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Association of prenatal perchlorate, thiocyanate, and nitrate exposure with neonatal size and gestational age

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Abstract

Background—Perchlorate and similar anions compete with iodine for uptake into the thyroid by the sodium iodide symporter (NIS). This may restrict fetal growth via impaired thyroid hormone production.

Methods—We collected urine samples from 107 pregnant women and used linear regression to estimate differences in newborn size and gestational age associated with increases in perchlorate, thiocyanate, nitrate, and perchlorate equivalence concentrations (PEC; measure of total NIS inhibitor exposure).

Results—NIS inhibitor concentrations were not associated with newborn weight, length, or gestational age. Each 2.62 ng/μg creatinine increase in perchlorate was associated with smaller head circumference (0.32 cm; 95% CI: −0.66, 0.01), but each 3.38 ng/μg increase in PEC was associated with larger head circumference (0.48 cm; −0.01, 0.97).

Conclusions—These anions may have effects on fetal development (e.g. neurocognitive) that are not reflected in gross measures. Future research should focus on other abnormalities in neonates exposed to NIS inhibitors.

Keywords

Perchlorate; Thiocyanate; Nitrate; NIS inhibitors; Prenatal; Birth weight

1. Introduction

Perchlorate (ClO_4^-) is an inorganic ion that is commercially produced for use as an oxidant in explosives, pyrotechnics, and rocket fuel [1,2]. Perchlorate can also form naturally

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through atmospheric reactions, and human exposure can occur via groundwater contamination and dietary intake of contaminated food crops [2]. In 2011 the United States Environmental Protection Agency (EPA) determined that perchlorate met the criteria for regulation as a drinking water contaminant, primarily based on the scientific evidence of perchlorate's effects on thyroid function [3–4]. Perchlorate competes with iodide for transport into the thyroid via the sodium/iodide symporter (NIS), thus inhibiting iodide uptake into the thyroid and possibly reducing thyroid hormone production [5–6].

Thiocyanate (SCN^-) and nitrate (NO_3^-) anions are also competitive inhibitors of thyroidal iodide uptake via the NIS [5], though their potencies for inhibiting iodide transport are only 1/15 and 1/240 that of perchlorate, respectively [4]. Thiocyanate is a by product of the breakdown of hydrogen cyanide in cigarette smoke, and it is also produced during the digestion and metabolism of some plant foods [7]. Nitrates also occur naturally in many plants [8]. and are common components of agricultural fertilizers and sewage which can contaminate drinking water sources [9].

Pregnant women and their fetuses comprise a particularly vulnerable population for whom the inhibition of iodide uptake can have substantial consequences. Fetal growth, particularly fetal brain development, is largely dependent on the bioavailability of iodide for thyroid hormone production in both the mother and fetus [10]. The NIS is also expressed in the placenta, thus providing a route for iodide transport from maternal to fetal circulation. NIS expression in placenta, however, can also facilitate transport of perchlorate, thiocyanate, and nitrate into the fetal compartment [11]. leading to fetal exposure, which may threaten thyroid function and fetal growth. Impairments in maternal thyroid hormone production during pregnancy may also contribute to shortened gestation and an increased risk of preterm birth [12–14]. Though several studies have examined the relationship between prenatal exposure to NIS inhibitors and neonatal thyroid hormones, these studies have relied mainly on ecologically based exposure measures such as contaminant concentrations in local drinking water [15–21]. Few studies have examined the association between fetal growth and prenatal exposure to NIS inhibitors.

Previously, we reported strong positive correlations between maternal urinary concentrations of NIS inhibitors and concentrations in cord blood and amniotic fluid [22]. suggesting that maternal urinary measures of these NIS inhibitors may be effective surrogates for fetal exposures. Though our prior study did not show an association between fetal growth and NIS inhibitor concentrations in cord blood, the study population consisted of healthy, iodine-replete pregnant women undergoing elective cesarean sections.

To date, no study has examined the relationship between maternal urinary levels of NIS-inhibitors and fetal growth, nor has any study examined this relationship among pregnant women at high-risk for alterations in fetal growth. Therefore, we conducted a prospective study of the association between NIS inhibitor concentrations in maternal urine samples throughout pregnancy and anthropometric measures at birth among a sample of women at risk for adverse pregnancy outcomes. We hypothesized that maternal urinary levels of perchlorate, thiocyanate, and nitrate during pregnancy would be negatively associated with neonatal weight, length, head circumference, and gestational age.

2. Methods

2.1. Study population

The study population and protocol for this study have been described previously [23]. Briefly, we recruited 107 pregnant women (9–39 weeks gestation at enrollment) between December 2008 and July 2010 from the High-Risk Obstetric Clinic at Robert Wood Johnson University Hospital. Having a previous preterm delivery was the most common indication for a high-risk pregnancy among the sample (33%), followed by hypertension (19%) and diabetes (16%; Table 1). Subjects were at least 18 years old and expecting singleton infants. All subjects provided informed consent prior to participation in the study, which was approved by the Institutional Review Board at the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School. The Centers for Disease Control and Prevention (CDC) involvement was limited to analyzing coded specimens and interpreting results.

2.2. Study protocol

All mothers initially provided a detailed medical history and information on household product use, occupation, hobbies, diet, and demographic variables. At each subsequent clinic visit, mothers provided a clean-catch urine sample. The total number of clinic visits and urine samples per subject ranged from 1 to 12 (mean ± standard deviation: 4 ± 3), and 84 (79%) subjects provided multiple samples throughout their pregnancy. There was an average of 20 days (±16) between samples among women who completed multiple study visits.

2.3. Quantification of urinary iodide and NIS inhibitors

Perchlorate, thiocyanate, nitrate, and iodide were analyzed by isotope dilution and ion chromatography/tandem mass spectroscopy (IC–MS/MS) using previously published methods with minor modifications [24]. Briefly, 0.250 mL of urine samples were diluted to 1.0 mL with aqueous internal standard solution containing stable isotope labeled perchlorate ($Cl^{18}O_4^-$), thiocyanate ($SC^{15}N^-$), nitrate ($^{15}NO_3^-$), and iodide (^{129}I). Urinary creatinine was measured based on enzymatic reaction using the Roche Creatinine Plus assay (Roche Product Application #11775685216v19).

Samples were vortex mixed and queued for injection (25 μL). Each analytical batch consisted of a blank, calibration standards, and four quality control (QC) samples (two QC low and two QC high). Analyte quantification was based on the peak area ratio of the analyte to stable isotope-labeled internal standard. The assay limit of detection was 0.05 ng/mL for perchlorate, 20 ng/mL for thiocyanate, and 700 ng/mL for nitrate. Reported results met the accuracy and precision guidelines of the quality assurance/quality control program of the Division of Laboratory Sciences, National Center for Environmental Health, CDC [25,26].

We also calculated a perchlorate equivalence concentration (PEC) for each urine sample by summing the product of the molar concentrations of each NIS inhibitor and its respective potency factor. The PEC calculation is based on evidence that thiocyanate and nitrate possess only 1/15 and 1/240, respectively, of the potency of perchlorate to inhibit iodide

transport at the NIS [5]. Perchlorate, thiocyanate, nitrate, and iodide levels are reported as creatinine-adjusted concentrations, whereas PEC was calculated using molar concentrations.

2.4. Neonatal measurements

Gestational ages were determined based on the best obstetric estimate in the medical record, using either sonographic dating or date of implantation. These were all found to be consistent with physical examination of the infant. Birth weight, length, and head circumference were measured during the first hour of life, after drying but before the first feeding. Length and head circumference were determined by certified neonatal nurses, using flexible tape measures.

2.5. Statistical analyses

We calculated descriptive statistics for infant and maternal characteristics and maternal risk factors for adverse pregnancy outcomes, and distributions of all maternal exposure variables and neonatal outcome measurements. We then calculated mean pregnancy concentrations of urinary perchlorate, thiocyanate, nitrate, iodide, and PEC by averaging the concentrations across all samples for each subject. We also calculated mean concentrations of all urinary analytes within the second trimester only and within the third trimester only for each subject. Because very few women (n = 8) provided urine samples during their first trimester, we did not calculate mean first-trimester exposures. We then computed Pearson correlation coefficients between all whole-pregnancy, second-trimester, and third-trimester average urinary concentrations of the NIS inhibitors and iodide, weighted by the number of urine samples per mother.

For our main analyses, we used weighted multivariable linear regression to estimate the changes in infant weight (g), length (cm), head circumference (cm), and gestational age (weeks) associated with interquartile range (IQR) increases in mean pregnancy concentrations of each analyte. All regression models were weighted by the number of urine samples per subject. We ran separate regression models using each analyte as the primary exposure for each of the four outcomes. All weight, length, and head circumference models included maternal smoking (sometimes/often [once per month to daily] vs. never/rarely [never to 1–2 times only]), nulliparity, infant gender, and gestational age, as these covariates were found to be associated with one or more of the maternal exposure variables and one or more of the outcome variables. All gestational age models included maternal race/ethnicity and paternal employment status (employed vs. unemployed). These models were run among all 107 subjects, and among only those subjects with term births (≥37 completed weeks of gestation; n = 81).

We also conducted sensitivity analyses to examine whether mean maternal urinary NIS inhibitor concentrations in either the second or third trimester were associated with infant measurements. Again, we used weighted multivariable linear regression to estimate the changes in infant measurements associated with IQR increases in mean second- and third-trimester maternal concentrations of each analyte, including the same covariates as described above. We also ran these regression models using analyte concentrations from the

last urine sample before delivery as the exposure of interest. All statistical analyses were conducted using SAS version 9.3 (SAS Institute, Inc., Cary, NC).

3. Results

Subject characteristics and maternal risk factors for adverse pregnancy outcomes are shown in Table 1. There was a nearly equal distribution of white, black, and Hispanic mothers, and more than half had some college education. Most mothers reported never smoking during their pregnancy, and most had at least one previous pregnancy. Previous preterm delivery was the most common indication for referral to the high-risk clinic. None of the mothers reported having a thyroid disease of any kind.

Table 2 shows the distributions of the number of urine samples per subject, mean urinary perchlorate, thiocyanate, nitrate, iodide, and PEC concentrations in the whole pregnancy, second trimester, third trimester, and last sample before birth, and infant outcome measurements. All four analytes were detected in 100% of the urine samples for all subjects. There were moderate-to-strong positive correlations of whole-pregnancy, second-trimester, third-trimester, and last-sample perchlorate concentrations with nitrate and iodide (r_s = 0.30–0.64, $p < 0.05$; Table 3). Nitrate was also moderately correlated with urinary iodide and thiocyanate over three of the four time periods analyzed (r_s = 0.30–0.33, $p < 0.05$). The composite measure of NIS inhibitor concentrations, PEC, was significantly correlated with thiocyanate and nitrate concentrations(r_s = 0.19–0.35, $p < 0.05$), but not with urinary perchlorate or iodide. The results of the main regression analyses estimating changes in infant measurements and gestational age per IQR increases in mean whole pregnancy analyte concentrations are shown in Table 4. Interquartile range increases in perchlorate, thiocyanate, nitrate, and PEC concentrations were not associated with decreases in infant weight, length, or gestational age among all subjects or among term births (37–41 weeks). Each 2.62 ng/μg creatinine increase in mean whole-pregnancy perchlorate concentration was associated with a 0.32 cm decrease (95% CI: −0.66, 0.01) in infant head circumference among all subjects, and a 0.38 cm decrease (95% CI: −0.74, −0.03) among term births. Each 3.38 ng/μg increase in PEC was associated with a 0.48 cm increase (95% CI: −0.01, 0.97) in head circumference among all infants, and a 0.38 cm increase (95% CI: −0.20, 0.96) among term births (Table 4).

Table 5 shows the results of sensitivity analyses estimating changes in infant outcomes per IQR increases in maternal exposures in the second and third trimesters and in the last urine sample before delivery among all subjects. Only 40 mothers provided urine samples in the second trimester, whereas nearly all (n = 104; 97) provided at least one sample in the third trimester. The changes in infant weight, length, head circumference, and gestational age associated with increases in analyte concentrations were generally slightly smaller or unchanged compared to the changes associated with mean whole-pregnancy concentrations (Table 5). However, each 3.00 ng/μg increase in third trimester mean PEC concentration was associated with a 0.53 cm increase (95% CI: 0.06, 1.00) in head circumference. Additionally, each 3.29 ng/μg increase in last sample PEC concentration was associated with a 0.36 cm increase (95% CI: −0.04, 0.75) in infant head circumference, and each 28,459

ng/μg increase in last sample nitrate concentration was associated with a 0.33 cm increase (95% CI: −0.002, 0.66) in head circumference (Table 5).

4. Discussion

In this prospective study of fetal growth and maternal urinary concentrations of inhibitors of thyroidal iodine uptake, increased maternal urinary concentrations of perchlorate, thiocyanate, and nitrate were generally not associated with differences in newborn weight, length, or gestational age. Though we did observe increased mean maternal concentrations of perchlorate during pregnancy to be associated, as hypothesized, with smaller infant head circumference, most of our analyses did not show a negative association between NIS inhibitors and neonatal size. Also, contrary to our hypothesis, we found increases in third trimester and mean pregnancy PEC, a composite measure of NIS inhibitor exposure, to be associated with increases in neonatal head circumference.

To our knowledge, no previous study has examined the relationship between newborn growth measurements and these anion concentrations in maternal urine. Among the few studies that have looked at differences in fetal growth in relation to maternal NIS inhibitor exposure, maternal exposure classification has been primarily based on the concentrations of NIS inhibitors in drinking water, with no direct assessment of biological toxicant concentrations [16,18,22]. Bukwoski et al. observed up to a 156% (95% CI: 44–445%) greater odds of intrauterine growth restriction (<2500 g at birth) among infants born to mothers whose residence at the time of delivery was in a community with higher concentrations of nitrate in the drinking water, compared to areas with the lowest nitrate levels [18]. Tellez et al. [22] and Amitai et al. [16] observed no differences in weight, length, head circumference, or gestational age among the newborns of women residing in cities with mean drinking water perchlorate levels ranging from 0.5 ng/mL to 340 ng/mL. However, the use of ecological data as proxies for individual exposure levels may have introduced some degree of bias due to exposure misclassification. The likelihood of misclassification, though, would not be expected to vary based on neonatal outcomes, and so the results of those studies may be underestimates of the effect of NIS inhibitors on fetal growth.

The negative relationship between maternal perchlorate and infant head circumference is consistent with a causal model in which perchlorate inhibits iodide uptake in the maternal and/or fetal thyroid, thereby reducing thyroid hormone production and impairing fetal growth. Fetal serum thyroid hormone (T4) concentration rises gradually until about 36 weeks of gestation [27]. and fetal T4 production is responsible for an increasing amount of the biologic activity of thyroid hormone in the fetus from mid-gestation onward [10]. The placenta is largely permeable to perchlorate, with fetal doses calculated at up to 82% of maternal dose in late gestation. Animal studies suggest that the fetal thyroid is more sensitive to the suppressive effects of perchlorate than the adult thyroid [28]. At the same time, the placenta remains weakly permeable to the passage of maternal T4 even in the third trimester. Therefore, impaired fetal or maternal thyroid function secondary to perchlorate exposure could contribute to relative hypothyroidism in the fetus, which is associated with impaired growth, maturation, and brain development [29,30]. Animal models have shown an

association between maternal hypothyroidism and decreased brain size [31]. and Blazer et al. [32] have reported decreased head circumference in the children of hypothyroid mothers despite therapeutic supplementation. In contrast, large head size has been noted in several reports on infants with congenital hypothyroidism [33–35]. The interaction of perchlorate, thyroid hormone levels, and growth of the brain and calvarium is likely a complex phenomenon that is dependent on timing, duration, and variability of exposure, as well as interaction with other NIS inhibitors such as nitrate.

We previously examined the relationship between cord blood concentrations of NIS inhibitors and newborn size among a sample of healthy mothers with full-term pregnancies [23]. In that study, we did not observe trends of decreasing newborn weight, length, or head circumference across increasing quartiles of cord blood perchlorate, thiocyanate, nitrate, or PEC. For example, compared to the lowest quartile of cord blood perchlorate (≤0.095 μg/L), the upper quartiles (0.095–0.139 μg/L; 0.139–0.223 μg/L; ≥0.223 μg/L) were not associated with significant decreases in infant birth weight (2nd quartile: −99 g (95% CI: −332, 135); 3rd quartile: −100 g (−306, 106); 4th quartile: 19 g (−189, 227); p for trend 0.98). There was, however, a pattern of increasing head circumference across increasing quartiles of cord blood nitrate concentrations. Compared to the lowest quartile of cord blood nitrate (≤1900 μg/L), the second (1900–2480 μg/L), third (2480–3310 μg/L), and fourth (3310–4530 μg/L) quartiles were associated with 0.06 cm (95% CI: −0.65, 0.78), 0.12 cm (−0.60, 0.84), and 0.76 cm (0.03, 1.48) increases, respectively, in neonatal head circumference (p for trend = 0.06). This is similar to the results of the current analysis of maternal urinary PEC and infant head circumference. Here, we observed a 0.48 cm increase (95% CI: −0.01, 0.97) in head circumference per 3.36 ng/μg increase in maternal urinary PEC, the combined measure of NIS inhibitor concentrations. However, our previous study did not show a trend of increasing head circumference across increasing quartiles of cord blood PEC (2nd quartile: 0.78 cm (95% CI: 0.05, 1.52); 3rd quartile: 0.25 cm (−0.46, 0.96); 4th quartile: 0.32 cm (−0.39, 1.03); p for trend = 0.72). Though the results of the current PEC analysis appear to contradict the negative relationship between perchlorate and head circumference, it should be noted that maternal urinary PEC concentrations were more strongly correlated with maternal thiocyanate and nitrate than with perchlorate concentrations in this sample. Though thiocyanate is a known metabolite of cigarette smoke, it is also produced during the digestion of various plant foods. Given that nitrate is also consumed via plant foods, it may be possible that higher PEC concentrations in this sample are associated with greater fruit and vegetable consumption. This could potentially explain the positive relationship observed between PEC and head circumference, since maternal diets higher in fruits and vegetables have been associated with greater infant birth measurements, including head circumference [36–40]. However, this study did not collect sufficient dietary data to assess this relationship. It should also be noted that although the current methods were partly based on previous work showing an association between anion concentrations in maternal urine and the fetal compartment, that relationship was established among healthy mothers. It is unclear whether a similar relationship is present among high-risk pregnancies such as those examined here.

The lack of associations between NIS inhibitors and most birth outcomes measured in this study could be attributable to sufficient levels of maternal iodine intake in this sample.

Iodine deficiency is generally diagnosed across whole populations as a median urinary iodide concentration <100 ng/mL, though this cut-off level is often used to categorize individuals in studies of NIS inhibitor effects. Blount et al. analysis of data from the National Health and Nutrition Examination Survey (NHANES) showed a negative relationship between urinary perchlorate concentrations and serum thyroxine (T4) only among adolescent and adult females with urinary iodine concentrations <100 ng/mL [41]. The distribution of mean pregnancy urinary iodide concentrations in this sample (median = 156 ng/μg creatinine; range 45–1097 ng/μg) indicates that only about one quarter of the mothers in this study fell below the 100 ng/mL threshold. Therefore, sufficient thyroid hormone production in these mothers and their fetuses may have been maintained via adequate iodine nutrition. We did observe a greater negative effect on neonatal length of mean pregnancy thiocyanate concentrations among mothers with <100 ng/μg mean urinary iodide (1.72 cm [95% CI: −3.95, 0.50] decrease per IQR increase vs. 0.17 decrease [−0.77, 0.43] among mothers >100 ng/μg iodide; interaction $p = 0.03$). We also observed a greater negative effect on gestational age of mean pregnancy PEC among mothers with higher iodide concentrations (≥100 ng/μg:1.02 wk [−1.96, −0.08] decrease per IQR increase vs. <100 ng/μg: 0.18 wk [−1.07, 0.70] decrease; interaction $p = 0.04$). However, given the large number of statistical tests conducted, it is possible that those interactions and our other significant findings may be due to chance alone. Additionally, since there are currently no established reference ranges for urinary concentrations of these NIS inhibitors, it is unclear whether our current and previous results are due to maternal concentrations being below a biologically relevant threshold.

Although this study had several strengths, including biological measures of maternal NIS inhibitor exposures, urine samples collected at multiple time points during pregnancy, and urinalysis conducted at the CDC perchlorate laboratory, there are several limitations that should be considered when making inference. First, the power to detect a meaningful change in infant growth measures associated with maternal NIS concentrations may have been limited by the relatively small sample size of this study. However, our results suggest no clear pattern of response across any maternal exposures or infant outcomes, and so it is unlikely that our sample size prevented us from observing significant associations.

Second, this study was a secondary analysis of urine samples obtained for a separate study of prenatal phthalate exposure and gestational age [24], and so there was no *a priori* protocol in place to ensure uniformity in the timing or number of samples collected across subjects. Although most subjects (79%) provided multiple samples throughout their pregnancy, the majority of samples (78%) were obtained in the third trimester and there was wide variability in the number of samples per subject (range: 1–12). Given that the fetus is entirely dependent on circulating maternal thyroid hormones during the first 13–15 weeks of gestation [11], the inhibition of maternal thyroid hormone production by NIS inhibitors in the first trimester may be particularly detrimental to fetal growth. However, only eight subjects (7%) in this study provided at least one urine sample during their first trimester, and so we were unable to assess the association between first trimester NIS inhibitor exposures and neonatal outcomes. The lack of maternal NIS inhibitor concentrations during what may be the most relevant period of exposure may have biased our effect estimates toward the null

and could explain the lack of any pattern of response across exposures or outcomes. Future studies should collect more urine samples during the first trimester in order to examine the association between earlier prenatal NIS inhibitor exposures and fetal growth. Future studies should also concurrently measure maternal and/or neonatal T4 or TSH levels to determine whether observed associations between NIS inhibitors and neonatal size are mediated by changes in thyroid function. Further, there may have been error in whole pregnancy maternal exposure measurements given that the majority of subjects (n = 66; 61.7%) only provided urine samples during their third trimester. However, there were no differences in infant outcomes between mothers who provided samples during multiple trimesters versus those whose samples were from their third trimester only. This non-differential exposure misclassification may have biased our effect estimates toward no association between maternal exposures and infant outcomes.

Finally, there may also be some degree of outcome misclassification in this study, given that we used weight, length, and head circumference at birth as surrogate measures of fetal growth. We did not measure fetal size *in utero* at the time of each urine sample, and so we could not assess whether maternal urinary NIS inhibitor concentrations were associated with the trajectory of fetal growth throughout gestation.

5. Conclusions

We did not observe the hypothesized pattern of decreased neonatal size associated with exposure to increased prenatal concentrations of inhibitors of thyroidal iodide uptake. These anions may have more subtle effects on fetal development (e.g. neurocognitive effects) that are not reflected in such gross measures as those examined in this study. Future research of prenatal NIS inhibitor exposure should focus on other developmental abnormalities in infants and utilize multiple maternal samples during the most critical prenatal developmental periods.

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Table 1

Characteristics of study subjects.

	N (%)
Infant gender	
Male	59 (55)
Female	48 (45)
Preterm birth (≤36 weeks; study pregnancy)	26 (25)
Maternal age	
20–24	20 (19)
25–29	28 (26)
30–34	17 (16)
35–39	29 (27)
≥40	13 (12)
Maternal race/ethnicity	
White	33 (31)
Black	27 (25)
Hispanic	35 (33)
Other	11 (10)
Missing	1 (1)
Maternal education	
<High school graduate	20 (19)
High school graduate	26 (24)
Some college	27 (25)
College degree	33 (31)
Missing	1 (1)
Maternal employment status	
Employed	50 (47)
Unemployed	51 (48)
Missing	6 (6)
Smoked during pregnancy	
Never	86 (82)
Rarely	3 (3)
Sometimes	5 (5)
Often	11 (10)
Number of previous pregnancies	
0	17 (16)
1	29 (27)
2	21 (20)
3	21 (20)
≥4	19 (18)
Indication for high-risk pregnancy	
Congenital anomaly	4 (4)

	N (%)
Previous preterm delivery	35 (33)
Diabetes	17 (16)
Hypertension	21 (19)
Urinary infection	8 (7)
Febrile illness	14 (13)
Missing	8 (7)

Table 2

Distributions of maternal exposures and infant outcomes.

				Percentiles					
	N	Mean ± SD	Min.	5th	25th	50th	75th	95th	Max.
Urine samples per mother, no.	107	4 ± 3	1	1	2	3	6	10	12
Average time between samples, days	84	19.6 ± 15.5	6.0	7.0	11.2	15.8	19.8	53.5	105.0
Time from last sample to birth, days	107	10 ± 18	0	0	2	6	11	34	117
Mean whole pregnancy concentrations, ng/μg									
Perchlorate[a]	107	4.4 ± 2.8	0.7	1.6	2.6	3.7	5.3	10.5	20.1
Nitrate	107	51,674 ± 21,356	2197	28,763	35,911	50,839	60,970	86,837	158,304
Thiocyanate	107	1484 ± 2004	124	202	479	967	1505	4551	14,769
PEC[b]	107	4.8 ± 2.5	0.5	1.8	3.1	3.9	6.5	8.4	13.7
Iodide	107	190 ± 142	45	63	99	156	240	395	1097
Mean 2nd trimester concentrations, ng/μg									
Perchlorate	40	5.5 ± 9.9	0.8	1.4	2.5	3.3	4.4	12.5	64.4
Nitrate	40	52,675 ± 41,078	2197	23,389	35,699	45,865	55,235	89,310	279,618
Thiocyanate	40	1117 ± 1126	122	193	518	817	1156	4121	5533
PEC	40	5.6 ± 3.8	0.8	1.7	2.8	4.8	7.0	12.7	20.8
Iodide	40	166 ± 119	39	50	88	132	222	464	562
Mean 3rd trimester concentrations, ng/μg									
Perchlorate	104	4.5 ± 3.8	0.7	1.4	2.5	4.0	5.4	8.6	31.0
Nitrate	104	51,582 ± 20,659	4110	29,174	39,221	50,867	62,316	92,471	127,976
Thiocyanate	104	1528 ± 2124	83	202	474	940	1601	4501	14,769
PEC	104	4.8 ± 2.6	0.5	1.8	3.1	4.0	6.1	8.2	16.7
Iodide	104	194 ± 165	45	56	99	153	250	395	1425
Last sample concentrations, ng/μg									
Perchlorate	107	4.5 ± 5.5	0.7	1.2	2.4	3.4	5.2	9.1	54.1
Nitrate	107	51,244 ± 28,837	2197	26,379	32,511	43,507	60,970	103,971	184,943
Thiocyanate	107	1520 ± 2283	90	168	434	864	1619	5097	16,536
PEC	107	4.4 ± 2.8	0.5	1.3	2.4	3.9	5.7	9.4	16.7
Iodide	107	268 ± 791	42	47	91	149	251	395	8011

				Percentiles					
	N	Mean ± SD	Min.	5th	25th	50th	75th	95th	Max.
Infant measures									
Gestational age, weeks	107	37.6 ± 2.6	23	33	37	38	39	40	41
Weight, grams	107	3102 ± 747	670	1805	2625	3195	3665	4180	4985
Length, cm	107	48.4 ± 4.7	19.5	43.2	47.0	49.5	50.8	54.2	56.0
Head circumference, cm	105	33.0 ± 2.5	22.0	29.2	32.0	33.0	34.5	36.0	39.4

[a] Perchlorate, thiocyanate, nitrate, and iodide concentrations are creatinine-adjusted (ng/μg creatinine);

[b] PEC: perchlorate equivalence concentration (molar concentrations) = (perchlorate) + (nitrate/240) + (thiocyanate/15).

Table 3

Spearman correlations between whole pregnancy (n = 107), 2nd trimester (n = 40), 3rd trimester (n = 104), and last sample (n = 107) concentrations of NIS inhibitors and iodide in maternal urine.

	Thiocyanate	Nitrate	PEC	Iodide
Perchlorate				
Whole pregnancy[a]	0.11	0.47[b]	−0.05	0.58[b]
2nd trimester	0.12	0.64[b]	0.05	0.30
3rd trimester	0.09	0.46[b]	−0.08	0.61[b]
Last sample	0.14	0.40[b]	−0.11	0.57[b]
Thiocyanate				
Whole pregnancy	–	0.28[b]	0.27[b]	0.02
2nd trimester	–	0.04	0.35[b]	−0.01
3rd trimester	–	0.30[b]	0.26[b]	0.04
Last sample	–	0.32[b]	0.18	0.12
Nitrate				
Whole pregnancy	–	–	0.20[b]	0.30[b]
2nd trimester	–	–	0.23	0.12
3rd trimester	–	–	0.19[b]	0.33[b]
Last sample	–	–	0.27[b]	0.31[b]
PEC				
Whole pregnancy	–	–	–	−0.16
2nd trimester	–	–	–	−0.29
3rd trimester	–	–	–	−0.19
Last sample	–	–	–	−0.17

[a] Whole pregnancy, 2nd trimester, and 3rd trimester correlations;

[b] $p < 0.05$.

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Table 4

Changes in infant weight, length, head circumference, and gestational age associated with interquartile range (IQR) increases in mean whole pregnancy exposures.

		All subjects			Term births		
	IQR (ng/μg)	***N***	**β (95% CI)**	***p***	***N***	**β (95% CI)**	***p***
Weight, grams[a]							
Perchlorate[b]	2.62	107	−63.7 (−171.5, 44.1)	0.24	81	−68.0 (−189.2, 53.2)	0.27
Thiocyanate	1026	107	−27.3 (−92.4, 37.8)	0.41	81	−23.5 (−93.2, 46.3)	0.51
Nitrate	25,059	107	6.6 (−122.1, 135.3)	0.92	81	85.3 (−106.1, 276.7)	0.38
PEC[c]	3.38	107	20.5 (−139.9, 180.9)	0.80	81	9.5 (−186.6, 205.7)	0.92
Iodide	141	107	32.9 (−58.5, 124.2)	0.48	81	30.5 (−68.0, 129.1)	0.54
Length, cm							
Perchlorate	2.62	107	−0.06 (−0.91, 0.79)	0.89	81	0.03 (−0.96, 1.01)	0.96
Thiocyanate	1026	107	−0.17 (−0.68, 0.34)	0.51	81	−0.05 (−0.61, 0.51)	0.85
Nitrate	25,059	107	−0.12 (−1.13, 0.89)	0.82	81	0.14 (−1.40, 1.69)	0.86
PEC	3.38	107	0.09 (−1.17, 1.36)	0.88	81	0.07 (−1.50, 1.65)	0.93
Iodide	141	107	0.15 (−0.57, 0.87)	0.68	81	0.15 (−0.64, 0.95)	0.70
Head circ., cm							
Perchlorate	2.62	105	−0.32 (−0.66, 0.01)	0.06	80	−0.38 (−0.74, −0.03)	0.03
Thiocyanate	1026	105	0.05 (−0.15, 0.26)	0.60	80	0.08 (−0.13, 0.29)	0.46
Nitrate	25,059	105	0.07 (−0.33, 0.47)	0.73	80	0.02 (−0.56, 0.61)	0.92
PEC	3.38	105	0.48 (−0.01, 0.97)	0.06	80	0.38 (−0.20, 0.96)	0.19
Iodide	141	105	0.03 (−0.26, 0.31)	0.86	80	−0.00 (−0.30, 0.29)	0.98
Gestational age, weeks[d]							
Perchlorate	2.62	104	0.34 (−0.12, 0.79)	0.15	80	0.12 (−0.12, 0.36)	0.32
Thiocyanate	1026	104	0.02 (−0.21, 0.25)	0.87	80	−0.02 (−0.13, 0.10)	0.76
Nitrate	25,059	104	−0.16 (−0.73, 0.42)	0.59	80	0.07 (−0.33, 0.47)	0.73
PEC	3.38	104	−0.46 (−1.10, 0.18)	0.15	80	−0.19 (−0.55, 0.17)	0.30
Iodide	141	104	0.08 (−0.30, 0.47)	0.67	80	0.00 (−0.20, 0.19)	0.97

[a] Weight, length, and head circumference models include maternal smoking, nulliparity, infant gender, and gestational age.

[b] Perchlorate, thiocyanate, nitrate, and iodide concentrations are creatinine-adjusted (ng/μg creatinine).

[c] PEC: perchlorate equivalence concentration (molar concentrations)=(perchlorate)+(nitrate/240)+(thiocyanate/15).

[d] Gestational age models include maternal race/ethnicity and paternal employment status; all models weighted by number of urine samples per subject.

Table 5

Changes in neonatal measurements and gestational age per interquartile range increases in exposures in the 2nd and 3rd trimester and the last sample before delivery.

		Weight (grams)[a]			Length (cm)			Head circumference (cm)			Gestational age (weeks)[b]		
	IQR (ng/μg)	*N*	β (95% CI)	*p*	*N*	β (95% CI)	*p*	*N*	β (95% CI)	*p*	*N*	β (95% CI)	*p*
2nd trimester[c]													
Perchlorate[d]	1.94	40	−0.6 (−44.9, 43.7)	0.98	40	−0.07 (−0.38, 0.25)	0.67	39	−0.03 (−0.15, 0.09)	0.60	40	0.03 (−0.26, 0.32)	0.81
Thiocyanate	638	40	111.5 (−110.4, 333.3)	0.31	40	1.10 (0.45, 2.65)	0.16	39	0.15 (−0.46, 0.77)	0.62	40	0.05 (−0.57, 0.68)	0.86
Nitrate	19,536	40	−27.4 (−123.7, 69.0)	0.57	40	−0.15 (−0.84, 0.53)	0.65	39	−0.02 (−0.29, 0.24)	0.86	40	0.10 (−0.59, 0.78)	0.77
PEC[e]	4.16	40	−37.4 (−256.9, 182.2)	0.73	40	0.06 (−1.49, 1.62)	0.93	39	−0.0002 (−0.61, 0.61)	0.99	40	−0.26 (−1.59, 1.08)	0.70
Iodide	134	40	18.6 (−150.2, 187.3)	0.82	40	−0.14 (−1.33, 1.05)	0.81	39	0.28 (−0.18, 0.74)	0.22	40	−0.24 (−1.30, 0.83)	0.66
3rd trimester													
Perchlorate	2.93	104	−49.2 (−150.4, 52.1)	0.34	104	0.02 (−0.78, 0.83)	0.96	102	−0.19 (−0.50, 0.13)	0.25	101	0.20 (−0.15, 0.55)	0.27
Thiocyanate	1127	104	−33.6 (−99.0, 31.9)	0.31	104	−0.21 (−0.73, 0.31)	0.43	102	0.05 (−0.16, 0.26)	0.62	101	0.01 (−0.18, 0.20)	0.92
Nitrate	23,095	104	37.6 (−90.1, 165.1)	0.56	104	−0.11 (−1.13, 0.90)	0.82	102	0.14 (−0.27, 0.54)	0.50	101	−0.25 (−0.69, 0.20)	0.28
PEC	3.00	104	60.8 (−90.7, 212.2)	0.43	104	0.31 (−0.89, 1.52)	0.61	102	0.53 (0.06, 1.00)	0.03	101	−0.28 (−0.78, 0.22)	0.27
Iodide	151	104	43.6 (−41.7, 128.9)	0.31	104	0.26 (−0.42, 0.94)	0.44	102	0.06 (−0.21, 0.33)	0.66	101	0.08 (−0.21, 0.36)	0.60
Last sample													
Perchlorate	2.87	107	−1.1 (−51.3, 49.2)	0.97	107	0.07 (−0.36, 0.51)	0.74	105	−0.01 (−0.17, 0.16)	0.91	104	0.04 (−0.21, 0.28)	0.76
Thiocyanate	1185	107	−41.3 (−105.6, 23.0)	0.21	107	−0.37 (−0.93, 0.19)	0.19	105	−0.07 (−0.28, 0.14)	0.52	104	0.03 (−0.23, 0.29)	0.81
Nitrate	28,459	107	68.9 (−29.3, 167.3)	0.17	107	0.38 (−0.48, 1.24)	0.38	105	0.33 (−0.002, 0.66)	0.05	104	−0.17 (−0.64, 0.30)	0.48
PEC	3.29	107	75.1 (−45.4, 195.5)	0.22	107	0.13 (−0.93, 1.18)	0.81	105	0.36 (−0.04, 0.75)	0.08	104	−0.002 (−0.93, 0.20)	0.20
Iodide	160	107	14.4 (−5.5, 34.2)	0.15	107	0.12 (−0.06, 0.29)	0.19	105	0.04 (−0.03, 0.10)	0.25	104	−0.01 (−0.10, 0.09)	0.88

[a] Weight, length, and head circumference models adjusted for maternal smoking, nulliparity, infant gender, and gestational age.

[b] Gestational age models adjusted for maternal race/ethnicity and paternal employment status.

[c] 2nd and 3rd trimester models weighted by number of urine samples per subject.

[d] Perchlorate, thiocyanate, nitrate, and iodide concentrations are creatinine-adjusted (ng/μg creatinine).

[e] PEC: perchlorate equivalent concentration (molar concentrations) = (perchlorate) + (nitrate/240) + (thiocyanate/15).