Supporting Information

Perinatal exposure to perchlorate, thiocyanate, and nitrate in New Jersey mothers and newborns

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Table S1. Characteristics of the study participants (n=150 subjects)

CHARACTERISTIC	n*	%
Infant Gender		
Male	62	41
Female	51	34
Data Missing	37	25
Maternal Race		
White	62	41
Black	7	5
Hispanic	11	7
Other or Data Missing	70	47
Maternal Age		
20-29 years	28	19
30-34 years	61	41
35-39 years	35	23
≥ 40 years	9	6
Data Missing	17	11
Maternal Smoking During Pregnancy		
Never	139	93
Sometimes/Often	10	7
Data Missing	1	1
Exposure to environmental tobacco smoke?		
Never	121	81
Sometimes/Often	28	19
Data Missing	1	1
Gravidity (# pregnancies ≥ 20 Weeks gestational age)		
1	16	11
2	58	39
3	40	27
4 or more	36	24
Body Mass Index		
< 25	18	12
25-29.9	44	29
30-34.9	40	27
≥ 35	41	27
Data Missing	7	5
Take Daily Prenatal Vitamin?		
Never	39	26
Sometimes/Often	111	74
Drink Bottled Water?		
Never	11	7
Sometimes/Often	139	93

*Number of subjects for total study; some subjects did not provide complete sample sets.

Table S2. Distribution of newborn measurements for pregnancies with matching cord blood analyte measurements.

Outcome	n	Min	25th	50th	75th	Max	Reference range (5th – 95th)*
Head Circumference (cm)	115	31.60	34.30	35.00	35.75	39.00	32.15 – 38.52
Birth weight (g)	120	2430	3225	3553	3890	4895	2527 - 4340
Length (cm)	115	45.50	49.50	50.80	52.85	57.20	45.57 – 54.31

*reference range percentiles from http://www.cdc.gov/GrowthCharts/

Sample Collection

Maternal urine (MU) and blood (MB) samples were collected in the preoperative holding area within an hour of surgery and before intravenous drip began. Blood samples (10–30 mL) were drawn into 10-mL Vacutainer serum separator tubes (Becton-Dickinson). After blood was collected, patients were taken to the operating room where anesthesia was administered. Immediately after placement of a Foley catheter into the bladder, maternal urine (10–30 mL) was aspirated from the collection port into a sterile cup, transferred later into a glass bottle (Qorpak, Bridgeville, PA; 30 mL) and stored frozen at -70 °C. Maternal urine was collected from 34 of the study participants. As the cesarean incision was extended laterally and the intact membranes were identified, an 18-gauge intravenous catheter sheath without the needle was directly inserted through the membranes and approximately 10–30 mL of amniotic fluid (AF) was collected. In some instances, this process was not feasible or the membranes were grossly ruptured, so amniotic fluid was collected by means of a fluid gush into a sterile cup. Notation was made if the fluid appeared contaminated with maternal blood. Neither collection method nor blood contamination of amniotic fluid was associated with altered analyte levels. Amniotic fluid was subsequently transferred from the sterile cup into a glass bottle (Qorpak, 30 mL) and stored frozen at -70 °C. Following fetal delivery the umbilical cord was clamped and 30–60 mL of cord blood (CB) were aspirated directly from the umbilical vein after cleaning the cord. Cord blood specimens were collected using 21-gauge needles with 30-mL syringes. Syringe-collected cord blood samples were transferred into serum separator tubes. All cord blood samples were obtained within 15 min of delivery. Study personnel were present at each delivery to ensure correct sampling procedures were followed. Maternal pregnancy characteristics (age, race or ethnicity, income, occupation, hobbies, tobacco smoke exposure, bottled water use and prenatal vitamin use) and neonatal outcome data (birth weight, length and head circumference) were recorded.

Sample Processing and Analysis

Following collection of blood into serum separator tubes, samples were stored upright for 30–60 min at room temperature to allow clot formation. The clotted specimens were centrifuged at 3000 rpm for 15 min. Aliquots of serum (1–3 mL) were transferred into cryovials containing 125–375 µL 1M phosphoric acid, and stored at -70 ^{o}C until analysis. Perchlorate was measured by ion chromatography tandem mass spectrometry (*1*).

Perchlorate dose estimates

Perchlorate doses were estimated based on a published method of extrapolating serum perchlorate levels from dosing studies (*2*). The equation used is listed below:

$$\log Dose = \left(\frac{\left(\frac{\log MaternalSerumPerchlorate(\mu g / L)}{99.5} - 1.052 \right)}{0.9193} \right)$$

Dose estimates of non-persistent toxicants based on a single biological sample are not necessarily representative of typical exposure. Given the episodic and varied nature of perchlorate exposure and the relatively short half life of perchlorate in the body (~8 hrs), the levels of perchlorate in blood are likely to vary with time (*3*). Thus distributions of dose estimates based on spot samples are most precise at the central tendency, and are likely to overly extend the extremes of the distribution.

Calculation of perchlorate equivalence concentration (PEC)

Comparison of NIS-inhibitors in maternal blood with fetal iodide levels included the following potency factors based on an in vitro study of NIS-mediated competitive transport: perchlorate, 1; thiocyanate, 1/15; nitrate, 1/240 (*4*); these potency factors are based on a single in vitro study and thus include a degree of uncertainty. We calculated a perchlorate equivalence concentration variable (PEC) by summing the product of molar concentrations of each iodide uptake inhibitor and its corresponding potency factor (*4*):

$$PEC = \left(\frac{perchlorate}{99.45} \right) + \left(\frac{thiocyanate}{58.08 \times 15} \right) + \left(\frac{nitrate}{62.01 \times 240} \right)$$

Figure S1: Perchlorate distributions (µg/L) in maternal and fetal matrices

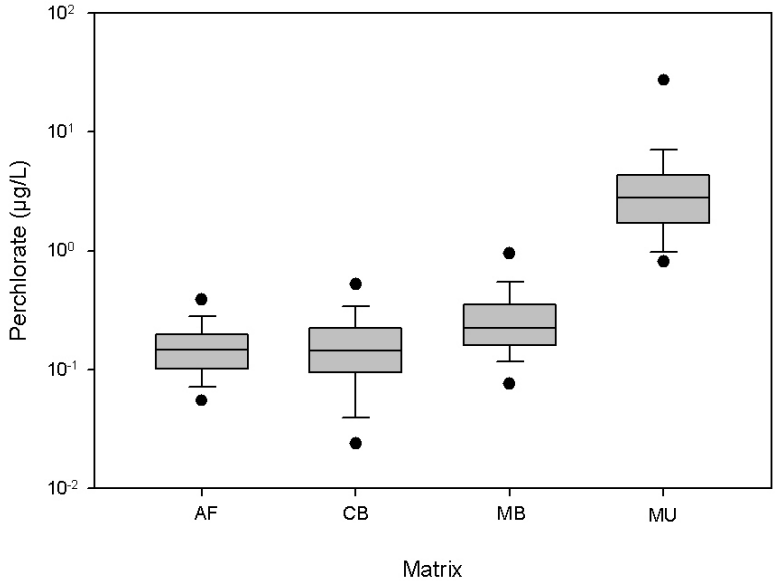


Figure S2: Thiocyanate distributions (µg/L) in maternal and fetal matrices

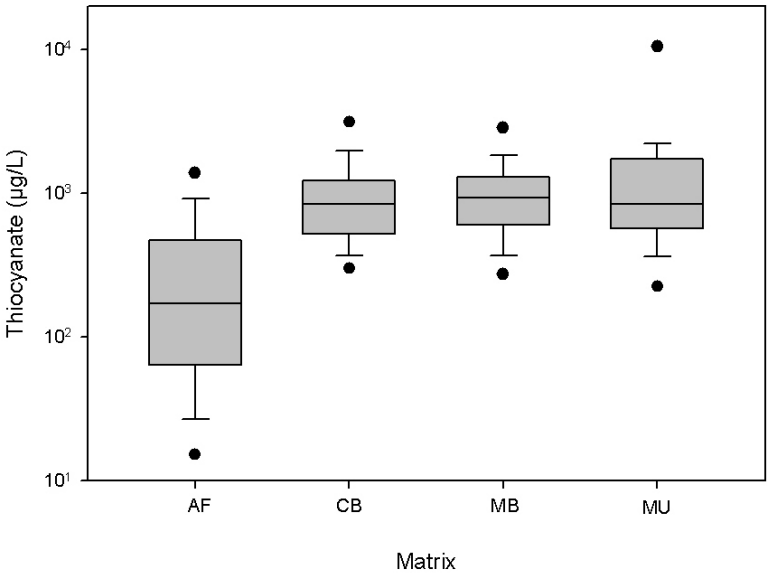


Figure S3: Nitrate distributions (µg/L) in maternal and fetal matrices

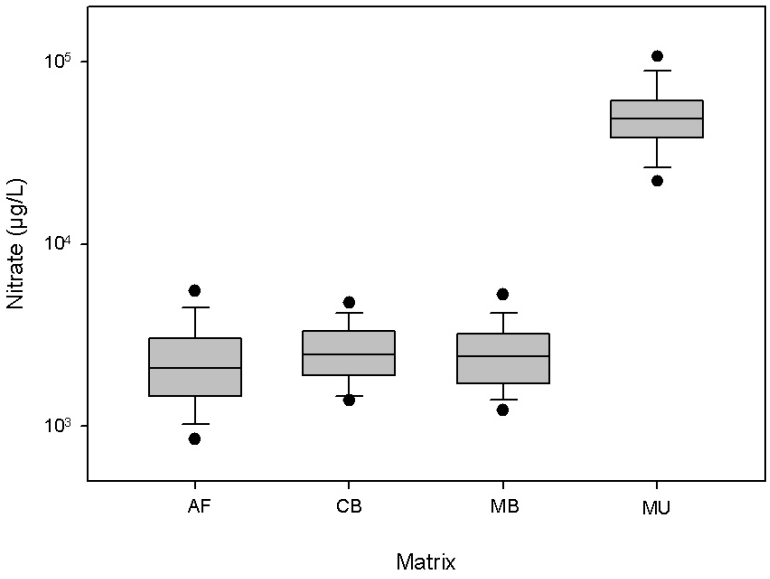


Figure S4: Iodide distributions (µg/L) in maternal and fetal matrices

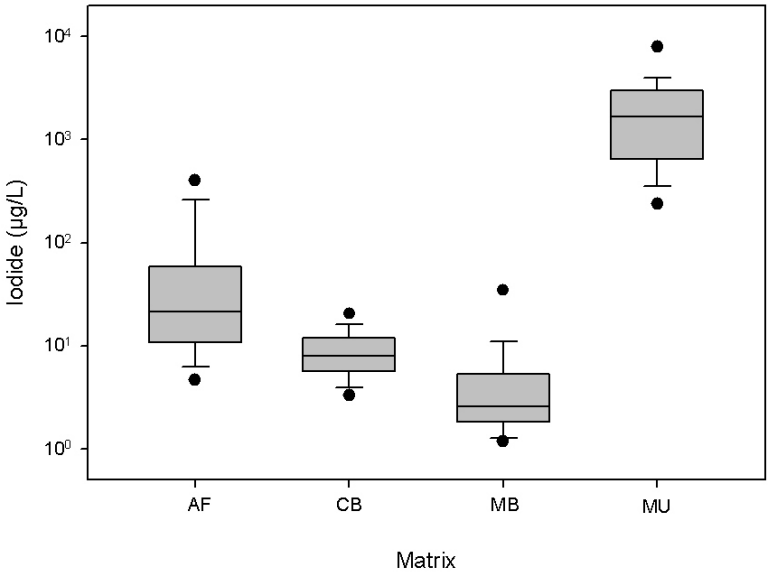
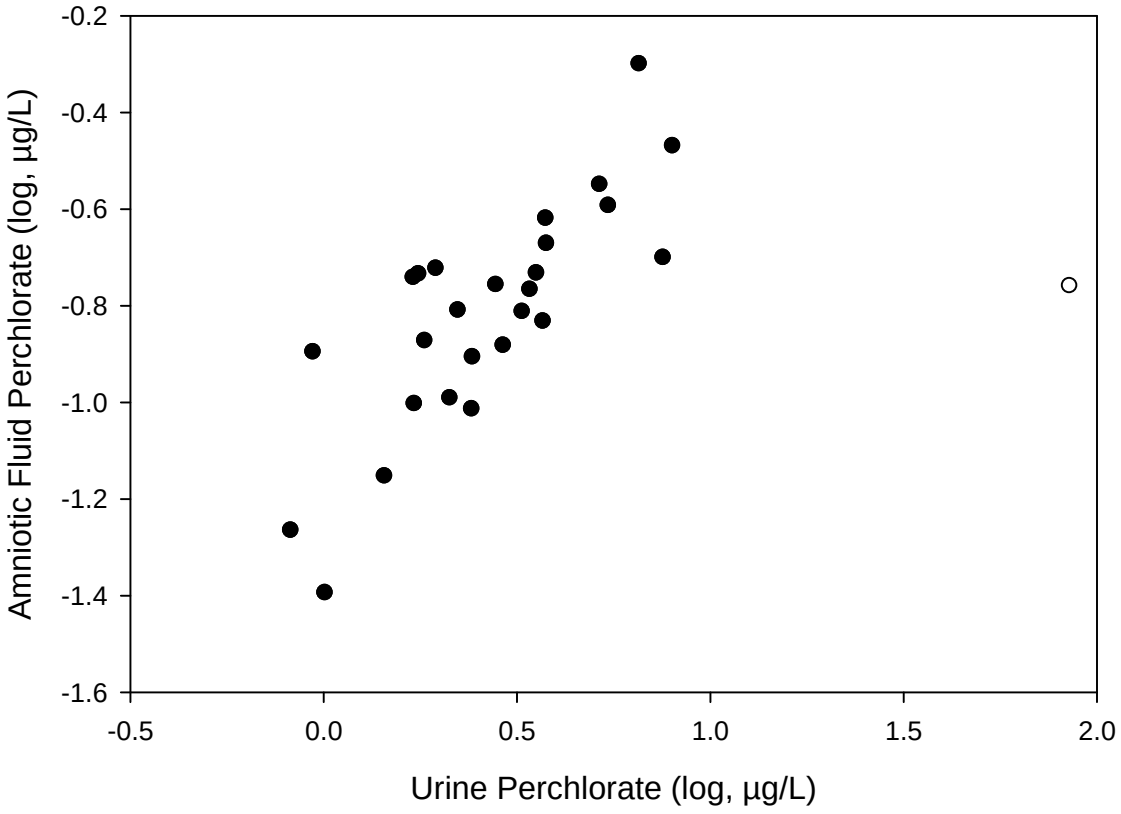


Figure S5: Correlation of perchlorate in amniotic fluid and maternal urine for 26 pairs of samples: r=0.57 for full data set. If an outlying point (○) is excluded r=0.92.



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1. Amitai Y, Winston G, Sack J, Wasser J, Lewis M, Blount BC et al. Gestational exposure to high perchlorate concentrations in drinking water and neonatal thyroxine levels. *Thyroid* **2007,** *17,*843-850.

2. Gibbs,J. A comparative toxicological assessment of perchlorate and thiocyanate based on competitive inhibition of iodide uptake as the common mode of action. *Hum.Ecol.RiskAssess.* **2006**, *12*, 157-173.

3. Merrill EA, Clewell RA, Robinson PJ, Jarabek AM, Gearhart JM, Sterner TR, Fisher JW. PBPK model for radioactive iodide and perchlorate kinetics and perchlorate-induced inhibition of iodide uptake in humans. *Toxicol.Sci* **2005**, *83*, 25-43.

4. Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P et al. Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. *Thyroid* **2004**, *14,* 1012-1019.