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Preterm Birth and Metal Mixture Exposure among Pregnant Women from the Navajo Birth Cohort Study

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Background: Preterm birth (PTB), defined as birth before 37 wk gestation, is associated with hypertension, diabetes, inadequate prenatal care, unemployment or poverty, and metal exposure. Indigenous individuals are more likely to have maternal risk factors associated with PTB compared with other populations in the United States; however, the role of environmental metals on PTB among pregnant Indigenous women remains uncertain. Previous research identified associations between PTB and individual metals, but there is limited investigation on metal mixtures and this birth outcome.

Objectives: We used a mixtures analysis framework to investigate the association between metal mixtures and PTB among pregnant Indigenous women from the Navajo Birth Cohort Study (NBCS).

Methods: Maternal urine and blood samples were collected at the time of study enrollment and analyzed for metals by inductively coupled plasma dynamic reaction cell mass spectrometry. Bayesian Profile Regression was used to identify subgroups (clusters) of individuals with similar patterns of coexposure and to model association with PTB.

Results: Results indicated six subgroups of maternal participants with distinct exposure profiles, including one group with low exposure to all metals and one group with total arsenic, cadmium, lead, and uranium concentrations exceeding representative concentrations calculated from the National Health and Nutrition Examination Survey (NHANES). Compared with the reference group (i.e., the lowest exposure subgroup), the subgroup with the highest overall exposure had a relative risk of PTB of 2.9 times (95% credible interval: 1.1, 6.1). Exposures in this subgroup were also higher overall than NHANES median values for women 14–45 years of age.

Discussion: Given the wide range of exposures and elevated PTB risk for the most exposed subgroups in a relatively small study, follow-up investigation is recommended to evaluate associations between metal mixture profiles and other birth outcomes and to test hypothesized mechanisms of action for PTB and oxidative stress caused by environmental metals. https://doi.org/10.1289/EHP10361

Introduction

There are >160,000 abandoned hardrock mines in the western United States.[1] An estimated 600,000 tribal community members live within 10 km of these abandoned mines, increasing the potential for exposure to multiple metals and metalloids (henceforth referred to as metal mixtures).[1] Traditional cultural practices, including the reliance on local resources, may increase contact with environmental metals in soil, water, or other environmental media more than is typically assumed in risk models.[2,3] On the Navajo Nation for example, there are >500 abandoned uranium (U) mines and residents are concerned that these sites contribute to health disparities and adverse birth outcomes.[4–6] Previous environmental research on the Navajo Nation has documented the occurrence of U, arsenic (As), and other co-occurring metals in soil and water resources.[7–10] The presence of metal mixtures in environmental media on the Navajo Nation has also been associated with a greater risk for cardiovascular disease; having multiple chronic diseases when combining cardiovascular, diabetes, and kidney disease; and autoimmune disease in exposed individuals.[11–15] Reliance on unregulated drinking water sources or provision of public drinking water that fails to meet Safe Drinking Water Act regulations[16] (i.e., maximum contaminant levels) set by the U.S. Environmental Protection Agency (EPA) may also lead to greater exposure to environmental metals, such as As, U, manganese (Mn), and others.[1,9,17] Furthermore, our previous analyses have shown that metal consumption in drinking water with concentrations of As and U even below the Safe Drinking Water maximum contaminant levels set by the U.S. EPA were associated with increased production of autoimmune markers.[14] These results and community concerns about the effects of environmental metal exposures for the health of future generations drives this examination of birth outcomes associated with a range of metal mixture exposures on the Navajo Nation.

In response to community concerns about the impact of exposure to U and other co-occurring metals on the health of future generations, the Navajo Birth Cohort Study (NBCS) was initiated in 2010.[18] The goal of the NBCS was to investigate associations between U exposure, birth outcomes, and child development. This study began recruiting pregnant women from across the Navajo Nation in 2013. The NBCS provides a platform to assess exposure to metals and metalloids during pregnancy among a cohort with a range of exposures to U and co-occurring metals and metalloids. As part of the study, urine, blood, and serum samples were collected from enrolled mothers and analyzed for metals. Previous results from the NBCS biomonitoring data have shown higher concentrations of metals and metalloids among pregnant Navajo women and men compared with the National Health and Nutrition Examination Survey (NHANES) medians.[15,19,20] Median urine U concentrations in pregnant Navajo women were 2.8 times greater than the median value for women in the United States[21]; median urine concentrations of Mn, cadmium (Cd), and lead (Pb) were

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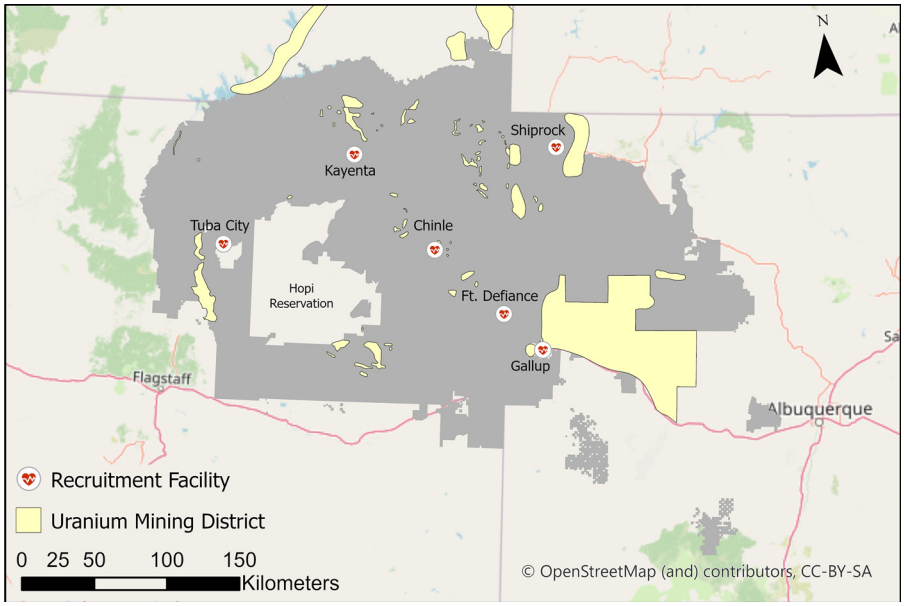

Figure 1. Map of the Navajo Nation (darker gray shaded area) located in the Southwestern United States. The lighter, yellow shaded areas on and adjacent to the Navajo Nation represent previous uranium mining areas.

also elevated compared with the U.S. population,[21] whereas serum zinc (Zn) concentrations were typically lower than World Health Organization (WHO) recommended sufficiency concentrations,[5] and urine iodine (I) measurements suggest population-level insufficiency.[22]

Risk of preterm birth (PTB), defined as birth before 37 wk gestational age, has social, economic, and environmental components. In the United States, PTB rates for all racial and ethnic groups collectively fell from 10.4% to 8.4% between 2007 and 2014 and then increased to 10.0% by 2018.[23,24] Indigenous peoples, however, consistently experience higher rates of PTB when compared with the national average in the United States.[25] Regional differences have also been observed indicating important differences among Indigenous communities due to geography, employment status, education, household income, and prevalence of chronic health conditions, such as hypertension and diabetes mellitus[25,26]; notably, contributions of environmental metals were not included in these previous investigations. Estimated frequency of PTB for Indigenous peoples in the United States ranges between 11%–14%,[27–30] including 11% for the Navajo Nation in 2017. Individuals from Indigenous communities are more likely to have maternal risk factors that are associated with PTB, such as hypertension, diabetes, inadequate prenatal care, and diminished employment opportunity and earnings.[31] Previous studies have found associations between PTB[32] and maternal exposure to U,[33] As,[34,35] thallium (Tl),[36] Pb, Mn, and Zn,[37] and mercury (Hg),[38] as well as Cd.[39] Literature suggests an inconsistent association between PTB and Hg or As (assessed individually), whereas associations with Pb and Cd are well documented and consistent.[40] Although many of these studies investigated associations between individual metals, few have investigated associations with metal mixtures and PTB.[24,41] It remains challenging to quantify joint effects of multiple exposures on health outcomes.[42–46] Cluster analysis has emerged as one method for classifying individuals into subgroups with similar characteristics (e.g., exposure profiles) and has been used to evaluate exposure–response relationships to chemical mixtures measured in blood[47] and air samples.[48] A clustering approach enables investigation of the aggregate effect of multiple chemicals on an outcome of interest and is useful when exposure covariates are correlated.[49] There is limited work investigating the combined effects of biomarkers of metal mixtures exposure and PTB. The purpose of this paper was to investigate associations between exposure to metal mixtures during pregnancy and PTB, one of the outcomes of concern among the participating communities. We hypothesized that greater exposure to metal mixtures, as evidenced by biomonitoring results, would be associated with higher probability of PTB.

Methods

Study Area and NBCS

The Navajo Nation encompasses >70,000 km^2 of Utah, Arizona, and New Mexico in the Four Corners area of the Southwestern United States (Figure 1). Pregnant Navajo women included in the present analysis were recruited for the NBCS between February 2013 and June 2018. Trained Navajo staff recruited women during pregnancy confirmation visits to obstetric/gynecology clinics at participating Indian Health Service (IHS) and Public Law 638 (PL 638) hospital facilities on the Navajo Nation. Inclusion criteria included *a*) a clinically confirmed pregnancy, *b*) maternal age between 14 and 45 years of age, *c*) residence on the Navajo Nation for a minimum of 5 y, *d*) willingness to deliver at one of six participating HIS or PL 638 hospital facilities, and *e*) willingness to allow follow-up evaluations during the baby's first year of life. Enrollment targeted women during their first trimester (<13 wk gestation), but women could enroll at any time during their pregnancy. All women provided written informed consent for the study protocol, which was approved and overseen by the University of New Mexico Institutional Review Board and the Navajo Nation Human Research Review Board. Publication of this manuscript adheres to all Principal Investigator (PI) expectations set by the Navajo Nation Human Research Review Board.

Biological Sample Collection

Spot urine, blood, and serum sample collection and storage was conducted by trained hospital or clinic staff following U.S. Centers for Disease Control and Prevention (CDC) National Center for Environmental Health's (NCEH) Division of Laboratory Sciences (DLS) (Atlanta, Georgia) recommendations and NHANES protocols.[50] Biomonitoring samples were

collected during the appointment when the participant enrolled in the study. Blood and urine samples were stored at −80 °C in a freezer until transferred on dry ice to freezer storage facilities at the University of New Mexico (Albuquerque, New Mexico), where they were also stored at −80 °C. Samples were shipped overnight on dry ice to the CDC NCEH's DLS for analysis.

Biomonitoring Sample Preparation and Analysis

Urine concentrations of antimony (Sb), As (total), barium (Ba), beryllium (Be), Cd, cobalt (Co), cesium (Ce), I, Pb, Mn, Hg, molybdenum (Mo), platinum (Pt), strontium (Sr), tin (Sn), Tl, tungsten (W), and U, were measured using inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS),[51–55] as were blood concentrations of Cd, Mn, total Hg, and Pb, and serum concentrations of Zn, selenium (Se), and copper (Cu).[56,57] The limit of detection (LOD) for these elements in urine, blood or serum ranged from 0.002 to 24.48 ng/mL, depending on the analyte and biological media (Table S1). Urine creatinine concentrations were determined using Enzymatic by Roche/Hitachi Modular P Chemistry Analyzer[58] and analyzed at the CDC NCEH's DLS.

To prepare the biomonitoring data (24 elements measured in blood, serum, and urine) for statistical analysis, several steps were completed, including assessing their reliability (using proactive and reactive quality assurance processes to check for missingness, completeness, and verifying unexpected values with the laboratory) for including in statistical analyses and creatinine adjustment for urine analytes. Here, we excluded Be and Pt because <10% of participants had measurable concentrations of these chemicals. In addition, I was excluded because it is an unreliable individual-level biomarker.[59,60] Once biomonitoring variables were selected, creatinine-corrected concentrations of elements measured in urine were computed.

Medical Record Abstraction and Enrollment Survey

Medical record abstractions were completed for all 765 participants enrolled in the NBCS during the study period (February 2013 through June 2018). Participants lacking biomonitoring ($N = 225$), with no information about prenatal risk factors recorded by the provider in their medical records ($N = 76$), and participants with a multiparous pregnancy ($N = 6$) were excluded from the present analysis. In addition, participants were excluded from analysis if the gestational age of the fetus at delivery ($N = 24$) or if the labor onset method (spontaneous or induced, $N = 17$) was not recorded in the medical records. After these exclusions, 417 women were included in the present analysis. Additional information was extracted from medical records, including prepregnancy height and weight to calculate prepregnancy body mass index (BMI) (in kilograms per meter squared), the estimated due date (based on ultrasound imaging), and child's date of birth to calculate gestational age at enrollment and length of gestation period. During prenatal care appointments, IHS medical providers evaluated mothers for medical and sociodemographic risk factors known to increase risk of PTB.[26] These risk factors included *a*) maternal age was <16 or >35 years of age ($N = 88$), *b*) being a lifetime cigarette smoker ($N = 37$), and *c*) consumption of alcohol during pregnancy ($N = 35$). Low frequency of smoking and alcohol consumption in this cohort are supported by other investigations.[61,62] Using information for these factors collected by IHS providers as part of their standard care, we calculated a prenatal risk score for this analysis to account for factors reported previously to be associated with PTB.[63,64] Owing to the small sample size for individual factors, each variable was recorded as a binary variable (not reported by the provider in the medical record = 0, or, yes, reported by the provider in the medical record = 1) and summed. Similar to Coker et al., in the present analysis the summed value was used for statistical modeling.[49]

An enrollment survey was also administered by trained staff to collect sociodemographic and other information. Relevant enrollment survey questions included the following: date of birth and current age; smoking history (defined as having smoked 100 cigarettes in her lifetime—no or yes); maternal educational achievement (completed high school—no or yes); employment status (unemployed or employed); annual household income (<$10,000; $10,000–$40,000; or >$40,000); gravity (*N*) and parity (*N*).

Summary Statistics

Correlations among metals exposure variables were determined using Spearman's rho (ρ) and visualized as a heat map. Summary statistics (median and 25th and 75th percentile values) were calculated using the NADA package (version 1.5.6) for R because some observations were less than the LOD, also known as left-censored data.[65] Robust Regression on Order (ROS) statistics, a left-censored statistical method, was employed for this analysis.[66–68] The robust ROS method fits a linear regression to the uncensored observations, and then values of individual censored observations are predicted using the regression equation based on their normal scores. The predicted values are then combined with the uncensored observations and summary statistics are calculated. In the present analysis, values for any biomonitoring measurement less than the LOD were imputed using the robust ROS method and were not assumed to be zero. This method was selected because previous literature has suggested summary statistics calculated using this approach are less biased than using single imputation.[69] If <30% of the individuals in a subgroup had detectable concentrations, then summary statistics were not calculated for that particular metal or metalloid.[66] For comparison, we calculated representative values for the measured analytes using information from the NHANES cycles 2011–2012, 2013–2014, and 2015–2016. We calculated 6-y weighted values to account for complex sampling design and weighting, limited to females 14–45 years of age. Calculations were completed using R (version 3.5.1; R Development Core Team) following CDC recommendations.[70] Any tests for significant differences among groups were evaluated at an alpha-level of 0.05.

Bayesian Profile Regression

Bayesian Profile Regression (BPR) was used to identify clusters of participants with similar exposure profiles to metals and metalloids, as well as to estimate joint effects of coexposure profiles. Compared with conventional cluster analysis methods, such as *k* means cluster or hierarchical clustering, BPR offers several advantages. First, BPR is a data-driven type of cluster analysis whereby a Dirichlet process mixture model is employed so that the number of clusters does not need to be assigned prior to analysis.[71] Because BPR is also set in a Bayesian framework with Markov chain Monte Carlo (MCMC) estimation, this method allows for simultaneously estimating the associations of exposure profile clusters with an outcome of interest (e.g., PTB). In addition, BPR allows for categorical variables and missing values in the clustering covariates, which is generally not possible with conventional clustering methods.[71,72] Finally, BPR includes options for performing variable selection to identify important clustering variables and performing predictions using "pseudo" exposure profiles. These procedures are performed during the model fitting process and in post-processing steps, including a dissimilarity matrix that identifies optimal number of clusters and Bayesian model averaging to appropriately address uncertainty. Thus, BPR has been increasingly used for environmental and epidemiological research.[9,49,73–75] Additional information about BPR can be found in other recent publications.[72,76,77]

Clustering covariates. In this study, all metal and metalloids deemed eligible (see the section "Biomonitoring Sample Preparation and Analysis") for statistical analysis were fit as covariates for clustering. Prior to fitting these exposure measurements in BPR, the continuous concentration value of each biomonitoring variable was reclassified into categorical variables of exposure. Fitting the covariates as categorical exposures, such as quantiles, was justified because the values were highly nonnormally distributed and are on different scales. For instance, owing to left-censoring, total blood Hg, inorganic blood Hg, and urine Mn were classified into binary indicators of exposure that represent nondetectable and detectable concentrations. In addition, urine Hg was classified into tertiles because approximately one-third of the observations were less than the LOD. The remaining biomonitoring variables were classified into quartiles. All observations less than the LOD were assigned to the lowest quantile of exposure.

Estimating cluster associations with PTB. The relationship between the metals and metalloids exposure clusters (ECs) and PTB was modeled using BPR, with the adjusted association between cluster and PTB estimated at each iteration of BPR's MCMC algorithm. The selection of confounding variables was guided by a directed acyclic graph presented in Figure S1. To control for confounding, we performed BPR using a model that adjusted for maternal education, annual household income, gestational age (in weeks) of the enrollment biomonitoring sample, enrollment hospital facility location as a proxy for unmeasured regional variation in environmental metals and other factors, and the maternal prenatal risk score (based on smoking, alcohol, and maternal age as described in the section "Medical Record Abstraction and Enrollment Survey"). The continuous confounding variables, such as gestational age in weeks, was mean centered to facilitate MCMC convergence and to facilitate interpretation of cluster effect posterior distributions provided by the full BPR model.

The posterior distribution of the adjusted probability of PTB was obtained from the MCMC sweeps from the BPR model. Relative risk (RR) of PTB was determined by calculating the posterior distribution of the probability of PTB for each cluster divided by the posterior distribution of the probability of PTB for the lowest EC. The 95th credible interval (CrI; lower 2.5% and upper 97.5%) was determined using the distribution of this ratio for each EC. In this way, cluster assignment uncertainty was retained throughout the analysis. BPR was implemented using the PReMiuM package (version 3.1.3)[72] for R (version 3.5.1; R Development Core Team) with default priors, a burn-in of 20,000 iterations and 100,000 MCMC sweeps. Options available in the PReMiuM package, such as variable selection to generate latent selection weights and predictions with "pseudo exposure profiles" (described below), were also applied during this analysis. The variable selection method has been described in detail elsewhere.[72,76] To aid interpretation, the RR of PTB was calculated directly from the BPR output to account for uncertainty as the ratio of preterm probability for each EC relative to the EC with the lowest joint exposure levels (EC 1), similar to Coker et al.[49]

Characterization of exposure profile clusters. For each metal and each EC, the median quantile score (calculation described in the section "Clustering covariates") was calculated and plotted as a heat map to simplify display of the joint distribution of the metal exposure variables.[49,78] Quantile scores of 1 were considered "low" exposure; a score of 2, "moderately low"; a score of 3, "moderately high"; and a score of 4, "high." For the variables that were split into nondetect and detect (total blood Hg, inorganic blood Hg, and urine Mn), the ranking indicates absence or presence; for urine Hg approximately one-third of samples had concentrations less than the LOD, so tertiles were calculated to represent low, moderate, and high exposure. In addition, the median concentrations for each EC were compared with calculated NHANES values, and a similar visual was created (see the section "Summary Statistics" for description of 6-y weighted NHANES summary statistics calculated for the present analysis), indicating whether the EC median value was less than, similar to, or greater than the NHANES median.

Prediction scenarios. BPR also supports simultaneous implementation of predictive scenarios to investigate the role of individual exposures or specific combinations of exposures on probability of the modeled outcome. We leveraged this capability to predict the posterior probability of PTB for different exposure prediction scenarios (i.e., pseudo exposure profiles). Observing how each pseudo exposure profile is allocated across all iterations informs our understanding of PTB probability for select metals and joint exposures of interest. Because the BPR method has the capability to handle missing information, the values of undefined covariates therefore reflected the population average effect and covariate pattern observed in the main sample.[77] For example, if we are interested in observing the effect of total urinary As in the upper end of the distribution of exposure, we can specify a pseudo exposure profile with only urine total As assigned at the fourth quartile, and the remaining biomarkers are undefined [set as "not applicable" (NA) or "missing"]. The model then allocates the pseudo exposure profile to a cluster based on the defined exposure variable and the population average effect for the undefined variables. Therefore, at each MCMC iteration, the pseudo exposure profiles are allocated to a cluster in the mixture model and the predicted probability of PTB is estimated. The posterior distribution of the predicted probability of PTB can then be evaluated for each pseudo exposure profile.

Here, we explored the predicted effects of different exposure scenarios focused on the following biomarkers: urine total As, U, Pb, Cd, Tl, Sb, and Hg, as well as blood total Hg and blood inorganic Hg. Different metal exposure combinations were also investigated, including total As+U, total As+U+Hg (blood or urine measurements). These combinations were selected based on previous environmental literature suggesting the widespread occurrence of As and U in environmental media on the Navajo Nation,[9,79] as well as potential Hg in home exposure owing to heating behaviors of Navajo Nation residents.[80,81] For comparison, one pseudo exposure profile of all lowest exposure quantiles was defined as a reference group.

Sensitivity Analyses

We compared BPR to other statistical approaches, including random forest,[82] principal components analysis (PCA),[83] and generalized additive models (GAMs)[84] in evaluating the relationship between metal exposures and PTB. These approaches to estimating the health effects of mixtures were described previously, and their strengths and weaknesses were compared.[85,86] Multiple hypotheses testing was adjusted for using the Benjamini–Hochberg method, which controls for the false discovery rate.[87] Multiple sensitivity analyses were completed to complement the BPR analysis. We conducted an unadjusted BPR model and compared it with results from the fully adjusted model (see the section "Estimating cluster associations with PTB" for model adjustments) that controls for confounding in the main analysis, We also compared supervised results with unsupervised clustering results and evaluated the impact of including the same metals in different biological samples (i.e., urine vs. blood) on the BPR ECs. The same model parameters were used for the sensitivity analysis as with the fully adjusted BPR model—a burn-in of 20,000 iterations and 100,000 MCMC sweeps. Furthermore, we compared sociodemographic results between groups of individuals excluded and included in the present analysis, and investigated differences between spontaneous and medically induced PTBs.

Results

Study Participant Demographics

Statistical summaries of demographic and socioeconomic information were calculated for 417 maternal study participants (Table 1). The mean maternal age at study enrollment was 27.1 y, and the median prepregnancy BMI score and gestational age at enrollment were 28.6 kg/m^2 and 25.6 wk, respectively. Enrollment survey results indicated that 20% of individuals reported smoking >100 cigarettes during their lifetime. Most enrolled women had at least a high school education, but two-thirds were unemployed and 58% reported an annual household income of <$40,000. We observed statistically significant differences for some variables when stratified by PTB (no or yes), including gestation length (in weeks) [no, PTB: 39.4 (95% CrI: 38.7, 40.1) wk; yes, PTB: 36.0 (95% CrI: 34.6, 36.6); $p < 0.001$] and gravity (no, PTB −3.0 (95% CrI: 1.0, 4.0); yes, PTB 3.5 (95% CrI: 2.2, 5.0); $p = 0.02$). No differences were observed for other sociodemographic factors, including maternal age, gestational age at study enrollment, prepregnancy BMI, smoking status, material education, employment status, annual household income, or enrollment location. Comparison of demographic and socioeconomic information between individuals included in the analysis ($N = 417$) and those excluded ($N = 364$) (detailed in the section "Medical Record Abstraction and Enrollment Survey") indicated no significant difference for maternal age, prepregnancy BMI, gravity, parity, prenatal risk score, gestation length (in weeks), smoking status, maternal educational achievement, or maternal employment status (Table S2). Those participants included in this analysis had a higher annual household income (e.g., annual household income of >$40,000: excluded, 10.9%; included, 20.1%; $p = 0.008$), enrolled later in the pregnancy [excluded, −21.9 (95% CrI: 12.9, 31.3) wk; included, −25.6 (95% CrI: 15.4, 33.1) wk; $p = 0.044$], and had a slightly lower frequency of PTB (excluded, −12.4%; included, 7.2%; $p = 0.022$), whereas other sociodemographic variables were not statistically significantly different.

Summary Statistics—Joint Distribution of Metals

Spearman's ρ values ranged from −0.15 to 0.84 (values are reported and depicted in Figure S2 and Table S3). Moderately strong-to-strong positive Spearman correlation coefficients were observed for the majority of urine metals, including Mn, Ba, Sr, Mo, W, Ce, Tl, Co, Pb, Sb, As, Sn, Cd, and U. Strong positive correlations were observed between inorganic Hg (blood) and total Hg (blood), as well as with urine Hg. Blood and serum Se were positively correlated and negatively correlated with eight urine metals. Serum Cu was negatively correlated with most urine metals, although most coefficient values were weak.

BPR ECs

The fully adjusted BPR model indicated an optimal clustering of six subgroups sized 89 (21.3% of participants), 33 (7.9% of participants), 47 (11.3% of participants), 52 (12.5% of participants), 71 (17.0% of participants), and 125 (30.0% of participants), respectively (Table 2). The optimal number of subgroups was consistent when the number of iterations was varied (from 100,000 to 500,000). Upon review of the MCMC post-processing visualization, a strong clustering signal was observed with

Table 1. Summary of sociodemographic characteristics [median (25th, 75th percentiles) or *N* (% of total responses)] of maternal Navajo Birth Cohort Study participants (2013–2018), including stratification by preterm birth (PTB).

Characteristics	All participants ($N = 417$)	No PTB ($N = 387$)	PTB ($N = 30$)	*p*-Value[a]
Maternal age (y)	27.1 (23.0–32.1)	27.0 (22.8–32.0)	30.2 (25.1–32.8)	0.082
Gestational age enrollment (wk)	25.6 (15.4–33.1)	25.7 (15.8–33.5)	24.4 (15.1–28.5)	0.092
Gestation length (wk)	39.3 (38.3–40.1)	39.4 (38.7–40.1)	36.0 (34.6–36.6)	<0.001
Prenatal risk score				0.8
0	328 (79)	305 (79)	23 (77)	
≥1	89 (21)	82 (21)	7 (23)	
Gravity (*N*)	3.0 (1.0–4.0)	3.0 (1.0–4.0)	3.5 (2.2–5.0)	0.02
Parity (*N*)	1.0 (0.0–2.0)	0.0 (0.0–2.0)	3.0 (2.2–4.0)	0.17
Prepregnancy BMI (kg/m^2)	28.6 (24.9–33.0)	28.5 (24.7–32.8)	30.1 (26.0–35.0)	0.13
Maternal lifetime smoking status (ever smoked 100 cigarettes in lifetime)				
No	282 (80)	260 (80.0)	22 (85)	0.7
Yes	70 (20)	66 (20)	4 (15)	
Not reported (*N*)	65	61	4	
Maternal education (completed high school)				
No	80 (21)	274 (79)	20 (74)	0.8
Yes	294 (79)	73 (21)	7 (26)	
Not reported (*N*)	43	40	3	
Unemployed				
No	125 (33)	116 (33)	9 (33)	>0.9
Yes	250 (67)	232 (67)	18 (67)	
Not reported (*N*)	42	39	3	
Annual household income				
<$10,000	59 (20)	58 (21)	1 (5)	0.09
$10,000–$40,000	111 (38)	98 (35)	13 (62)	
>$40,000	124 (42)	117 (43)	7 (33)	
Not reported (*N*)	123	114	9	
Enrollment location				
Chinle	154 (37)	143 (37)	11 (37)	0.7
Fort Defiance	25 (6.0)	24 (6.2)	1 (3.3)	
Gallup	75 (18)	70 (18)	5 (17)	
Kayenta	19 (4.6)	18 (4.7)	1 (3.3)	
Shiprock	35 (8.4)	30 (9.6)	5 (17)	
Tuba City	109 (26)	102 (26)	7 (23)	

Note: %, percentage; BMI, body mass index.
[a]Determined using Wilcoxon rank sum test; Pearson's chi-squared test; or Fisher's exact test.

Table 2. Summary of mean posterior probability and relative risk of preterm birth (PTB) from the fully adjusted Bayesian Profile Regression model for maternal Navajo Birth Cohort Study participants (2013–2018).

BPR EC	*N* (%)	Observed PTB (frequency) [*N* (%)]	Mean posterior probability of PTB (95% CrI)[a]	RR (95% CrI)	Probability $EC_i > EC1$
1	89	3 (3.4)	0.043 (0.017, 0.08)	1.00 (Ref)	—
2	33	1 (3.0)	0.051 (0.013, 0.115)	1.47 (0.27, 3.90)	54.64
3	47	2 (4.3)	0.06 (0.022, 0.115)	1.72 (0.44, 4.17)	67.54
4	52	4 (7.7)	0.092 (0.043, 0.153)	2.62 (0.81, 5.95)	90.4
5	71	7 (9.9)	0.098 (0.052, 0.155)	2.77 (0.97, 6.03)	94.28
6	125	13 (10.4)	0.101 (0.064, 0.145)	2.89 (1.1, 6.11)	96.8

Note: The fully adjusted BPR used logistic regression to model association EC-specific association with PTB, adjusted for maternal education, household income, gestational age (in weeks) at the time of biomonitoring sample collection, enrollment hospital facility location as a proxy for unmeasured regional variation in environmental metals, and the prenatal risk factor score (based on smoking, alcohol, and maternal age). —, Not applicable; BPR, Bayesian Profile Regression; CrI, credible interval; EC, exposure cluster; PTB, preterm birth; Ref, reference; RR, relative risk.

[a]EC 1 represented individuals with low exposures for all metals; ECs 2 and 5 had moderately low exposure for most metals; ECs 3 and 4 comprised individuals with moderately high exposures to most metals. EC 6 comprised individuals with the highest overall exposures. All ECs, briefly summarized here, were compared with summary statistics calculated using the National Health and Nutrition Examination Survey, Cycles 2011–2012, 2013–2014, and 2015–2016, limited to females 14–45 years of age.

relatively narrow CrIs, giving confidence in the best clustering allocation (see Figure S3 and Table S4).

Of the six identified subgroups, EC 1 (described subsequently as the reference subgroup) represented individuals with low exposures for all metals, including >70% of individuals with inorganic blood Hg concentrations less than the LOD (Table 3). The median concentration of other metals in EC 1 were in the first quantile (Figure 2A). Compared with NHANES (6-y weighted value using cycles 2011–2012, 2013–2014, and 2015–2016), the median concentrations for all urine metals in this EC were lower (Figure 2B), and the median concentration of most serum and blood metals were lower. The median serum Cu concentration was higher than the median NHANES concentration, whereas median serum Zn concentrations were less than NHANES and the WHO sufficiency level of 70 μg/dL, a pattern consistent across all ECs. Two other subgroups (EC 2 and 5) had moderately low exposure for most metals; individuals in subgroup EC 2 are distinguished from EC 5 by detectable concentrations of urine Hg, inorganic blood Hg, and total blood Hg. Median urine concentrations of total As, Cd (EC 2 only), Ce, Pb, Sb, and Tl concentrations were lower than the median NHANES concentration; Mn, Mo, and W concentrations were similar; and Ba (EC 2 only), Co, urine Hg (EC 2 only), Sn, Sr (for EC 2 only), and U were higher. ECs 3 and 4 comprised individuals with moderately high exposures to most metals. For these ECs, median urine metal concentrations were all higher than median NHANES values except total As, Pb, and Tl, which had similar concentrations, and urine Hg, which was higher for EC 3 and lower for EC 4. EC 4 are distinguished from EC 3 owing to lower concentrations of urine Hg and total blood Hg. EC 6 comprised individuals with the highest overall exposures. Compared with NHANES, all median urine metal concentrations were higher for individuals in EC6, except urine and blood Hg. We compared demographic characteristics among the ECs, and no differences were observed except for maternal education (e.g., EC 1, −47% less than high school education, vs. EC 6, −69% less than high school education; $p = 0.007$, Table S5).

The variable section method employed through BPR generated a latent selection weight for each variable, indicating a high probability (>0.90) that most urine metals (with the exception of Sn) supported the observed clustering pattern (Table S4). Total Hg and inorganic Hg concentrations in whole blood also had latent selection weights >0.90, suggesting that these exposure variables strongly support the clustering pattern. The latent selection weights calculated from the MCMC runs indicated that blood and serum metal measurements of Cd, Pb, Se, and Zn did not contribute significantly to the clustering pattern. In addition, when the MCMC post-processed output was reviewed, none of the CrIs for these metals were lower or higher than the overall group average, further suggesting limited contribution to the clustering (Figure S3A–C; summary statistics are presented in Table S6). These same metals were not strongly correlated with creatinine-corrected urine measurements.

We conducted multiple sensitivity analyses to further examine these results. A sensitivity analysis was conducted to confirm that including the same metals in different biological samples had minimal impact on the ECs. The fully adjusted BPR model was rerun excluding measurements for elements measured in blood that were also measured in urine (i.e., Cd, Mn, Pb, and Se) (cosine similarity = 0.9995; Table S7). Furthermore, we reran the BPR analysis without adjustment for confounders and observed minimal changes in PTB RR for all ECs (Table S8). The PTB RR for EC 6 remained greater than for the lowest exposure group (95% CrI did not cross the null) with minimal change in PTB RR for the other ECs (Table S8). We also observed that 98.6% of individuals ($N = 401$) were in the same EC regardless of clustering with or without PTB being defined as the outcome (Table S9). These results suggest a reasonable level of stability in BPR cluster allocation regardless of clustering with or without an outcome and inclusion/exclusion of confounders.

To further explore the BPR results, we examined the similarity of the BPR clusters with clusters identified using a random forest analysis and observed some similarity (cosine similarity = 0.813; Table S10). Furthermore, the random forest approach classified all subjects with PTB into two clusters (one cluster with a single observation, and a second cluster with 29 observations) (Table S11). We applied PCA, which showed that the first component (PC1) accounts for 20% of the total variation and the second component (PC2) accounts for 10% of the total data variation. Metals that contribute to the first component with the largest loadings included Ce, Tl, Sr, and total As. Metals that contribute to the second component with the largest loadings included Ba, Mn, and Hg. The scatter plot of PC1 and PC2 color-coded by the BPR clusters with overlayed variable loadings is described in Figure S4, and loading scores are presented in Table S12. PC1 and PC2 were significantly different (evaluated using Kruskal–Wallis test) among the six BPR clusters.

Probability of PTB

The percentage of PTBs for the 417 pregnancies included in the analysis was 7.2% ($N = 30$). Individual ECs presented a range of PTB mean posterior probabilities including 4.3% (95% CrI: 1.7%, 8.0%) for EC 1 up to 10.1% (95% CrI: 6.4%, 14.5%) for EC 6 (Table 2). The RR of PTB (calculated directly from the BPR output to account for uncertainty) is the ratio of preterm probability for each EC relative to the EC with the lowest joint exposure levels (EC 1). When compared with EC 1 (the lowest exposure group), subgroup EC 6 had a PTB RR of 2.9 times (95% CrI: 1.1, 6.1). The median concentration of urine metals for individuals classified into EC 6 was higher than respective medians calculated using

Table 3. Select summary statistics for blood, serum, and urine biomonitoring results [median and interquartile range (25th, 75th percentiles) for each metal] of maternal Navajo Birth Cohort Study participants (2013–2018) stratified by exposure cluster (generated using Bayesian Profile Regression).

Media	Metal	Units	Total N (% $N <$ LOD)	Exposure cluster						NHANES[a]
				1	2	3	4	5	6	
Blood	Cadmium	µg/L	401 (1.5)	0.3 (0.22–0.39)	0.29 (0.24–0.44)	0.28 (0.22–0.41)	0.33 (0.23–0.4)	0.32 (0.24–0.43)	0.29 (0.22–0.45)	0.30 (0.15–0.69)
	Manganese	µg/L	401 (0.0)	18 (14–24)	20 (15–23)	18 (14–22)	19 (16–24)	20 (16–24)	18 (15–23)	12 (6.6–24)
	Lead	µg/dL	401 (0.0)	0.34 (0.25–0.41)	0.35 (0.24–0.48)	0.31 (0.25–0.39)	0.35 (0.26–0.43)	0.33 (0.26–0.53)	0.35 (0.26–0.47)	0.63 (0.32–1.4)
	Selenium	µg/L	401 (0.0)	170 (160–180)	170 (170–180)	170 (160–180)	170 (170–180)	170 (160–180)	170 (160–180)	212 (123–412)
	Mercury (inorganic)	µg/L	401 (72.6)	<LOD (<LOD–<LOD)	0.36 (0.33–0.5)	0.35 (0.2–0.45)	<LOD (<LOD–<LOD)	<LOD (<LOD–<LOD)	<LOD (<LOD–<LOD)	0.25 (<LOD–0.50)
	Mercury (total)	µg/L	401 (37.4)	<LOD (0.14–0.34)	0.56 (0.4–0.9)	0.45 (0.39–0.67)	0.071 (0.033–0.14)	<LOD (<LOD–0.14)	0.3 (<LOD–0.45)	0.70 (0.31–1.9)
Serum	Copper	µg/dL	414 (0.0)	240 (220–260)	240 (200–250)	230 (200–250)	240 (210–260)	240 (210–260)	230 (200–260)	146 (83–273)
	Selenium	µg/L	414 (0.0)	110 (100–110)	110 (98–120)	110 (100–120)	110 (98–110)	100 (100–110)	110 (100–120)	139 (81–271)
	Zinc	µg/dL	414 (0.0)	62 (54–74)	59 (53–67)	62 (54–72)	64 (56–74)	60 (53–67)	62 (53–71)	89 (50–168)
Urine	Arsenic (total)	µg/g creatinine	402 (0.0)	1.6 (1.2–2.4)	4.1 (2.9–6.7)	5.7 (4.3–7.1)	6.2 (4.8–8.1)	4.2 (3.3–5)	9 (6.7–13)	5.9 (3.5–11)
	Barium	µg/g creatinine	408 (0.0)	0.88 (0.46–1.9)	3 (1.8–5.3)	3.6 (2–6.4)	4 (2.7–7.9)	1.8 (1–3.2)	6.2 (3.6–10)	1.3 (0.76–2.2)
	Cadmium	µg/g creatinine	408 (11.5)	0.048 (<LOD–0.081)	0.097 (0.079–0.16)	0.27 (0.16–0.32)	0.18 (0.096–0.29)	0.12 (0.07–0.23)	0.43 (0.23–0.71)	0.13 (0.076–0.23)
	Cobalt	µg/g creatinine	408 (0.0)	0.29 (0.13–0.4)	0.65 (0.49–1)	1 (0.77–1.4)	1 (0.82–1.4)	0.61 (0.42–0.83)	1.5 (1.1–2.2)	0.49 (0.33–0.76)
	Cesium	µg/g creatinine	408 (0.0)	1.3 (1–1.8)	3.5 (2.7–4.4)	5.1 (4.7–6.1)	4.6 (3.6–5.8)	3.7 (2.7–4.6)	7.9 (6.9–9.8)	4.2 (3.1–5.8)
	Mercury	µg/g creatinine	403 (50.1)	0.079 (<LOD–0.14)	0.46 (0.34–0.66)	0.55 (0.32–1.1)	<LOD (<LOD–<LOD)	<LOD (<LOD–<LOD)	0.18 (0.07–0.52)	0.25 (0.13–0.51)
	Manganese	µg/g creatinine	406 (33.7)	0.053 (<LOD–0.14)	0.15 (<LOD–0.25)	0.2 (0.14–0.28)	0.21 (0.14–0.29)	<LOD (<LOD–0.15)	0.31 (0.22–0.48)	0.14 (0.083–0.24)
	Molybdenum	µg/g creatinine	408 (0.0)	13 (6.3–20)	34 (25–46)	62 (46–71)	50 (37–80)	41 (27–52)	96 (69–130)	38 (26–57)
	Lead	µg/g creatinine	408 (1.5)	0.082 (0.057–0.11)	0.19 (0.16–0.22)	0.28 (0.23–0.34)	0.27 (0.21–0.33)	0.17 (0.12–0.23)	0.48 (0.38–0.72)	0.25 (0.17–0.40)
	Antimony	µg/g creatinine	408 (13)	0.023 (<LOD–0.032)	0.036 (0.03–0.056)	0.082 (0.054–0.11)	0.06 (0.048–0.089)	0.046 (0.032–0.057)	0.12 (0.086–0.16)	0.050 (0.034–0.077)
	Tin	µg/g creatinine	407 (1.0)	0.34 (0.21–0.66)	0.86 (0.67–1.3)	2.2 (0.98–5.1)	1.2 (0.63–2.6)	0.93 (0.64–1.7)	2.7 (1.4–5.4)	0.44 (0.26–0.83)
	Strontium	µg/g creatinine	405 (0.0)	55 (21–90)	150 (110–210)	220 (180–270)	270 (210–370)	110 (79–160)	350 (230–540)	100 (64–150)
	Thallium	µg/g creatinine	408 (2.0)	0.045 (0.032–0.067)	0.093 (0.074–0.12)	0.17 (0.13–0.2)	0.16 (0.12–0.21)	0.13 (0.09–0.18)	0.24 (0.18–0.3)	0.18 (0.12–0.25)
	Tungsten	µg/g creatinine	408 (6.9)	0.04 (0.018–0.062)	0.067 (0.059–0.1)	0.13 (0.064–0.22)	0.11 (0.072–0.19)	0.073 (0.048–0.12)	0.23 (0.13–0.4)	0.070 (0.041–0.13)
	Uranium	µg/g creatinine	408 (2.9)	0.0043 (0.0026–0.0091)	0.014 (0.0081–0.029)	0.014 (0.01–0.022)	0.017 (0.011–0.036)	0.0089 (0.0067–0.017)	0.028 (0.018–0.05)	0.006 (0.003–0.010)

Note: If <30% of subgroup observations were greater than the LOD, then the percentile concentrations were not calculated; if the calculated value was less than the method LOD, then "<LOD" was used in the above table. LOD, limit of detection; NHANES, National Health and Nutrition Examination Survey.

[a]NHANES cycles 2011–2012, 2013–2014, and 2015–2016 6-y weighted values were calculated to account for complex sampling design and weighting, limited to females 14–45 years of age. Calculations were completed using R (version 3.5.1; R Development Core Team) following Centers for Disease Control and Prevention recommendations.[71]

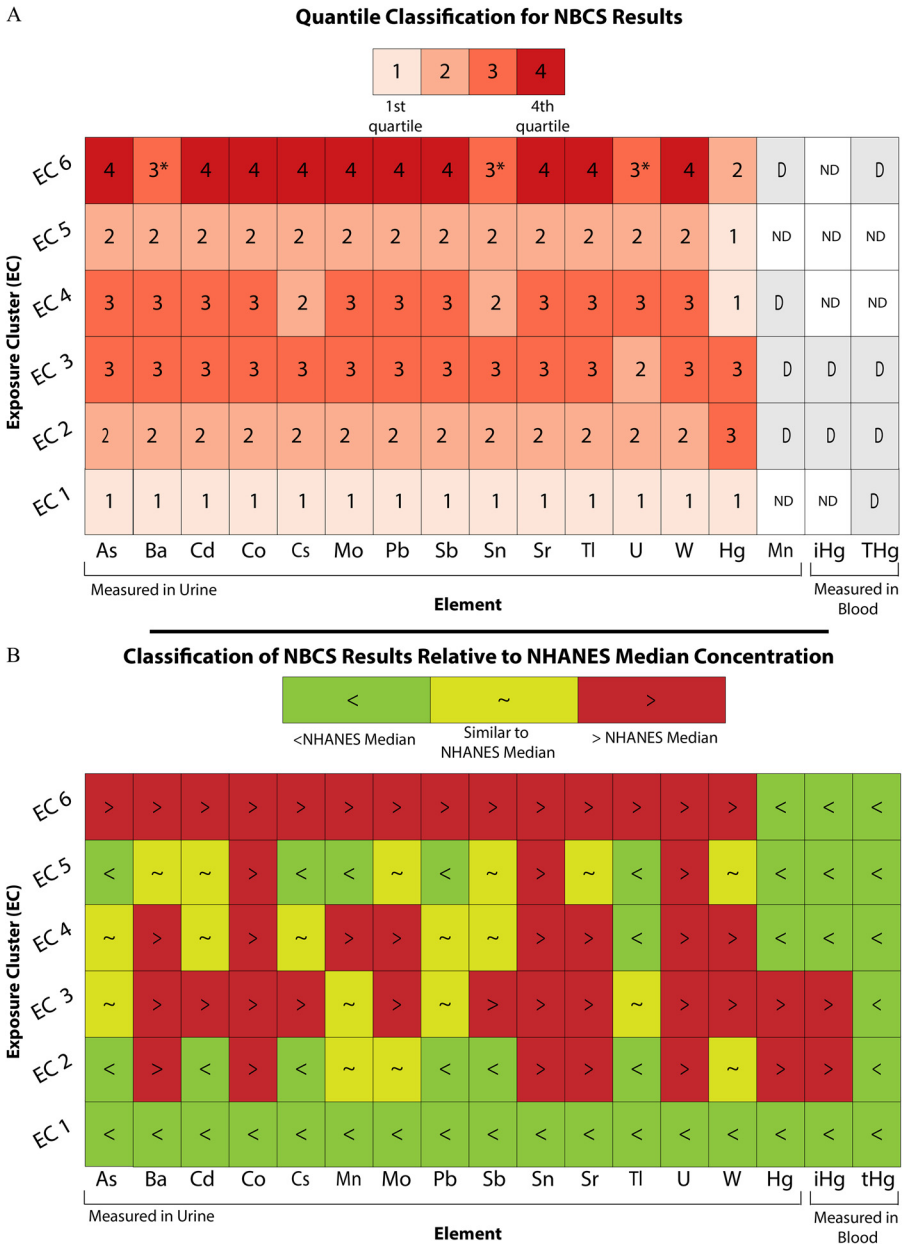

Figure 2. Classification of maternal Navajo Birth Cohort Study (NBCS) participants into exposure clusters (ECs) by (A) quantiles of measured metal and metalloid biomarkers and (B) relative to median metal biomarker concentrations measured in NHANES. (A) Each column represents an individual element and each row represents a distinct subgroup (or EC). The numeric values in each box represents a quantile score (1 is the lowest, and 4 is the highest). iHg, THg, and Mn were classified as not detected or detected. Asterisks indicate that that particular element was classified into tertiles. (B) Each column represents an individual element and each row represents a distinct subgroup (or EC). The colors and symbols correspond to the NBCS median concentration relative to the NHANES median for the same analyze; green (<) indicates that the NBCS median is less than the NHANES median; yellow (~) indicates that the NBCS median is similar to the NHANES median; and red (>) indicates that the NBCS median is greater than the NHANES median. Seven analytes were not visualized here because the variable selection method suggested very low contribution for the observe clustering outcome; this included blood Cd, Mn, Pb, and Se and serum Cu, Se, and Zn. See Table 3 for summary statistics. Note: Ba, barium; Cd, cadmium; Ce, cesium; Co, cobalt; Cu, copper; D, detected; iHg, inorganic mercury; NHANES, National Health and Nutrition Examination Survey; Mg, mercury; Mn, manganese; Mo, molybdenum; ND, not detected; Pb, lead; Sb, antimony; Se, selenium; Sn, tin; Sr, strontium; Tl, thallium; THg, total mercury, U, uranium; W, tungsten; Zn, zinc.

NHANES data from cycles 2011–2012 through 2015–2016 (Table 3 and Figure 2B). EC 6 had nondetectable concentrations of total and inorganic blood Hg and the lowest exposure tertile for urine Hg, whereas EC 5 had detectable total blood Hg concentrations but inorganic blood Hg concentrations that were less than the LOD, as illustrated in Figure 2. The remaining ECs (ECs 2, 3, 4, and 5) did not suggest elevated RR of PTB compared with the reference group (Table 2).

For comparison with the BPR results, the GAM analysis identified metals associated with PTB that were significant at the 0.05 nominal significance level, including Pb, Tl, W, Hg, Cd, Sb, and Sr (Figure S5 and Table S13). However, after correcting for multiple comparisons of the metals, none of the metals showed a statistically significant association. Although we observed some differences in patterns of association between spontaneous and medically induced PTBs by ECs, statistical power to evaluate

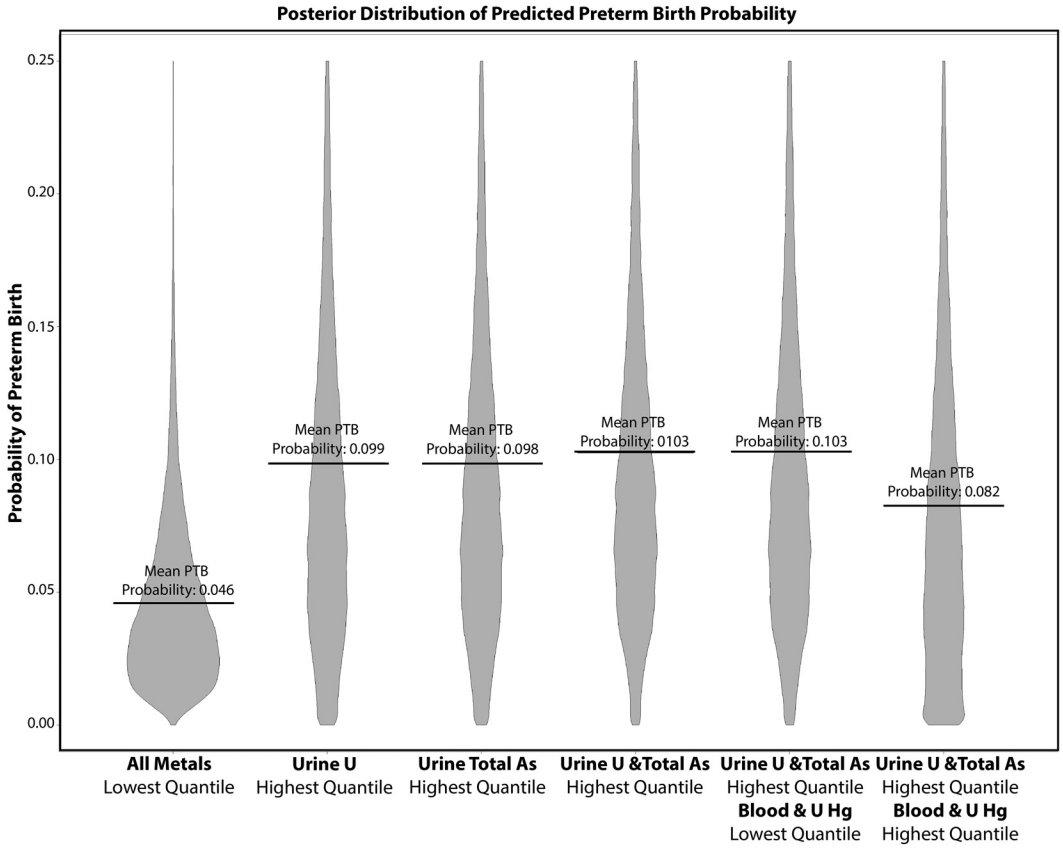

Figure 3. Pseudo profiles generated from the Bayesian Profile Regression analysis for maternal Navajo Birth Cohort Study participants (2013–2018). The figure illustrates the posterior distribution of PTB risk for individual elements (urinary total As and urine U), and combinations of urine total As+U, and urine total As+U+Hg (urine Hg, total blood Hg, and inorganic blood Hg). The posterior distribution of predicted PTB is plotted by the quantile score of each metal or metal mixture. The black line overlapping each bean plot represents the mean predicted probability of PTB. Numeric data represented in this figure are also provided in Table S15. Note: As, arsenic; Hg, mercury; PTB, preterm birth; U, uranium.

differences was limited owing to the small sample sizes for each category (Table S14).

Predictive Scenarios

The prediction scenarios indicated generally higher posterior probability of PTB associated with most metals as the concentration increased from lowest to highest quantile. For example, when total urine As was evaluated, the mean ± standard deviation (SD) posterior probability of PTB was 0.06 ± 0.069 for the first quantile and 0.098 ± 0.078 for the fourth quantile. This trend was observed for Ba, Cd, Co, Ce, Mn, Mo, Pb, Sb, Sn, Sr, Tl, W, and U. Conversely, lower mean posterior probability of PTB were observed for higher inorganic blood Hg, total blood Hg, and urine Hg quantiles.

The mean posterior predicted probability of PTB was 0.046 ± 0.033 when all metals with a latent selection weight >0.5 were included at their lowest quantile score. In contrast, when As and U were at the highest quantile simultaneously, the mean posterior predicted probability of PTB was 0.103 ± 0.073, slightly higher than either total As or U alone at the highest exposure quantile (0.098 ± 0.078 and 0.099 ± 0.086, respectively).

When Hg quantiles were changed from lowest to highest and other metals were held at their highest quantiles, the mean posterior predicted probability of PTB was lower. When concentrations of total As and U were both in the fourth quantile and all Hg analytes in blood and urine were not detectable, the mean posterior probability of PTB was 0.103 ± 0.081. In contrast, when concentrations of total As and U were both in the fourth quantile and all Hg analytes in blood and urine were at the highest exposure quantile scores, the mean posterior probability of PTB lowered to 0.082 ± 0.069 (Figure 3). The mean posterior predicted probability of PTB for 17 metals and combinations are reported in Table S15.

Discussion

The primary aim of this study was to evaluate the association of metal mixture exposure with overall PTB using BPR as the analytic framework. Overall, 7.2% ($N = 30$) of singleton births for enrolled NBCS mothers in this analysis occurred before 37 wk gestational age; this percentage is less than the PTB rate for singleton pregnancies in the United States, which were 7.74% in 2014 and 8.02% in 2016.[24] The overall PTB frequency on the Navajo Nation is between 10% and 11% (2016–2017) and is lower than other Indigenous communities in the United States.[88] We observed a PTB percentage of 9.2% for all NBCS participants before exclusion for missing critical covariates and biomonitoring information, which is commensurate with overall numbers for the Navajo Nation. We hypothesized a positive concentration–response relationship between increasing aggregate exposure to multiple metals and increased risk of PTB. Using blood, serum, and urine biomonitoring measurements of 24 metals, distinct patterns of exposure were observed. The reference subgroup (EC 1) had low exposures for all measured metals ($N = 89$, 21.3% of cohort) vs. one subgroup that had higher exposure (EC 6, $N = 125$, 29.9% of cohort). The high-exposure subgroup (EC 6) had an RR of PTB 2.9 times (95% CrI: 1.1, 6.1) greater than the reference group. Although not significantly different in the lower exposure clusters, this trend was observed when comparing

the rank order of exposure and PTB across the six ECs; ECs 2–5 were not significantly elevated relative to the reference group, so we focus discussion on EC 6.

The individuals classified into EC 6 had the overall highest metal exposures, as illustrated in Figure 2A. The median concentrations of all metals, except Hg, were in the third or fourth quantiles for this EC. Concentrations of metals previously associated with PTB (As, Cd, Pb, and U) exceeded median and 75th percentile NHANES concentrations. We also created GAMs with smooth splines to evaluate the concentration–response relationship between metals and PTB (Figure S4). We observed linear and nonlinear relationships for metal associations; however, after correcting for multiple comparisons of the metals included in the model, none of the individual metals showed statistically significant association in this sample set.

Potential sources of metals exposure for Navajo Nation residents include abandoned mine waste, either through ingestion of metals found at elevated concentrations in groundwater,[10,79,89] ingestion of locally grown or raised food,[22,90,91] or inhalation of airborne materials.[92] In some instances, Safe Drinking Water Act variances were issued for public water systems working to meet national primary drinking water standards for As and U (https://echo.epa.gov/). Cigarette smoking is an unlikely exposure source for Pb and Cd because 80% of maternal NBCS participants reported being never-smokers (smoked <100 cigarettes in their lifetime). Although the study was not designed to assess secondhand smoke exposure, some enrolled fathers did complete an enrollment survey. Previous results reported elsewhere indicated that 43% of enrolled NBCS fathers reported smoking 1–2 cigarettes/d, mostly during work hours and off-reservation.[15] Survey results indicated that only 1.4% of fathers reported smoking in their home, further suggesting less opportunity for secondhand smoke exposure.[93] This remains an opportunity for more targeted follow-up investigation.

Although the PTB RR for EC 5 was not statistically significantly greater than that of the reference group (PTB RR = 2.77; 95% CrI: 0.97, 6.03) the Hg exposure for this EC presented differently compared with the other ECs. Urine Hg concentrations (representative of predominately inorganic Hg exposure[94]) were in the first tertile and inorganic blood Hg species were not detectable, suggesting limited exposure to inorganic Hg. Potential sources of Hg exposure for Navajo residents that participated in this study include three coal-burning power plants on or adjacent to the Navajo Nation.[80,95] It is important to note that all three power stations were operational throughout recruitment of participants included in this analysis and that the Navajo Generating Station ceased operations after recruitment of participants included in the present analysis concluded. Other potential Hg sources include coal burning for home heating,[96] which 40.3% of NBCS participants reported, and dental amalgams.[97] No data from this study were available to assess the association between dental amalgams and urine or blood Hg although the relationship has been suggested for other populations in the United States. Seafood and fish consumption are unlikely Hg sources because seafood is culturally prohibited, and a diet and nutrition analysis of NBCS participants indicated minimal consumption of these foods.[22]

Of the 125 individuals in EC 6 who had elevated concentrations of most metals compared with NHANES and the NBCS distribution overall, 13 women had had a PTB (Table S13). Oxidative stress has been linked with environmental metals and has been associated with conditions during pregnancy that may also contribute to PTB.[98] Previous work suggests that if oxidative stress increases early in pregnancy, it can result in poor placentation, leading to preeclampsia or intrauterine growth restrictions.[99] Later in pregnancy, oxidative stress may activate maternal endothelium, leading to preeclampsia[100]; damage membranes, resulting in premature rupture[101]; cause cervix shortening, leading to spontaneous labor[102]; or lead to fetal growth restrictions, necessitating a medically induced labor preterm.[103] Previous work with the NBCS participants reported a significant association between 8-iso-prostaglandin $F_{2\alpha}$, a marker of oxidative stress, and urine total As concentrations but not with urine U in univariable or multivariable regression models. This analysis also suggested that Zn modified the relationship between As and oxidative stress biomarkers.[19] A subsequent investigation using a two-step approach to assess associations between metal mixtures and oxidative stress identified association between Ba, Ce, and Tl with oxidative stress markers.[20] Other literature also indicates a link between particulate matter released during solid fuel burning [including coal, which releases 10 times more particulate matter $\leq$2.5 μm in aerodynamic diameter ($PM_{2.5}$) than other solid fuels burned in Navajo homes[104]] and oxidative stress effects.[105] These investigations suggest a link between oxidative stress and environmental contaminants and a potential mechanism for PTB among the study population.

The pseudo exposure profiles for individual metals indicated an increasing posterior probability of PTB as the quantile score increased from 1 (lowest) to 4 (highest). A small additive effect was observed when combinations of metals were evaluated, suggesting some support for our hypothesis. However, we observed that individuals with greater concentrations of Hg in urine or blood (second or third tertiles) were allocated into subgroups with lower PTB risk. Although not a mechanistic study, similar observations regarding inverse association between Hg exposure and PTB have been reported in other studies, although the results were not conclusive and the effects attenuated in fully adjusted models that included maternal educational attainment, age, and living with a partner/spouse.[106,107] These previous studies investigated total blood Hg[106] and urine Hg[107] (similar to the present study) but did not measure inorganic Hg specifically. More detailed investigations into the form (inorganic vs. organic) and potential mechanisms of action are necessary to better explain this negative association.

Limitations

There are important limitations to the present study, principally related to the availability of exposure data for maternal participants. The exclusion criteria applied for this analysis resulted in 7.2% ($N = 30$) of included births being classified as preterm, which is slightly lower than the 9.2% PTB in the overall sample before exclusion. Absence of information from an enrollment biomonitoring sample was the most important factor for excluding participants from the present analysis. The results presented here suggest (Table S2) that selected participants (when compared with NBCS participants excluded from analysis) had a higher household annual income and a later gestational age at enrollment but no other significant differences in socioeconomic or demographic information. Furthermore, this prospective study did not specifically target PTB as the outcome of interest. Consequently, there was a small number of PTBs, which does limit the statistical power to detect associations between metals exposure and PTB. Nonetheless, results from this study should be interpreted cautiously in light of potential selection bias, potentially limiting the generalizability of these findings.

Our approach emphasized the joint effect of multiple metals exposures and does not enable us to investigate explicitly the effects of individual metals on PTB. BPR is well suited for use when the exposure variables are strongly correlated, as was observed for urine metal measurements. However, the blood and serum measurements were weakly correlated with creatinine-corrected urine measurements. The uncorrelated nature of these

metals across media likely contributed to their limited role in defining the clustering pattern, limiting our ability to assess their influence on PTB risk in the context of our analysis. This limitation is particularly important for the micronutrients (such as Se and Zn) included in the present analysis, given that the BPR results suggest they have limited impact on the clusters and PTB outcome. We hypothesize that is a function of the low correlation between blood/serum and urine measurements. It is important to note that exposure risk may not be strictly additive with respect to metal mixtures and BPR is not designed to assess the structure of the exposure–response surface. Other mixture analysis frameworks, such as the Bayesian Kernel Machine Regression or the Elastic Net,[41] are better suited for identifying synergy or interactions between individual metals and micronutrients and PTB (among other birth outcomes) or for exploring the structure of the exposure–response surface. In addition, the pseudo exposure profile results should be interpreted cautiously owing to the elongated shape of the posterior distribution, illustrated in Figure 3. The observational data set likely has limited information on some of these specific joint patterns of exposure and provides less informative predictions when compared with more commonly observed patterns in the cohort, such as those presented in the "allLow" bean plot in Figure 3. Last, we did not include speciated As measurements in this analysis and chose to include analytes that were measured in samples from nearly all enrolled women.

Summary of Key Findings

This study is distinct from previous studies because it investigated the joint effects of metal exposures and PTB at environmental concentrations and is representative of exposure mixture patterns in this population. Study findings suggest that, among maternal participants, increased exposure to multiple metals was associated with higher probabilities of PTB. The lowest probability of PTB for NBCS maternal participants was associated with the lowest exposure group, whereas the highest probabilities of PTB were associated with exposure to metal mixtures at concentrations exceeding NHANES values. Compared with the lowest exposure group, the highest exposure group had a PTB RR of 2.9 times (95% CrI: 1.1, 6.1). Elevated urine metal concentrations suggest a need for further investigation into existing metals–PTB mechanistic hypotheses, such as oxidative stress, to explain the higher probability of PTB for subgroups with elevated metal mixtures exposure in this population. Follow-up investigation using mediation analysis or other mixture analysis frameworks are underway to address inflammation or oxidative stress associations with PTB and other birth outcomes among pregnant Navajo women. The clusters identified in our work are representative of patterns specific to the population studied. We recommend caution generalizing these results to other populations owing to the small number of PTB events documented in this birth cohort. The overall result of increased RR of PTB with increasing metals exposure provides a framework for assessing the generalizability of these findings to other populations.

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Data are from the NBCS and whose authors may be contacted at the University of New Mexico. Data will be made available to interested researchers pending approval for dissemination of sensitive data by both the University of New Mexico Human Research Protections Office and the Navajo Nation Human Research Review Board.

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