relationships by modeling dilution-standardized concentrations with untransformed, $\log_2$ transformed, and categorical terms based on tertiles. The untransformed and $\log_2$ transformed terms were scaled by their interquartile range (IQR) to allow comparison across analytes. Since we observed potential non-linear dose–response and better moder fit as indicated by lower AICs for the continuously modeled analytes, we report results from the $\log_2$-transformed and tertile-based models as primary and results from untransformed terms in supplementary tables. For analytes with DF ranging from 20 to 80 %, we modeled exposure using a binary indicator term (detected: values ≥ LOD; not detected: values < LOD). We did not conduct analyses of analytes with a low DF (<20 %). We imputed missing covariates using multiple imputation by chained equations (m = 10). We fit linear (GA, BW-GA z-score) or logistic (SGA, LGA, preterm, early term, late term) mixed models with a random effect for site. For binary outcomes, we fit separate logistic mixed models for each category of gestational age or birth weight compared to the referent category (term birth and appropriate for gestational age, respectively). Adjusted models included fixed effects for the following covariates: parity (multiparous, primiparous [ref]), maternal age (linear), calendar year of sample collection (linear), season of sample collection (winter [December to February], spring [March to May], summer [June to August], autumn [September to November; ref]), maternal education (graduate degree [ref]; some college or Bachelor's degree; high school or less), and cotinine as a biomarker of recent smoking (<LOD [0.1 ng/mL]; LOD-2.5 ng/mL; >2.5 ng/mL). Although we described concentrations by maternal self-reported race/ethnicity (Hispanic, non-Hispanic Asian, non-Hispanic Black, non-Hispanic others which include Native Hawaiian or other Pacific Islander, American Indian or Alaska Native, Multiple Race, or participants who selected "Other Race", non-Hispanic White), we did not adjust for race/ethnicity in outcome models, as we adjusted for education and ECHO site to block confounding pathways related to socioeconomic status. We focused on the estimation of effect estimates and corresponding 95 % confidence intervals (CIs) rather than null hypothesis testing, and described the trends of the estimates (Amrhein et al. 2019; Goodman 1993; Newman 2008; Poole 1987). We examined whether associations differed by child's sex using product term models and for the product terms, used an alpha level of 0.1 to highlight notable sex-differences.

3. Results

3.1. Descriptive characteristics

Our analytic population comprised a total of 1,231 participants from CiOB (n = 467), IKIDS (n = 297), and NYU CHES (n = 477; Supplementary Table 1). Of these 1,231 participants, most were non-Hispanic White, had some college or higher education, and gave birth in their 30 s (Table 1). Urine samples were collected from 2014 to 2020, with most collected in 2019 (42 %; Table 1). Majority of urine samples were collected during the second (68 %) or third (31 %) trimester (Table 1). Median GA at sample collection [min–max] was 23 [11–29] weeks for CiOB, 24 [20–30] weeks for IKIDS, and 25 [11–39] weeks for NYU CHES. The median GA at birth was 39.0 weeks, with 8 % preterm, 10 % early-term, and 9

% post-term births (Table 1). The median birthweight was 3,374 g, with 12 % of the births classified as SGA and 9 % as LGA (Table 1).

A total of 4 melamine/melamine analogs and 37 AAs (measured with 35 analytes) were detected in at least one sample (Table 2; Supplementary Table 2). Of these, 13 analytes were detected in ≥ 20 % of the participants, which included three melamine/melamine analogs and 11 AAs (Table 2). Cyanuric acid, melamine, and ammelide as well as one AA, aniline, were detected in 96–100 % of participants. Concentrations of analytes varied by certain sociodemographic characteristics, though patterns were not consistent across analytes (Supplementary Table 3). We observed low to moderate Spearman correlation coefficients between analytes (Fig. 1).

3.2. Melamine, melamine analogs, and birth outcomes

We observed longer GA with an IQR increase of $\log_2$ transformed melamine ($\hat{\beta}$: 0.082 [95 % CI: −0.012,0.177]), which was similar to the dose–response observed when using concentrations categorized by tertiles (2nd vs 1st tertile: 0.173 [−0.048, 0.394]; 3rd vs 1st tertile: 0.186 [−0.035, 0.407]; Fig. 2a; Supplementary Table 4). Similar trends were shown when the outcome was assessed using a binary indicator of post-term compared to term (1.22 [0.99, 1.50]; Supplementary Table 4).

While we did not observe sex-dependent associations for melamine, the highest tertile of ammelide group was associated with 0.310 [−0.007, 0.628] longer GA among females and 0.107 [−0.427, 0.212] shorter GA among males (Fig. 2a; Supplementary Table 4). When outcomes were assessed with binary indicators, an IQR increase in $\log_2$-transformed ammelide was associated with an early-term OR of 1.384 [1.004, 1.907] among males and 0.955 [0.758, 1.204] among females with an interaction term p-value of 0.06 (Supplementary Table 4). We observed similar associations for cyanuric acid (Supplementary Table 4). When combined exposure to melamine and melamine analogs was assessed using $\log_2$-transformed ΣMelamine, we observed near-null associations with wide CIs and no strong sex differences except for higher OR for early-term compared to term in males (1.354 [1.023, 1.793]) with an OR of 0.873 [0.647, 1.180] in females and a sex interaction p-value of 0.04 (Supplementary Tables 4).

Effect estimates for melamine and melamine analogs with BW-GA Z-scores were near-null with wide CIs for all analytes in crude and adjusted models (Fig. 2a; Supplementary Table 4). We did not observe evidence of sex-interaction when BW-GA Z-score was modeled continuously, however in binary-outcome models, cyanuric acid was associated with a lower OR of LGA compared to AGA among females with sex interaction p-values all below 0.09 (Supplementary Table 4).

3.3. Aromatic amines and birth outcomes

Among frequently detected AAs, we observed shorter GA with m/o-toluidine and 4,4-methylenedianiline among females (Fig. 3a) and greater BW-GA Z-scores with aniline in both sex and 4,4-methylenedianiline among females (Fig. 3b). An IQR increase in $\log_2$-transformed 4,4-methylenedianiline was associated with 0.083 [−0.218, 0.051] weeks

shorter GA in females and 0.071 [−0.059, 0.201] weeks longer GA in males with the sex interaction p-value of 0.1; correspondingly, higher 4,4-methylenedianiline was associated with higher odds of preterm birth in females only (Supplementary Table 5). Similar trends were observed for m/o-toluidine and GA (Supplementary Table 5). Aniline was associated with greater BW-GA Z-scores in 2nd (0.126 [−0.012, 0.265]) and 3rd tertile (0.135 [−0.006, 0.277]) as compared to the 1st tertile (Fig. 3b; Supplementary Table 5).

For infrequently detected AAs, we observed associations with p-toluidine and 3,4-dichloroaniline (Fig. 3 c-d; Supplementary Table 5). For example, detection of p-toluidine was associated with 0.144 [−0.332, 0.043] weeks shorter GA and 0.165 [0.047, 0.283] higher BW-GA Z-scores; we also observed associations with higher ORs of preterm and early-term compared to term births and LGA compared to AGA (Supplementary Table 5). For o-anisidine and 1,3-phenylenediamine, we observed sex-specific associations with continuously modeled GA and BW-GA Z-score, respectively Supplementary Table 5). An IQR increase in $\log_2$-transformed o-anisidine was associated with 0.106 [−0.086, 0.298] weeks longer GA in the total population, with a post-term versus term OR of 1.556 [0.978, 2.475] (Supplementary Table 5). The association with GA was stronger in females (0.280 [0.007, 0.552]) than in males (−0.059 [−0.326, 0.208]) with the sex interaction p-value of 0.08. Outcomes modeled with binary indicator terms showed that the ORs of post-term versus term birth were similarly high in both sexes except for the case of $\log_2$-transformed o-anisidine, where an IQR increase was associated with a preterm versus term OR of 1.995 [0.920, 4.324] among males but 0.799 [0.434,1.473] among females with a sex interaction p-value of 0.07 (Supplementary Table 5). When outcomes were modeled using binary indicator terms, we observed additional sex-specific association for 3-chloroaniline, with an OR for LGA versus AGA of 2.345 [1.398, 3.934] among females and 1.143 [0.683, 1.912] among males with the sex interaction p-value of 0.05 (Supplementary Table 5). When combined exposure to AAs was assessed using ΣAA, greater BW-GA Z-score (3rd vs 1st tertile: 0.117 [−0.025, 0.26]) and odds of LGA (1.693 [1.088, 2.635]) was observed in the highest tertile as compared to the lowest tertile (Supplementary Table 5).

Inferences were similar in sensitivity analyses using untransformed analyte concentrations (Supplementary Table 5).

4. Discussion

In this largest study of prenatal exposure to melamine, melamine analogs, and AAs and associations with birth outcomes (n = 1,231), we observed widespread exposure: 4 melamine/melamine analogs and 37 AAs were detected in at least one pregnancy urine sample. Eight of these analytes were detected in over 80 % of the participants, with near-universal detection (>95 %) of melamine, cyanuric acid, ammelide, and aniline. Higher levels of melamine during pregnancy were associated with small increases in the odds of LGA and post-term births. Several AAs were also associated with small increases in the odds of LGA, although the direction of associations with GA outcomes varied by analyte.

More melamine analogs and AAs were detected in the current study of 1,231 U.S. pregnant women from 3 ECHO sites (41 chemicals detected in at least 1 sample) as compared

to our prior study of 171 U.S. pregnant women from 9 cohorts (17 chemicals detect in at least 1 sample) (Choi et al. 2022). The current study expanded measurements to over 1000 samples but included only recently recruited participants from cohorts in 3 states (NY, IL, and CA), whereas the earlier study included more diversity in race/ethnicity, geography, and year of sample collection, which may have contributed to differences in analyte detection and concentrations. The median melamine concentration in this study (1.7 ng/mL) was comparable to that in our pilot (Choi et al. 2022) (1.6 ng/mL) and among non-pregnant women in the U.S. (1.6 ng/mL) (Zhu and Kannan 2019a). Ammelide was detected in 100 % of the current study population, at substantially higher levels (median: 4.1 ng/mL) than previously reported in our pilot (Choi et al. 2022) (13 % >LOD, median: <LOD) and in a study of 19 volunteers in NY (Zhu and Kannan 2019a) (99 % >LOD, median 0.84 ng/mL). Such widespread detection is alarming given the limited toxicological database and biomonitoring for ammelide (EFSA 2010) despite its potential toxicity (WHO 2009). The current study population also had more AAs detectable in urine samples (38 chemicals) than in our pilot study (Choi et al. 2022) (14 chemicals), with high levels of aniline (10.4 ng/mL) compared to our pilot study (Choi et al. 2022) (0.78 ng/mL) and a study of 58 pregnant women in Brazil (Souza et al. 2023) (1.38 ng/mL). Aniline is an end-stage metabolite of several AAs. Some AAs were less commonly detected in our study; for example, 2,4-diaminotoluene was detectable in only 2.3 % of our population, whereas detection was universal and higher in our pilot study (Choi et al. 2022) (median: 0.69 ng/mL) and in pregnant women in Brazil (Souza et al. 2023) (median: 25.2 ng/mL). Differences in the specific chemicals detectable and their levels by study population, even with similar detection limits and analytical chemistry approaches, suggest high variability in exposures to source products and calls for a need for routine biomonitoring.

Our findings indicate that prenatal exposure to melamine and its analog ammelide may result in small increases in gestational length. Interestingly, such relationships remained similar across all 3 ECHO sites with different prevalences of post-term births (estimates not reported), which supports our decision to pool estimates across sites and also suggests that our findings are likely not due to differences in medical practice by sites (e.g., geographic variations in C-sections (Henke et al. 2014)). While epidemiologic studies that focus on gestational exposure to melamine/melamine analogs and birth outcomes are lacking, animal studies have reported associations between gestational melamine and birthweight. Both decreased (Kim et al. 2011; Kim et al. 2023; Stine et al. 2014) and increased (An and Zhang 2016; Chu et al. 2017; Tayem et al. 2019) birthweight have been associated with prenatal melamine administration in rats. Low birthweight has been reported in high-dose melamine administration during organogenesis, whereas increases in birthweight were reported in lower doses (An and Zhang 2016; Chu et al. 2017; Tayem et al. 2019) and with administration throughout the entire pregnancy (An and Zhang 2016; Chu et al. 2017). The increased birthweight previously reported may in part be explained by increased gestational duration due to pregnancy melamine administration, since two out of three studies that reported increased fetal weight measured weights at the time of birth (An and Zhang 2016; Chu et al. 2017), rather than at a fixed gestation (Kim et al. 2011; Kim et al. 2023; Stine et al. 2014; Tayem et al. 2019), and slightly longer gestation is reported in one of the studies (Chu et al. 2017). Although imprecise, we observed small

increase in LGA with melamine, which is similar to the findings from animal studies on low doses. Our investigation of melamine analogs and sex-interaction contributes to the current understanding of melamine and melamine analogs on birth outcomes. Most previous studies of melamine and cyanuric acid with birth outcomes did not examine sex differences, despite that melamine and cyanuric acid reduce testosterone synthesis (Bolden et al. 2017; Chang et al. 2014; Sun et al. 2016) and prior studies report evidence of sex-specific associations with kidney outcomes (Day et al. 2024; Li et al. 2023). The underlying mechanisms for how melamine and melamine analogs exposure may affect birth outcomes remain unexplored, which calls for mechanistic studies to evaluate potential pathways, such as the disruption of metabolic systems. Further epidemiologic research on melamine and birth outcomes that focus on critical windows and exposure levels for LGA and post-term births can corroborate developmental impacts of melamine observed in the current study.

We also observed that some AAs were associated with shorter gestation (e.g., p-toluidine, o-anisidine among males, 1,3-phenylenediamine among females) and lighter birthweight for gestational age (e. g., 3,4-dichloroaniline). While direct comparison with previous literature is limited due to lack of studies that directly measured melamine and AAs in human biospecimen, our findings are in line with occupational epidemiologic studies that assessed exposure through job types or job-exposure matrix. Fetal growth restriction as indicated by LBW (Casas et al. 2015; Henrotin et al. 2015; Kersemaekers et al. 1997; Meyer et al. 2008) and SGA (Henrotin et al. 2015) was reported among hairdressers, occupationally exposed to high levels of AAs (Johansson et al. 2015) that are often included in hair products (IARC 2010); specifically women with occupational exposure to AAs as determined by job-exposure matrix, majority of which were hairdressers, had higher odds of giving birth to SGA as compared to women with no occupational exposue to AAs (Seidler et al. 1999). Further, our findings regarding 1,3-phenylenediamine are supported by an animal study that observed teratogenic effects of phenylenediamines (Marks et al. 1981). Since fetal growth is a general indicator of fetal health and predictive of long-term health, investigation of prenatal AAs exposure as risk factors for other adverse health outcomes during infancy and childhood is warranted.

Gestational exposure to some AAs (e.g., 3-chloroaniline, p-toluidine, aniline) were associated with slightly heavier birthweight, with the highest tertile of combined AAs exposure associated with greater BW-GA Z-score and LGA. We also observed longer gestational length with some AAs (e.g., o-anisidine and 1,3-phenylenediamine, 3-chloroaniline among females). A potential mechanism of action for AAs on LGA could be through disturbing the cardiometabolic system of pregnant women, since liver is a well-known target organ for multiple AAs and the cardiovascular system is another suspected target organ (IARC 2010). Heavier birthweight for gestational age and LGA are often not a included as an outcome in epidemiologic investigation. Our findings suggest the need to examine these endpoints in future research, particularly since LGA increases health concerns not only for babies (e.g., neonatal complications (Scifres 2021), obesity (Derraik et al. 2020)) but also their birthing parents (e.g., C-section (Campbell 2014)).

One strength of our study lies in its characterization of levels of melamine, melamine analogs, and multiple AAs in a large number of U. S. pregnant participants and

measurement of a comprehensive suite of AAs. This creates a new publicly available data that can be linked with other child health outcomes that may be more sensitive to these exposures. Further, our examination of both SGA and LGA enabled us to assess the potential impact of gestational melamine, melamine analogs, and AAs on LGA, which has been largely understudied. Our investigation of SGA and PTB was, however, based on a relatively small number of cases and was therefore limited in power. We were also limited to using single spot urine samples and the estimates were therefore subject to bias from exposure measurement error, which may have resulted in imprecise effect estimates. While there are no studies of intra-individual variability during pregnancy, a study of non-pregnant healthy volunteers suggest that urinary melamine and melamine analogs are likely to represent short-term, recent exposure (Zhu and Kannan 2019a). Since samples were collected in the 2nd trimester, a relevant exposure window that precedes the observed event, findings from our study suggest shortterm effects of melamine and AAs on birth outcomes. Additionally, our birth outcomes analyses were restricted to 3 ECHO sites with highly educated, predominantly non-Hispanic White women, a majority of whom gave birth in 2016–2019. Lastly, residual confounding is possible by factors such as co-occurring pollutants. The potential for confounding by phthalates, another well-characterized risk factor for birth outcomes, is low in our study population given low correlations ranging from −0.09 to 0.23. Tobacco smoke exposure, another well-characterized risk factor for birth outcomes, may raise concerns for confounding as it may contain AAs such as aniline, p-toluidine, and o-anisidine (Goniewicz and Czogala 2005; Stabbert et al. 2003). However, we adjusted for urinary cotinine concentration to address confounding and there is also a high proportion of non-detected cotinine levels in our study population (41 %), which shows that the exposure to environmental tobacco smoke and active smoking is low during pregnancy as reported in previous literature (Aurrekoetxea et al. 2013).

5. Conclusion

In this largest study of melamine, melamine analogs, and AAs in U.S. pregnant women, we observed frequent detection of several analytes. associations with birth outcomes including LGA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data statement

Select de-identified data from the ECHO Program are available through NICHD's Data and Specimen Hub (DASH). Information on study data not available on DASH, such as some Indigenous datasets, can be found on the ECHO study DASH webpage.

Data availability

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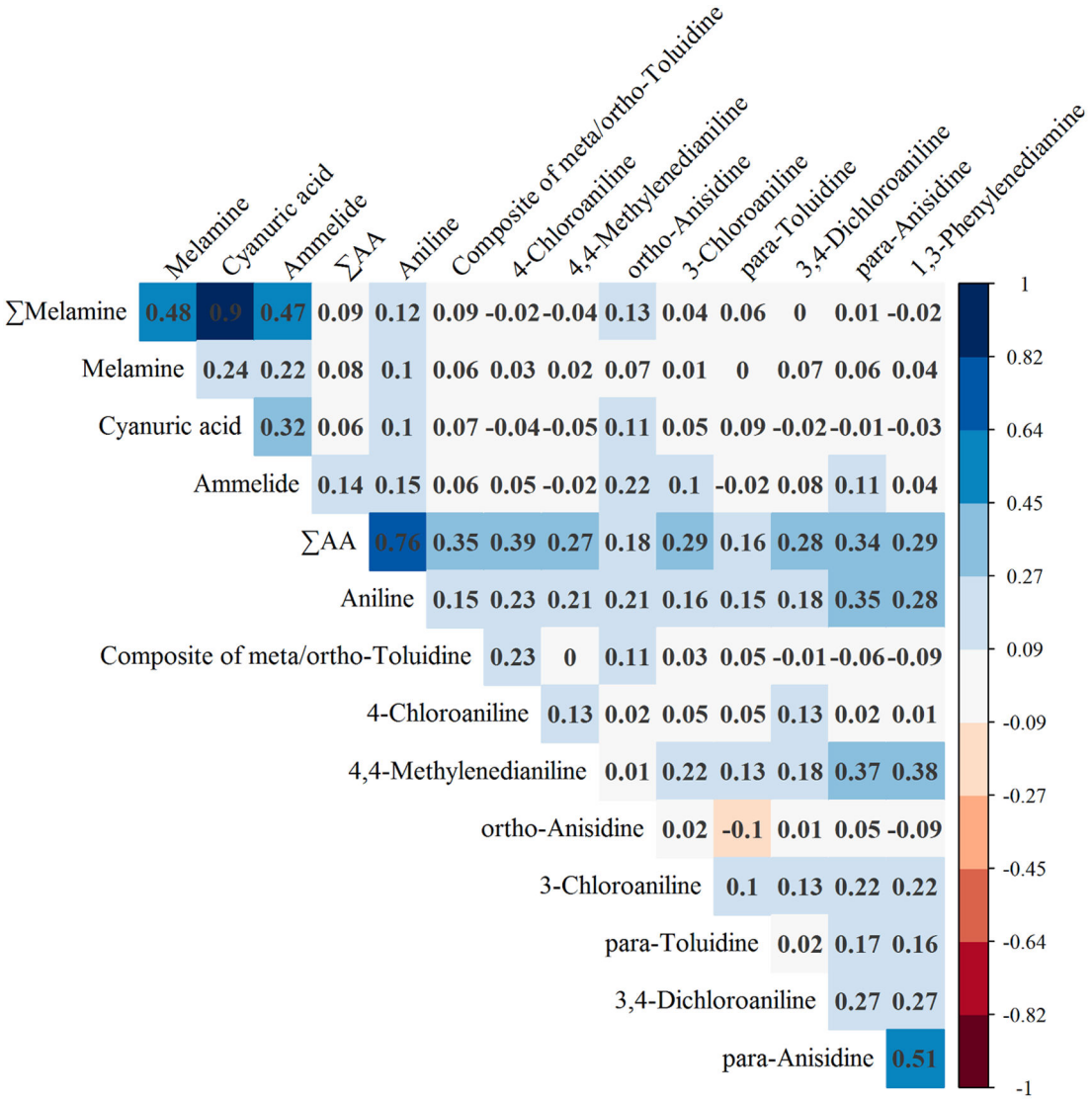


Fig. 1.

Spearman correlation coefficients of 13 urinary analytes (dilution-standardized) detected in ≥ 20 % of samples from 1,231 pregnant women from 3 ECHO sites. Abbreviations: AA, aromatic amine.

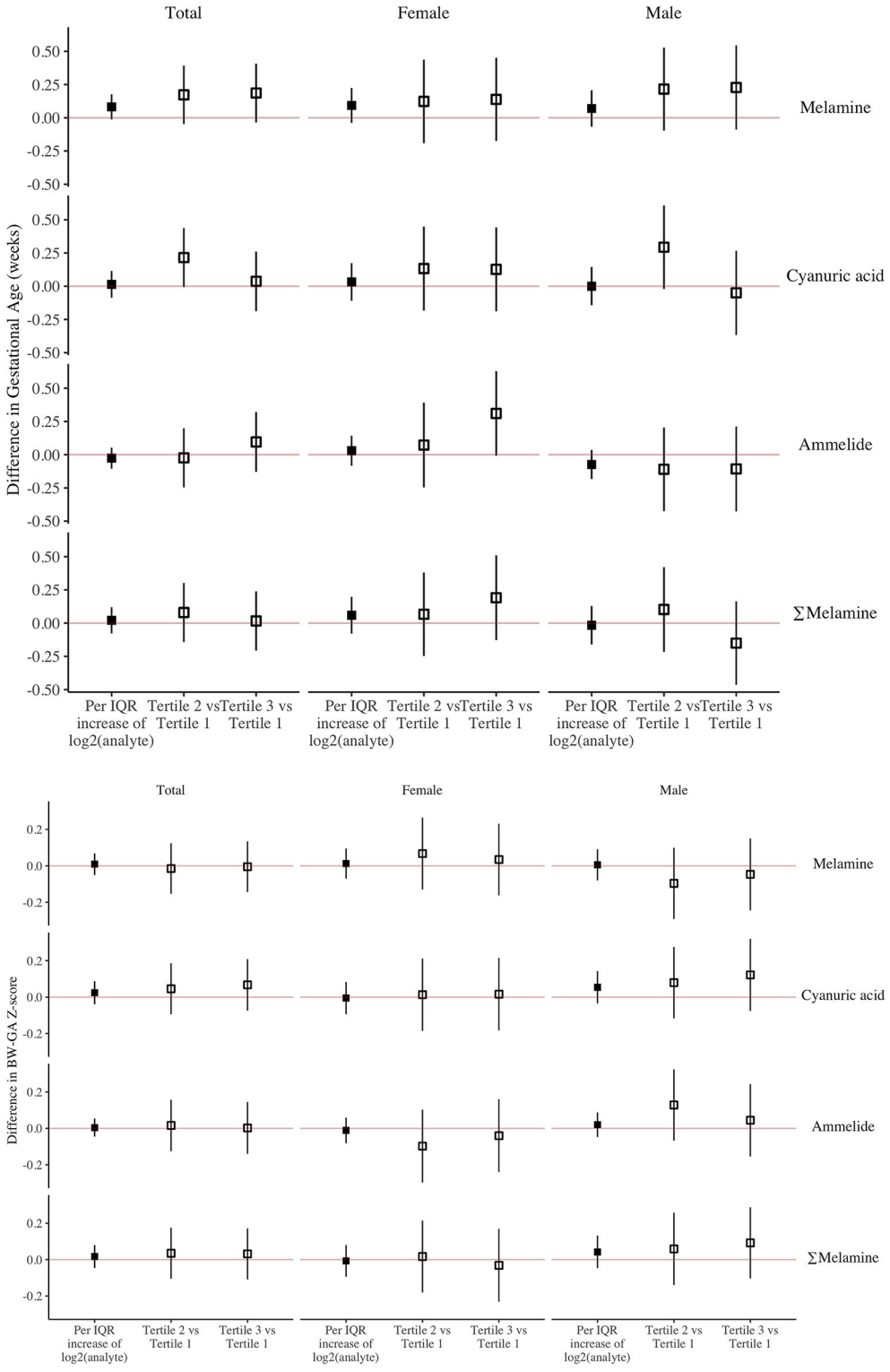
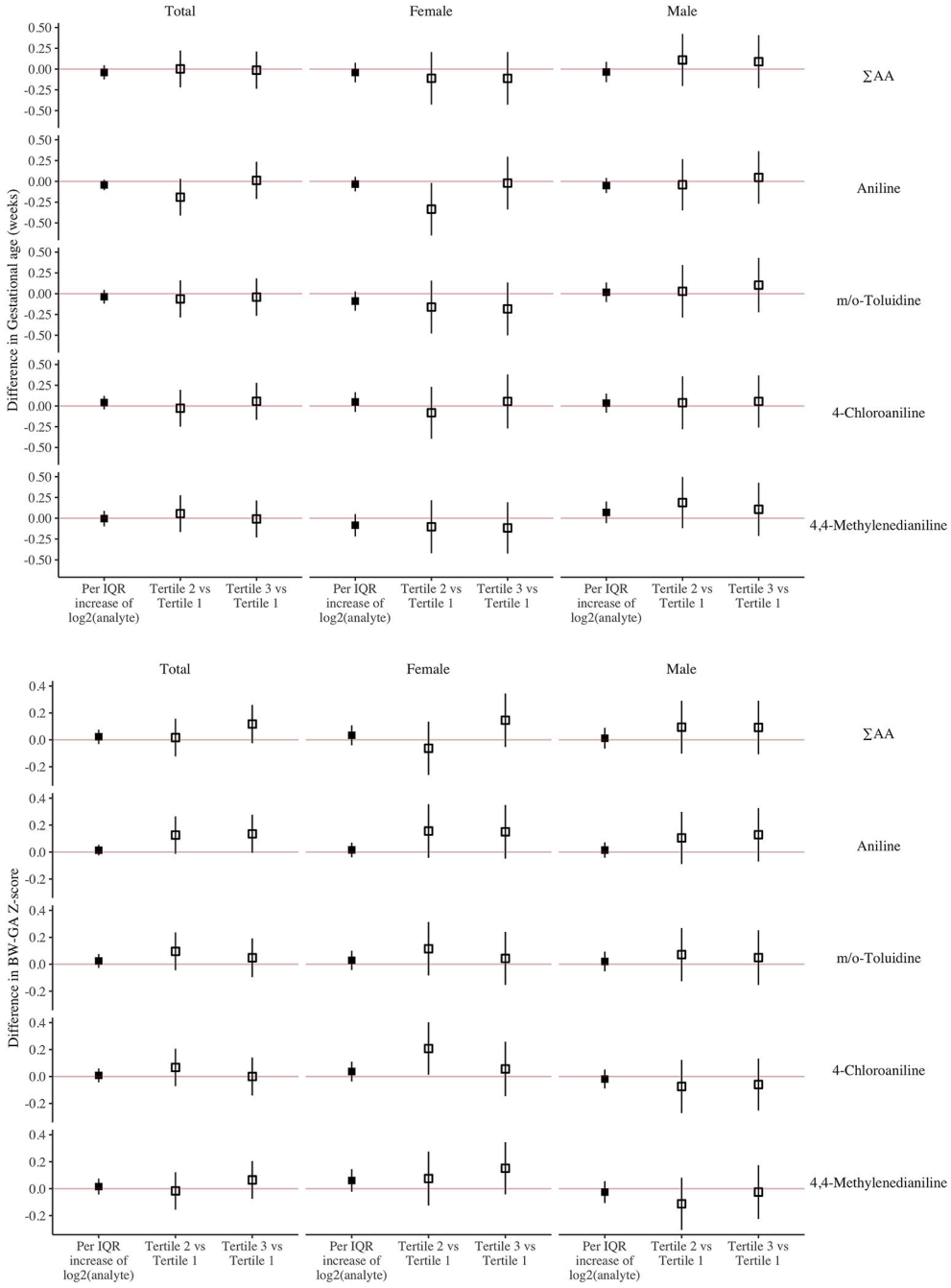


Fig. 2.

Adjusted beta coefficients of continuously modeled birth outcomes per contrast in gestational urinary melamine and melamine analogue concentrations in total and sex-stratified models. (a) Gestational age in weeks*. *Sex-interaction term p-values for

log2(analyte), tertile2 vs tertile 1, tertile 3 vs tertile 1: (melamine: 0.81; 0.68; 0.69; cyanuric acid: 0.75; 0.47; 0.43; ammelide: 0.19; 0.42; 0.06; ΣMelamine: 0.45; 0.87; 0.13). (b) BW-GA Z-score**. **Sex-interaction term p-values: (melamine: 0.91; 0.25; 0.57; cyanuric acid: 0.35; 0.64; 0.45; ammelide: 0.52; 0.11; 0.55; ΣMelamine: 0.43; 0.77; 0.38). Abbreviations: BW-GA Z-score, gestational age standardized birth weight Z-score; IQR, interquartile range.



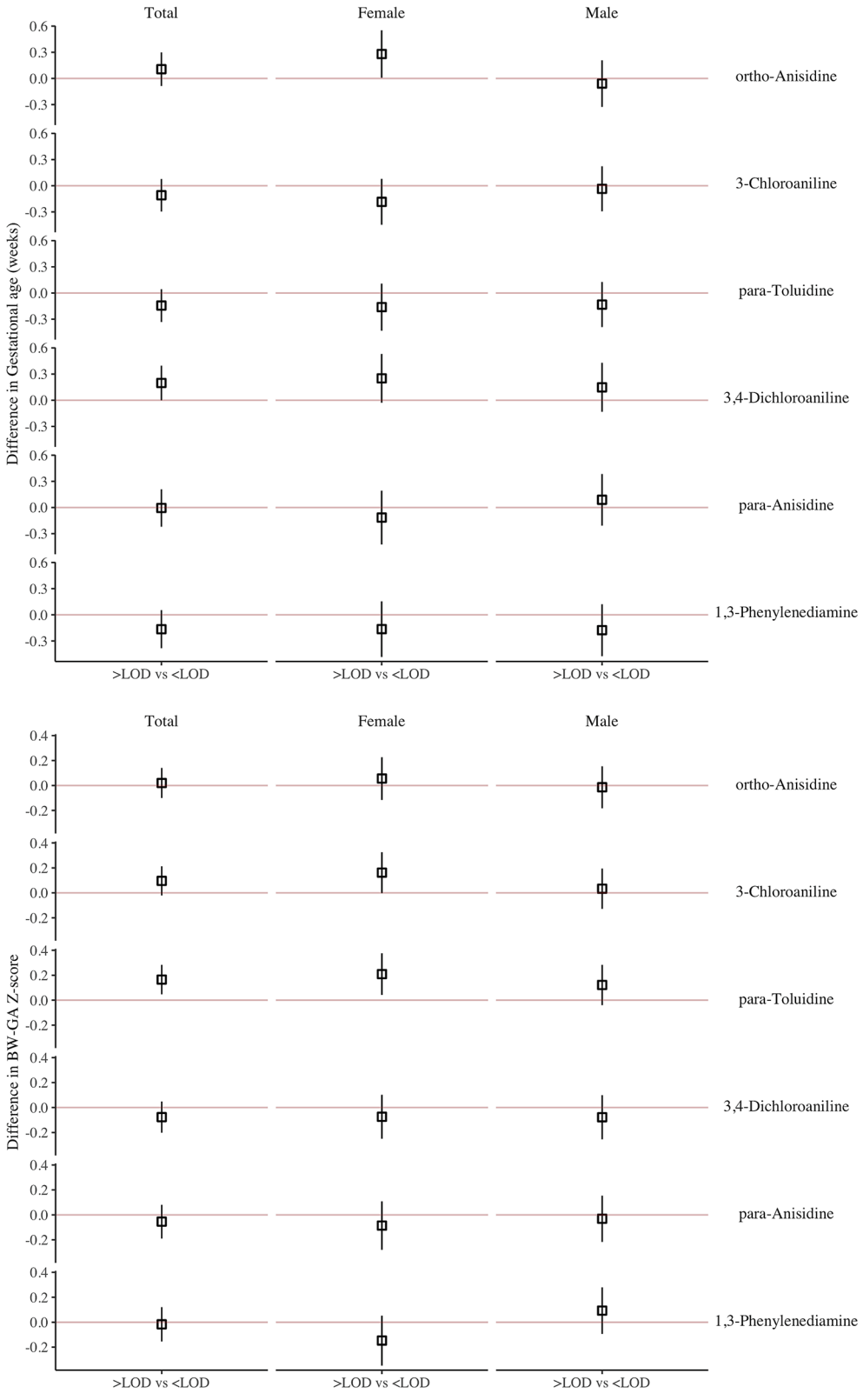


Fig. 3.

Adjusted beta coefficients of continuously modeled birth outcomes per contrast in gestational urinary aromatic amine concentrations in total and sex-stratified models. (a) Gestational age in weeks and frequently detected AAs*. *Sex-interaction term p-values for $\log_2$(analyte), tertile2 vs tertile 1, tertile 3 vs tertile 1: (ΣAA: 0.94; 0.33; 0.37; Aniline: 0.8; 0.19; 0.77; Composite of meta/ortho-Toluidine: 0.2; 0.4; 0.2; 4-Chloroaniline: 0.87; 0.59; 1; 4,4-Methylenedianiline: 0.1; 0.2; 0.32). (b) BW-GA Z-score and frequently detected AAs**. **Sex-interaction term p-values for $\log_2$(analyte), tertile2 vs tertile 1, tertile 3 vs tertile 1: (ΣAA: 0.69; 0.27; 0.7; Aniline: 0.98; 0.72; 0.88; Composite of meta/ortho-Toluidine: 0.87; 0.76; 0.97; 4-Chloroaniline: 0.29; 0.05; 0.41; 4,4-Methylenedianiline: 0.15; 0.18; 0.21). (c) Gestational age in weeks and infrequently detected AAs*. *Sex-interaction term p-values: (ortho-Anisidine: 0.08; 3-Chloroaniline: 0.42; para-Toluidine: 0.87; 3,4-Dichloroaniline: 0.61; para-Anisidine: 0.34; 1,3-Phenylenediamine: 0.96). (d) BW-GA Z-score and infrequently detected AAs*. *Sex-interaction term p-values: (ortho-Anisidine: 0.57; 3-Chloroaniline: 0.27; para-Toluidine: 0.45; 3,4-Dichloroaniline: 0.98; para-Anisidine: 0.68; 1,3-Phenylenediamine: 0.08). Abbreviations: AA, aromatic amines; BW-GA Z-score, gestational age standardized birth weight Z-score; LOD, limit of detection; IQR, interquartile range.

Table 1

Descriptive characteristics of 1,231 births from 3 Environmental influences on Child Health Outcomes (ECHO) sites.

Variable	Descriptions	N (%)
Covariates		
Maternal age at delivery (years)	< 25	71 (6 %)
	25 to < 30	179 (15 %)
	30 to < 35	616 (50 %)
	≥ 35	365 (30 %)
Maternal race/ethnicity (missing = <5)	Hispanic	338 (28 %)
	Non-Hispanic Asian	153 (13 %)
	Non-Hispanic Black	45 (4 %)
	Non-Hispanic Other *	35 (3 %)
	Non-Hispanic White	657 (54 %)
Maternal highest educational attainment (missing = 29)	High school or less	226 (19 %)
	Some college or Bachelor's degree	492 (41 %)
	Master's, professional, or doctorate degree	484 (40 %)
Maternal parity (missing = 10)	Multiparous	436 (36 %)
	Nulliparous	785 (64 %)
Maternal urinary cotinine during pregnancy (dilution-standardized)	Not detected (<LOD)	506 (41 %)
	Low (LOD–2.50 ng/mL)	305 (25 %)
	High (>2.50 ng/mL)	420 (34 %)
Season of sample collection	Winter (December–February)	279 (23 %)
	Spring (March–May)	342 (28 %)
	Summer (June–August)	323 (26 %)
	Autumn (September–November)	287 (23 %)
Year of sample collection	2014	106 (9 %)
	2015	131 (11 %)
	2016	189 (15 %)
	2017	130 (11 %)
	2018	130 (11 %)
	2019	529 (43 %)
	2020	16 (1 %)
Trimester at sample collection	First (0–13 completed weeks)	10 (1 %)
	Second (14–26 completed weeks)	840 (68 %)
	Third (27 + completed weeks)	381 (31 %)
Child's sex assigned at birth	Female	608 (49 %)
	Male	623 (51 %)
Birth outcomes		
Gestational age at delivery	Weeks, median [Q1, Q3]	39.0 [38.0, 40.0]
	Preterm (<37 weeks)	92 (8 %)
	Early term (≥37&<38 weeks)	123 (10 %)

Variable	Descriptions	N (%)
	Full term (≥38&≤40 weeks)	910 (74 %)
	Post-term (>40&≤42 weeks)	106 (9 %)
Birthweight	g, median [Q1, Q3]	3,374.0 [3,075.0, 3,705.0]
	BW-GA Z-score median [Q1, Q3]	0.1 [−0.6, 0.8]
	AGA (10–90th Aris percentile)	972 (79 %)
	SGA (<10th Aris percentile)	144 (12 %)
	LGA (>90th Aris percentile)	114 (9 %)

Abbreviations: AGA, appropriate for gestation age; LGA, large for gestational age; LOD, limit of detection; Q1, 25th percentile; Q3, 75th percentile; SGA, small for gestational age.

* includes Native Hawaiian or other Pacific Islander, American Indian or Alaska Native, Multiple Race, or participants who selected “Other Race”).

Table 2

Descriptive statistics of urinary concentrations of analytes detected in ≥ 20 % of samples from 1,231 pregnant women from 3 ECHO sites.

Analyte (unit)	n (%) > LOD	LODs	Dilution-standardized			Unstandardized		
			GM (GSD) *	Median [Q1, Q3]	Maximum	GM (GSD) *	Median [Q1, Q3]	Maximum
Melamine Panel								
Cyanuric acid (ng/mL)	1231 (100 %)	0.156, 0.080	18.8 (2.0)	17.8 [12.5, 26.1]	3455	17.1 (2.3)	17.2 [9.8, 28.9]	2870
Melamine (ng/mL)	1231 (100 %)	0.09, 0.03	2.0 (2.8)	1.7 [1.0, 3.1]	378	1.9 (3.1)	1.6 [0.9, 3.1]	490
Ammelide (ng/mL)	1206 (98 %)	0.135, 0.050	3.6 (2.4)	4.1 [2.7, 5.7]	98	3.3 (2.6)	3.7 [2.2, 6.0]	71
ΣMelamine (nmol/mL) **	NA	NA	0.2 (1.9)	0.2 [0.1, 0.3]	27	NA	NA	NA
Aromatic Amines Panel								
Aniline (ng/mL)	1179 (96 %)	0.1	10.4 (3.6)	11.5 [7.555, 18.5]	3341	9.5 (3.7)	11.3 [7.3, 17.1]	1380
Composite of m/o-toluidine (ng/mL)	1110 (90 %)	0.05	1.6 (4.0)	1.8 [0.964, 3.3]	137	1.4 (4.8)	1.8 [0.8, 3.7]	98
4-Chloroaniline (ng/mL)	1082 (88 %)	0.05	0.8 (4.3)	0.9 [0.456, 1.7]	290	0.8 (5.0)	0.9 [0.4, 1.8]	333
4,4-Methylenedianiline (ng/mL)	1012 (82 %)	0.030, 0.025	0.7 (6.5)	1.1 [0.318, 2.2]	534	0.6 (5.8)	1.1 [0.4, 1.9]	48
o-Anisidine (ng/mL)	809 (66 %)	0.030, 0.025	0.3 (6.9)	0.5 [<LOD, 1.2]	72	0.3 (7.2)	0.4 [<LOD, 1.3]	98
3-Chloroaniline (ng/mL)	553 (45 %)	0.05	0.2 (10.0)	<LOD [<LOD, 1.5]	270	0.2 (10.1)	<LOD [<LOD, 1.450]	252
p-Toluidine (ng/mL)	524 (43 %)	0.030, 0.025	NA	<LOD [<LOD, 1.5]	21	NA	<LOD [<LOD, 1.500]	44
3,4-Dichloroaniline (ng/mL)	363 (29 %)	0.1	NA	<LOD [<LOD, 1.7]	205	NA	<LOD [<LOD, 1.920]	68
p-Anisidine (ng/mL)	295 (24 %)	0.05	NA	<LOD [<LOD, <LOD]	206	NA	<LOD [<LOD, <LOD]	59
1,3-Phenylenediamine (ng/mL)	272 (22 %)	0.2	NA	<LOD [<LOD, <LOD]	67	NA	<LOD [<LOD, <LOD]	51
ΣAA (nmol/mL) **	NA	NA	0.2 (2.6)	0.2 [0.2, 0.4]	38	NA	NA	NA

* calculated for continuously modeled analytes (≥80 % above the LOD).

** calculated by summing the concentrations of frequently detected analytes scaled by their molecular weights.

Abbreviations: ECHO, Environmental influences on Child Health Outcomes; GM, geometric mean; GSD, geometric standard deviation; LOD, limit of detection; Q1, 25th percentile; Q3, 75th percentile; NA: not applicable.