PILOT STUDY OF PERCHLORATE EXPOSURE IN LACTATING WOMEN IN AN URBAN COMMUNITY IN NEW JERSEY

by

MARIJA BORJAN

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ABSTRACT OF THE DISSERTATION

Pilot Study of Perchlorate Exposure in Lactating Women in an

Urban Community in New Jersey

By MARIJA BORJAN

Dissertation Director:

Mark Gregory Robson, Ph.D., M.P.H.

Perchlorate is most widely known as a solid oxidant for missile and rocket propulsion systems and has been detected in drinking water, fruits, vegetables, and milk throughout New Jersey and most of the U.S. Perchlorate interferes with the uptake of iodine into the thyroid and may interfere with the development of the skeletal system and central nervous system of infants who ingest perchlorate. Therefore, it is important to understand the occurrence of perchlorate in breast milk. This study will allow us to acquire valuable information on human exposure to perchlorate through analyses of breast milk, urine, and drinking water. One hundred and six lactating mothers were recruited from the Eric B. Chandler health center in New Brunswick, NJ and provided consent to participate in this study. Each subject was asked to provide three sets of samples and complete a 24-hour dietary recall. Regression analyses showed that diet was the greatest predictor of perchlorate in breast milk and urine and that drinking water was not a major source of perchlorate. The average perchlorate level in drinking water samples was 0.168 ng/mL (n = 253), which is below New Jersey's Maximum Contaminant Level of 5 ng/mL. Perchlorate was detected in all breast milk samples provided (n = 276). The average perchlorate level in breast milk was 6.80 ng/mL. These findings are consistent with widespread perchlorate exposure in lactating women and infants, and that breast milk is a viable exposure route for infants. Based on the measured perchlorate levels in breast milk, we estimate that 25% of infants 0-6 months of age would exceed the EPA's reference dose of 0.0007 mg/kg/day. Breast-feeding is important in an infant's growth and development. Therefore, it is important to gain a better understanding of environmental contaminant concentrations in human breast milk and other sources of infant nourishment.

Acknowledgments

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List of abbreviations

BSDW: Bureau of Safe Drinking Water

DWEL: Drinking Water Equivalent Level

LOD: Limit of Detection

LOQ: Limit of Quantitation

MCL: Maximum Contaminant Level

MCLG: Maximum Contaminant Level Goal

NHANES: National Health and Nutrition Examination Survey

NIS: Sodium Iodide Symporter

NJDEP: New Jersey Department of Environmental Protection

NOEL: No Observable Effects Level

ppb: parts per billion [ppb = ng/mL]

RfD: Reference Dose

RSC: Relative Source Contribution

TH: Thyroid Hormone

T_4: Thyroxine

T_3: Triiodo-thyronine

UCMR: Unregulated Contaminate Monitoring Rule

US EPA: United Stated Environmental Protection Agency

US FDA: United States Food and Drug Administration

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Chapter 1

Introduction

Breast-feeding is important in an infant's growth and development. Not only does breast milk provide immunologic protection, it also contains nutrients needed by the infant. It has been shown that the risk for developing illnesses such as asthma, diabetes, arthritis, obesity, cardiovascular diseases, pneumonia, and some cancers is lower in children who have been breast fed as. (Mead *et al.* 2008; US FDA 2007a) However, environmental contaminants maybe found in breast milk, which raises significant public health issues. One contaminant is perchlorate, which is a concern among sensitive populations such as the fetus, nursing infants, and hypothyroid women. Environmental contaminants such as perchlorate can be transferred through breast milk from mother to baby.

Perchlorate is a known contaminant in drinking water, fruits, and vegetables. Once in the body, perchlorate is actively transported into the thyroid. Perchlorate competes with iodine for transport into the thyroid by the sodium (Na^+)/iodide (I^-) symporter (NIS) and can prevent the uptake of iodine into the thyroid, which is essential for thyroid hormone synthesis along with normal development, growth, and metabolism. A decrease in iodine levels can result in decreased thyroid hormone synthesis. Thyroid hormones are important determinants of metabolic activity, have an impact on a majority of the organ systems, and are important in the development of the skeletal system and the central nervous system. Iodide is not formed in the body and must be obtained from an outside source. Therefore, it is critical that infants consume an adequate amount of iodide through mother's breast milk.

The same mechanism that transports perchlorate into the thyroid is also present in the mammary gland, which allows for the active transport of perchlorate to the infant through breast milk. Infants and children go through rapid transformations in physical development, language development, and cognitive and motor capabilities, which make them more at risk from the effects of environmental contaminants. Endocrine functions in many organs are changing. Functional and structural changes in the digestive tract, reproductive system, lungs, and bones are occurring throughout infancy and childhood. The changes occurring in infants can influence the processing of environmental contaminants and can influence which organs and tissues in the body are affected. For example, renal clearance rate, which is the rate of chemical eliminated from the body (mL/min), is slower in infants compared to adults. In addition, infants consume more per unit of body weight than adults do. These factors may result in infants being exposed to a higher level of perchlorate, which can lead to hypothyroidism or neurodevelopment problems. (Kirk 2006; Klaassen *et al*. 2003; Landrigan *et al.* 2002; Robson 2007)

Given the possible public health impacts, I estimate total perchlorate exposure for lactating women based on perchlorate levels in maternal milk and urine. In addition, based on perchlorate levels in maternal milk I estimate total perchlorate exposure for infants consuming this breast milk. This research will also look at the influence of food products and drinking water on perchlorate levels in breast milk and urine. Thiocyanate and nitrate, which also competitively bind to the NIS, are also analyzed in the same samples.

Research Questions and Hypothesis

Research Questions

1) How much perchlorate is found in the breast milk of lactating women and therefore can be passed to the infant?
2) What factors influence the variation in maternal perchlorate dose?

Two primary hypotheses that will be tested in this dissertation:

1) Perchlorate levels in breast milk will be positively associated with levels of perchlorate in drinking water.
2) Perchlorate levels in breast milk will be negatively associated with iodide levels in breast milk.

Three other research questions and secondary hypotheses include:

3) *Question*: Will the mass of perchlorate excreted through breast milk be greater than the mass excreted through urine in the subject?

   Hypothesis: The mass of perchlorate excreted through breast milk is greater than the mass of perchlorate excreted in urine.
4) *Question*: If the major excretion route of perchlorate is breast milk, will iodide be excreted at a lower level in breast milk compared to urine?

Hypothesis: The mass of iodide excreted in breast milk will be lower compared to urine if breast milk is the major excretion route for perchlorate.

5) *Question:* Will the consumption of certain food products such as vegetables, fruits, and grains be positively associated with perchlorate levels in breast milk and urine?

   Hypothesis: Dietary intake of certain food products (vegetables, fruits, grains, and milk products) will be positively associated with perchlorate levels in breast milk and urine.

6) *Question*: Do the levels of perchlorate measured in breast milk samples lead to infants ingesting perchlorate at a level that exceeds the EPA reference dose?

   Hypothesis: The measured levels of perchlorate in breast milk likely leads to infants ingesting perchlorate at levels that exceed the EPA reference dose.

Chapter 2

Literature Review

Perchlorate (ClO_4^-) is an anion constituent of salt often linked with the cations ammonium, sodium, or potassium to form ammonium perchlorate (NH_4ClO_4), potassium perchlorate ($KClO_4$), and sodium perchlorate ($NaClO_4$). Perchlorate is a strong oxidizing agent and known to be very stable and nonreactive in aqueous environments. Perchlorate is highly soluble and mobile in water allowing it to follow the movement of water within plants and eventually accumulates in the leaves. Due to its negative charge and its low affinity for metal ions, perchlorate is poorly absorbed by sediment. (Gu 2007; ITRC 2005)

Perchlorate is most commonly known as a solid oxidant for missile and rocket propulsion systems (Urbansky 2002). Chilean nitrate-based fertilizers, fireworks, and road flares are also found to contain perchlorate. High concentrations of manmade perchlorate are often found in areas that entail manufacturing, testing, or disposal of solid rocket propellant; manufacturing of perchlorate compounds; and other processes that used perchlorate compounds as reagents. Perchlorate also occurs naturally in the environment. Low concentrations of natural sources of perchlorate are geographically limited to more arid environments. (ITRC 2005)

The primary route of human exposure to perchlorate is by ingestion of contaminated water and food. Dermal and inhalation exposure of perchlorate is unlikely in non-occupational settings. Perchlorate is fully ionized in water since it is an inorganic compound. In addition, perchlorate is incapable of moving through the skin because of its high polarity. Therefore, the chance for dermal absorption of perchlorate from bathing or cleaning is not likely. Individuals who work in the commercial production of ammonium perchlorate are primarily exposed to its dust by inhalation. (Gu 2007)

Once in the body, perchlorate can interfere with the uptake of iodine into the thyroid by competing for active transport by NIS, which can lead to a decrease in the production of thyroid hormones, and could eventually cause hypothyroidism (Figure 1). Exposure to perchlorate is a concern among sensitive populations such as the fetus, nursing infants, and hypothyroid women (Table 1). Adequate iodine intake is important to help reduce the potential effects of perchlorate.

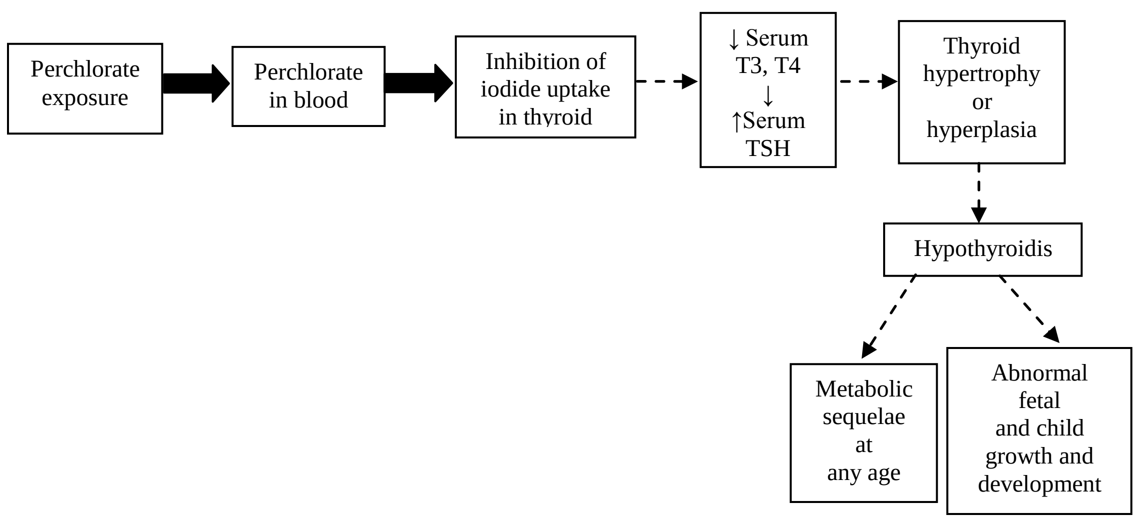


Figure 1. Mode of action model of perchlorate toxicity in humans (source: NAS 2005)

Table 1. Populations of potential concern

Potential Receptor of Concern	**Rationale**	**References**
Developing fetus	Thyroid hormones necessary for normal brain growth	Haddow *et al*. 1999, Howdeshell 2002, Heidel and Zoeller 2003, Lavado-Autric *et al*. 2003, Auso *et al*. 2003
Nursing infant	Exposure to perchlorate via human milk; brain still developing, thyroid hormones necessary for brain development	Clewell *et al*. 2003b, Tazebay *et al*. 2000
Children	Brain still developing; thyroid hormones necessary for brain development, as well as growth and metabolism	Giedd *et al*. 1999, Sowell *et al*. 1999, Thompson *et al*. 2000, Webster *et al*. 2003
Postmenopausal women	High rates of hypothyroidism	Hollowell *et al*. 2002, Surks *et a*l. 2004

(source: ITRC 2005)

Thyroid and Iodide Uptake

The thyroid is the endocrine gland that produces hormones used by the body to regulate physiological functions. Thyroxine (T_4) and triiodo-thyronine (T_3) are the two hormones produced by the thyroid gland. These hormones are important determinants of metabolic activity and have an impact on a majority of the organ systems. They are also important in the development of the skeletal system and the central nervous system in fetuses and infants. Iodine is essential for thyroid hormone synthesis along with normal development, growth, and metabolism. When the transport of iodide into the thyroid is inhibited, inadequate iodine levels in the thyroid lumen can result in the decreased synthesis of the thyroid hormones. (NAS 2005) Impaired thyroid function during pregnancy can result in neurodevelopmental effects in the fetus. (Haddow 1999)

The sodium (Na^+)/iodide (I^-) symporter (NIS) is a protein that mediates the transport of iodide into the thyroid. Not only does NIS transport iodide, it also actively transports other ions that have a similar shape and charge as iodide. Perchlorate can compete with iodide for NIS-mediated transport and thus inhibit the transport of iodide across membranes that contain NIS (e.g. basolateral surface of thyrocytes). NIS is also expressed in placenta and lactating breast tissue, raising concerns about active transport of perchlorate into the fetal compartment and breast milk. (NAS 2005)

Other nonthyroid tissues that actively accumulate iodide are the salivary gland, gastric mucosa, choroid plexus, ciliary body of the eye, and the lactating mammary gland. Active iodide transport is also mediated by the NIS in these tissues and are also susceptible to inhibition of iodide uptake by perchlorate and thiocyanate. (Dohan *et al*. 2003) Perchlorate that is not transported to the thyroid is cleared from the body unchanged, primarily via urinary excretion and milk secretion.

Other Iodide Uptake Inhibitors

Other anions that also inhibit iodide uptake by NIS include thiocyanate and nitrate. These anions are present in some food crops and water. Nitrate is naturally present in leafy vegetables and sometimes added to food as a preservative. Nitrate is also found in drinking water sources due to nitrate based fertilizers used for agricultural purposes. Thiocyanate is a metabolite of cyanide, which is found in tobacco smoke. Some foods that may contain thiocyanate include cabbage, broccoli, Brussels sprouts, turnips, cabbage, radishes, spinach, and tomatoes. (Steinmaus *et al.* 2007; Tonacchera *et al.* 2004; Kirk *et al.* 2007)

Steinmaus *et al*. (2007) found that in women with low urinary iodide levels, smokers had a greater association between urinary perchlorate and lower levels of serum T_4. This indicates that thiocyanate and perchlorate may interact to inhibit iodide uptake, and potentially further reduce thyroid hormone synthesis. These findings are consistent with an in vitro study of human NIS: Tonacchera *et al*. (2004) found that simultaneous exposure of perchlorate, thiocyanate, and nitrate inhibited iodide uptake in a simple additive manner.

Laurberg *et al*. (2004) looked at iodide levels in healthy, lactating women and their newborn infants. Urine cotinine was analyzed to determine smoking status. The study found that iodine levels in breast milk (smokers 26.0 ng/mL vs. nonsmokers 53.8 ng/mL) and the infant's urine (infants of smokers 33.3 ng/mL vs. infants of nonsmokers 50.4 ng/mL) were reduced due to smoke exposure. This effect may be caused by thiocyanate competing with iodide for NIS-mediated transport: smokers had higher levels of thiocyanate compared with non-smokers.

Breast Milk

Thyroid hormones are essential for infant neural development. As previously mentioned, an iodide transport protein similar to the NIS of the thyroid has also been identified in the mammary gland. Iodide is actively transported by the lactating mammary gland and secreted in the milk. According to Dohan *et al*. (2003), transport of iodide into the mammary gland usually take place during the later part of pregnancy and during lactation. Iodide is transported into the breast milk by the NIS, thus providing anions to the nursing infant for biosynthesis of their own thyroid hormones. This may create a greater public health risk than formerly thought because iodide uptake in the breast-fed infant can be directly inhibited by perchlorate. (Dohan *et al*. 2003, 2007)

Infants who are breast-fed depend on the iodide from their mother's milk for proper thyroid functioning and hormone synthesis. According to the Public Health Committee of the American Thyroid Association (2006), iodide is not produced in the body and must be obtained from an outside source (Figure 2). The Institute of Medicine (IOM) (2004) recommends 110µg/d of iodine for infants 0-6 months and 130µg/d for infants 7-12 months as an adequate intake of iodine. The IOM also recommends a dietary allowance of 290µg/d of iodine for lactating women.

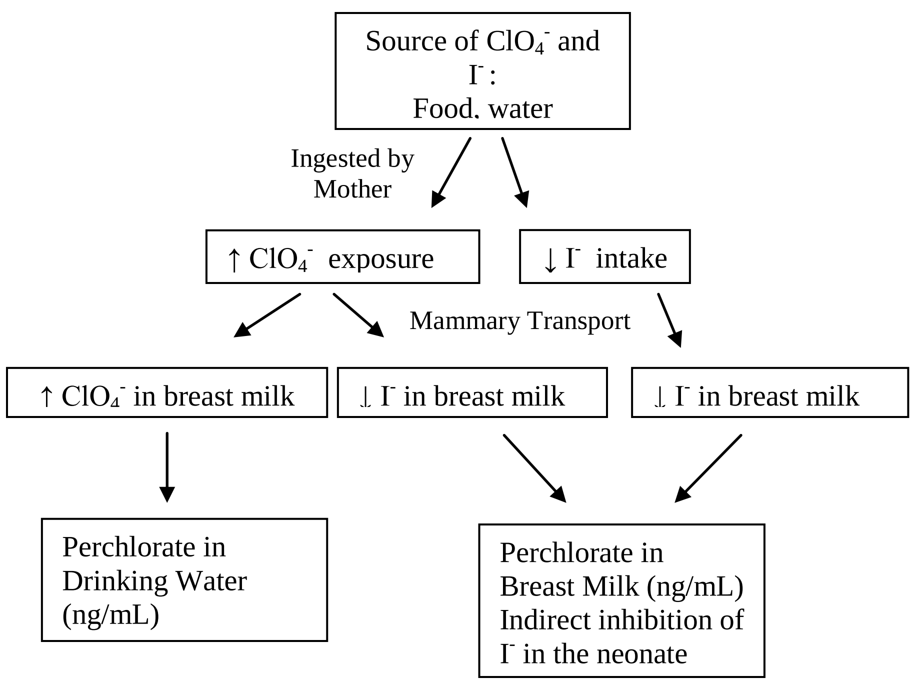


Figure 2. Mechanism of perchlorate interfering with neonatal iodide uptake

Kirk *et al*. (2007) analyzed breast milk samples from ten lactating women for perchlorate, iodide, and thiocyanate. Iodide ranged from 3.1-334 ng/mL and perchlorate ranged from 0.5-39.5 ng/mL. The authors conclude that iodine intake may be insufficient in breast-fed infants.

In a study of 57 lactating Boston-area women, 49 breast milk samples had detectable levels of perchlorate, ranging from 1.3-411 ng/mL. In 27 of the subjects, about 10 mL of breast milk was collected at the start of feed. In the remaining 30 women, breast milk samples were collected in 5 mL increments consecutively from start to finish, assessing any variation during a single feed. The investigators concluded that there was no significant correlation between perchlorate exposure and breast milk iodide concentration, but 47% of the lactating women's infants may be receiving an insufficient amount of iodide through breast milk. (Pearce *et al*. 2007)

Dasgupta *et al*. (2008) analyzed perchlorate, thiocyanate, and iodide excretion in breast milk and urine samples provided by 13 lactating women. Total excretion in breast milk ranged from 0.394-0.781 µg/day, 0.018-0.144 µg/day, and 0.086-0.464 µg/day for perchlorate, thiocyanate, and iodide, respectively. The data from this study also showed that only 1 of the 13 infants in this study may be receiving an adequate amount of iodine through breast milk and that over half of the infants are ingesting perchlorate above the reference dose.

Dietary Perchlorate

Perchlorate has been found in drinking water, leafy green vegetables, fruits, milk, and grains. According to the US EPA (2006), perchlorate has been found in the drinking water of more than 20 states (Figure 3). Perchlorate has also been detected in ground water and irrigation water and there is concern that fruits and vegetables irrigated with perchlorate-contaminated water may pose a public health risk. (Gu 2006)

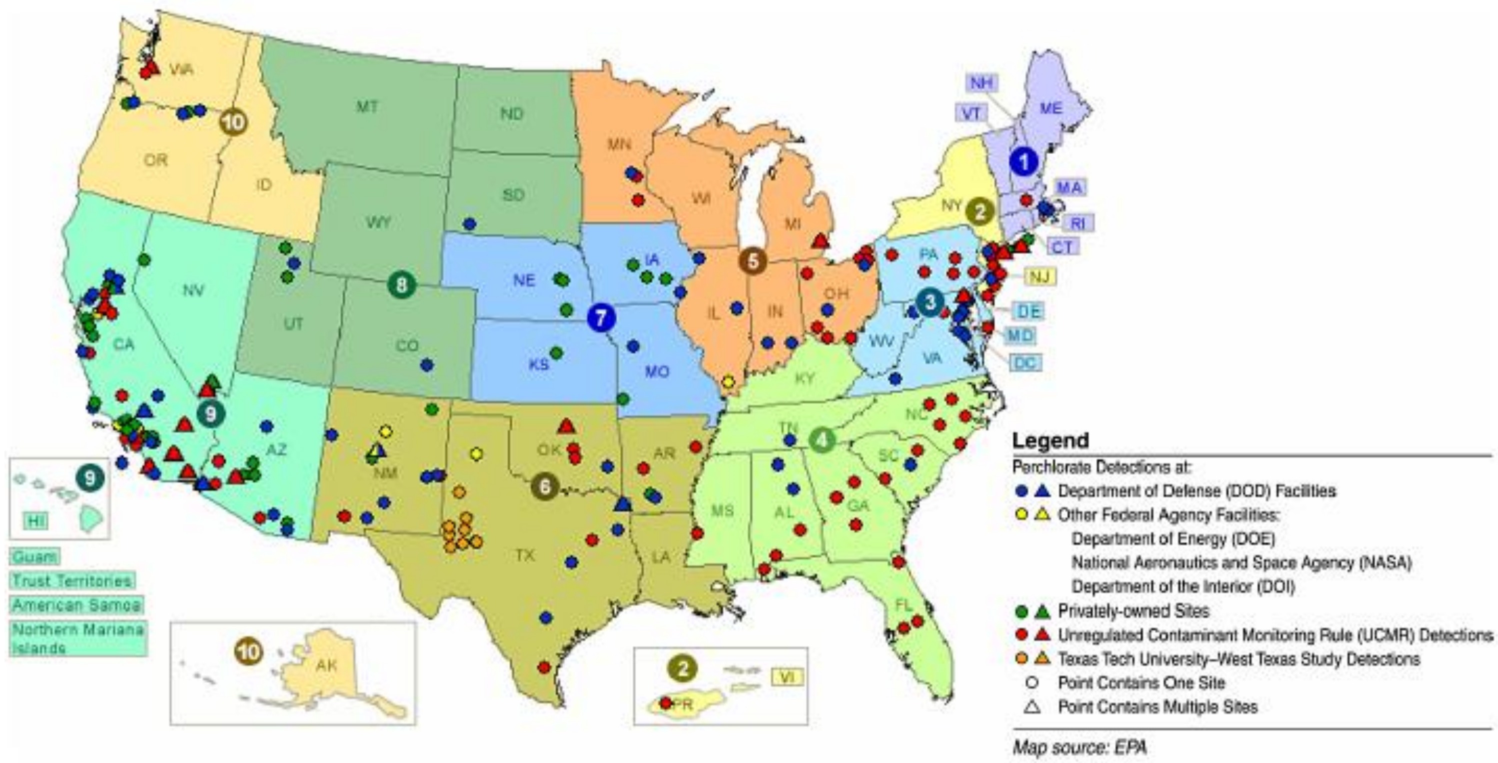


Table 3. National perchlorate detections in drinking water as of September 2004, EPA Regions are highlighted by color and region numbers (Source: US EPA 2007)

Numerous studies have been done on perchlorate contamination in the environment. These have shown that crops are often irrigated by perchlorate-contaminated water, causing not only fruits and vegetables to be contaminated, but also milk products from cows grazing on contaminated pastures. In the Southwestern U.S., the Colorado River water is contaminated with perchlorate due to a manufacturing plant that was previously located near the Las Vegas Wash and affects citrus fruit and lettuce irrigated by water from the river (Sanchez *et al.* 2005a; Sanchez *et al.* 2006). According to Sanchez *et al*. (2005a), 90% of the lettuce consumed by the country during the winter comes from this area. In these studies, exposure to perchlorate contaminated lettuce ranged from 0.45 to 1.8 µg/day and exposure to perchlorate from citrus fruit ranged from 0.005 to 1.20 µg/person/day. According to Sanchez *et al.* (2006), these levels are low in regards to the recommended reference dose set by the National Academy of Sciences.

In New Jersey, perchlorate has been detected in drinking water, fruits, vegetables, and milk. In the past, most perchlorate had been detected near the Colorado River in the southwest part of the U.S. Sanchez *et al*. (2005b) began conducting studies of leafy vegetables in North America outside the Colorado River area by collecting conventionally and organically grown lettuce and other leafy vegetables from production fields and farmer's markets. Lettuce samples were collected from Cumberland County, New Jersey during the fall. Samples were found to contain both nitrate and perchlorate. Of the 38 samples of conventionally grown lettuce, 22 were found to contain perchlorate levels above the minimum reporting level (MRL) (Sanchez *et al.* 2005b).

The US FDA (2007b) did a survey on perchlorate in food for 2004-2005 and analyzed milk and various domestic fruits and vegetables throughout the U.S. The samples were selected based on high water content, high consumption, and if the plants were known to be irrigated with perchlorate-contaminated water or grown in soil containing perchlorate. In the state of New Jersey, perchlorate was detected in romaine lettuce, tomatoes, spinach, broccoli, and whole milk. Table 2 shows the average perchlorate level detected in each food from different growers in New Jersey.

Table 2. New Jersey food found to contain perchlorate, 2004-2005

Food Analyzed	Location of Grower	Perchlorate (ppb)	US Average (ppb)
Romaine lettuce	Blairstown	7.07	10.3
	Bridgeton	4.46	
	Cedarville	10.1	
	Emerson	3.70	
	Newfield	14.2	
	Toms River	21.6	
Tomatoes, Beefsteak	Cedarville	5.58	13.7
	Chester	3.36	
	Swedesboro	ND	
Tomatoes, Plum	Glen Gardener	6.48	
Tomatoes	Cherry Hill	ND	
	East Brunswick	ND	
	Hillsborough	16.4	
	Jamesburg	1.04	
	Lakewood	2.60	
	Long Valley	1.30	
	Monmouth Junction	0.38*	
	Monroeville	ND	
	Thorofare	ND	
Spinach	East Windsor	8.15	115
	Vineland	40.9	
	Wayne	6.02	
Apple, Gala	Monroeville	ND	0.15
Broccoli	East Windsor	6.96	8.49
	Hackettstown	3.60	
Whole Milk	New Jersey	Range: 3.40-4.85	5.81

(Source: US FDA 2007b)

ND=Not Detected
*Estimate below limit of quantitation (LOQ) and above limit of detection (LOD)

- The estimated LOQ is 1.00 ppb for vegetables and fruits.
  The estimated LOD is 0.30 ppb for vegetables and fruits.
- The estimated LOQ is 0.50 ppb for water.
  The estimated LOD is 0.20 ppb for water
- The estimated LOQ is 3.00 ppb for milk.
- The estimated LOD is 1.00 ppb for milk

Through data obtained from human perchlorate studies, it has been established that 0.007 mg/kg/dy of perchlorate can be ingested without adversely affecting the uptake of iodine (US EPA 2005). Based on this data and an uncertainty factor of 10, the US EPA (2005) adopted its reference dose of 0.0007 mg/kg/day from the National Academy of Sciences review. The National Research Council (NRC) suggested that the Greer *et al*. (2002) study be used in determining the reference dose. Greer administered 0.007, 0.02, 0.1 and 0.5 mg perchlorate/kg body weight per day to a total of 37 healthy males and females over 14 days. Thyroidal radioiodide uptake was measured at 8 and 24 hours after administration of perchlorate to the subject. A No Observable Effects Level (NOEL) was estimated to be 0.007 mg/kg/day for inhibition of thyroidal radioiodide uptake. An uncertainty factor of 10 was applied to the lowest dose (0.007 mg/kg/day) in the Greer study to establish the reference dose of 0.0007 mg/kg/day. (Greer *et al*. 2002; NAS 2005; NJDEP 2005b)

According to the New Jersey Department of Environmental Protection (NJDEP), New Jersey currently has no perchlorate regulatory guidelines and recommends that water should not be consumed with perchlorate levels greater than 5 ppb, which is the unofficial maximum contaminant level (MCL) for perchlorate. The following equation explains the derivation of 5 ppb as the perchlorate MCL. (NJDEP 2005a, 2005b)

$$\frac{(0.0007 mg/kg/day)\times(67kg)\times(0.2)}{2 Liters/day} = 0.0047 mg/L = 5\mu g/L (or 5 ppb)$$

Where:

0.0007 mg/kg/day = reference dose

67 kg = assumed body weight of a pregnant adult

0.2 = relative source contribution factor (other sources of perchlorate, *i.e.* food)

2 Liters/day = average amount of tap water an adult consumes a day

Since pregnant women are a sensitive population, the health protective approach is to use an adult pregnant woman's body weight, 67 kg. The relative source contribution (RSC) factor, 0.2 or 20%, accounts for other sources of perchlorate exposure besides drinking water (i.e. diet).

Between 2001 and 2005, the US EPA's Unregulated Contaminate Monitoring Rule (UCMR) Program collected water samples in New Jersey to test for perchlorate and other contaminants. UCMR compiles data on suspected contaminants that may be in drinking water and do not currently have health based standards under the Safe Drinking Water Act. (US EPA 2006) UCMR found that perchlorate levels in certain areas of New Jersey exceeded 5 ppb (Table 2) for at least one sample during the four-year sampling period. According to the NJDEP, these areas have an ongoing perchlorate contamination problem. Some of the affected wells have been taken offline until funding is available for treatment or until perchlorate becomes regulated by New Jersey. (L. Bonnette, personal communication, September 5, 2008)

Based on the EPA data, NJDEP's Bureau of Safe Drinking Water (BSDW) did further sampling and selected sites that were based on UCMR results and sampled public water systems that were near military arsenals or areas that had been found to have high levels of nitrates. Over 300 samples were taken and only a few sites had perchlorate levels that exceeded the Minimal Reporting Level (MRL) of 4 ng/mL, or 4 ppb (Table 3).

Table 3. Public water systems sampled through the UCMR or by BSDW with results 4 ppb and greater

PW System Name	County	Township	Date Sampled	(ppb)	Sampled By
St. Marys School	Atlantic	Bueno Vista Twp	4/13/2005	6.8	BSDW
St. Marys School	Atlantic	Bueno Vista Twp	5/10/2005	5.5	BSDW
Park Ridge WD	Bergen	Park Ridge Boro	11/26/2002	5.1	UCMR
Park Ridge WD	Bergen	Park Ridge Boro	7/17/2003	13	UCMR
Park Ridge WD	Bergen	Park Ridge Boro	3/3/2004	5.1	BSDW
Park Ridge WD	Bergen	Park Ridge Boro	3/3/2004	23	BSDW
Park Ridge WD	Bergen	Park Ridge Boro	3/25/2004	5	UCMR
Park Ridge WD	Bergen	Park Ridge Boro	6/17/2004	21	BSDW
Park Ridge WD	Bergen	Park Ridge Boro	6/30/2004	5.6	BSDW
Vineland Water & Sewer Utility	Cumberland	Vineland City	12/3/2003	6	UCMR
Vineland Water & Sewer Utility	Cumberland	Vineland City	7/23/2003	6	UCMR
Montclair Water Bureau	Essex	Montclair Town	5/19/2003	5.3	UCMR
Montclair Water Bureau	Essex	Montclair Town	3/3/2004	6.1	BSDW
Middlesex WD	Middlesex	Woodbridge Twp	5/23/2001	7.1	UCMR
Middlesex WD	Middlesex	Woodbridge Twp	11/27/2001	5.2	UCMR

PW: Public Water
WD: Water Department
UCMR: Unregulated Contaminant Monitoring Regulation (US EPA)
BSDW: Bureau of Safe Drinking Water (NJDEP)

Chapter 3

Materials and Methods

Upon IRB approval, this study was presented to the community board of the Eric B. Chandler Health Center for approval to perform the study in their facility. A total of 106 breast feeding mothers were recruited from the Eric B. Chandler Health Center in New Brunswick, NJ. During prenatal and postnatal visits to the health center, mothers were made aware of the study by Chandler staff, members of the research team, and through advertisements that were posted in both English and Spanish throughout the health center.

Subjects were included in the study if they: 1) were expecting to give birth in less than a month and were planning to breast feed; 2) had recently given birth and were breast feeding; and 3) were 18 years of age or older. Subjects were excluded if they: 1) had a serious medical illness (acute or chronic) such as heart disease, diabetes, unstable hypertension, kidney disease, HIV, severe anemia, or hemolytic disease that were not being properly controlled; 2) had abnormalities of the breast, i.e. mastitis (inflammation of the breast); 3) were unable to understand the informed consent; and 4) the mother was about to change health care providers, move out of the area, or was unable to return to the clinic.

Mothers with anemia or iron deficiency, mild hypertension, preclampsia, kidney stones, gall bladder stones (cholelithiasis), hyperlipidemia (high cholesterol), and other medical conditions with a similar degree of severity were not excluded from the study.

Most of these disorders should not affect breast-feeding, the ability to sample or measure perchlorate, or risk the baby's or mother's health if they are breastfeeding.

Upon identification of a potential subject, one of the investigators or the research assistant explained the study procedure to the mother. After subjects' questions had been answered, the subject was required to sign an informed consent, which was witnessed and dated. A signed copy of the consent was provided to the subject. The consent form was available in both English and Spanish, and an interpreter was available for subjects who understood Spanish better than English. At all times, it was made clear to the subjects that the study did not involve active treatment and that participation is voluntary.

Once the subject signed the consent form, three appointments were scheduled for sample collection. All sample collections occurred at Chandler health center. The first appointment for sample collection occurred a week after delivery, and all three samples were obtained within a year. During the three separate sample collections, the subjects were asked to bring a sample of their primary source of drinking water. Eight mL of water were aliquoted into a labeled 10 mL cryo-vial (Table 4). While at the health center the mothers were provided a urine cup to collect a spot urine sample at each of the three visits. Upon urine collection, 8 mL of the urine was aliquoted into a labeled 10 mL cryo-vial for perchlorate analyses and 1 mL into a labeled 2 mL cryo-vial for creatinine analyses. Subjects were also asked to provide a sample of breast milk (8 ml) at each of the three collections. Manual breast pumps (Medela® Harmony Breastpump, model: 67186) were provided for sample collection and Chandler nursing staff assisted the mothers in using the breast pumps. All urine samples and breast milk samples were collected at Chandler. Samples were placed in labeled storage boxes and kept in a freezer in a separate allocated, secure area of the health center. At the end of the day, specimens were taken to the Environmental and Occupational Health Sciences Institute (EOHSI) and maintained at -20°C until shipment.

Table 4. Sample collection

Matrix	Amount Collected	Analytes Tested For
Primary drinking water	8 mL	Perchlorate
		Iodide
		Nitrate
Urine	8 mL	Perchlorate
		Iodide
		Nitrate
		Thiocyanate
Urine	1 mL	Creatinine
Breast Milk	8 mL	Perchlorate
		Iodide
		Nitrate
		Thiocyanate

* Water, urine, and breast milk were collected at each of the three assigned collection dates.

Subjects were also asked to complete a questionnaire during the first sample collection that provided demographic information along with information on breast feeding and drinking water consumption. At each sample collection a 24-hour dietary recall was completed by the subject. (Appendix A)

Subjects were compensated for participation in the study. Subjects were given $200 worth of gift certificates to a local supermarket: $50 after first sample collection, $50 after second sample collection, and $100 after third sample collection. Subjects were also allowed to keep the manual breast pump purchased for breast milk collection.

Breast milk, urine, and water samples were shipped to the Centers for Disease Control and Prevention (CDC) Laboratories in Atlanta, frozen on dry ice. Analyses were performed at CDC labs for perchlorate, as well as iodide, nitrate, thiocyanate, and urinary creatinine. Urine creatinine specimens were analyzed on the Roche Hitachi Mod P Chemistry Analyzer, using a Creatinine Plus Assay, which involves the combined use of creatininase, creatinase, and sarcosine oxidase. The oxidation of sarcosine produces hydrogen peroxide, which produces the resultant colorimetric indicator in the modified Trinder test. The method is described in detail in Roche's Creatinine Plus Product Application #04903773003. The lower detection limit for this method is 0.3 mg/dL for urine creatinine. The reported data are in mg/dL units.

Perchlorate, nitrate, thiocyanate, and iodide was measured in urine and breast milk using ion chromatography and tandem mass spectrometry as described by Valentin-Blasini *et al*. (2007). Analyses were conducted with a Dionex ICS-2500 ion chromatography system equipped with a GS50 gradient pump, EG50 eluent generator, AS50 autosampler, LC30 thermal compartment, and a 2-mm anion self-regenerating suppressor (ASRS Ultra II) operated in the external water mode (Dionex, Sunnyvale, CA). Peak Net 6 chromatography software was used for system control. The injection volume of 24 µL was separated using an IonPac AS-20 column (2mm×250 mm, Dionex). Analyte ions were measured using a Sciex API 4000 triple quadrupole mass spectrometer (MDS/Sciex, Concord, ON, Canada) with electrospray interface. These methods are described in detail by Valentin-Blasini *et al*. (2007). These analyses met the rigorous accuracy and precision specifications of the quality control/quality assurance program of the Division of Laboratory Sciences, National Center for Environmental Health, CDC (similar to rules outlined by Westgard *et al*. (1981)).

Statistical Analyses

This is a cross-sectional study design using convenience sampling; subjects were selected at the convenience of the investigator instead of randomly selecting the subjects to assure that individuals in general population have an equal probability of being selected. A database has been created in Microsoft Access to store collected data. All statistical analysis was carried out using SPSS version 15.0.

Descriptive statistics were utilized to describe and summarize the basic aspects of the data and to provide simple means with standard deviations and frequencies. Tests of normality were also checked. Pearson product-moment correlation coefficient and linear regression, along with matched-pair *t*-tests, were used to test the strength of certain relationships such as associations of food types and drinking water with the level of perchlorate and determining significant predictors of breast milk iodine concentrations. Since there is little information on the levels of perchlorate in breast milk, and this is a pilot study using convenience sampling methods, power calculations are not appropriate at this stage. Variables analyzed in this study are listed below in Table 5.

Table 5. Study variables

Variable	Description	Measure
Age		Ordinal
Race/ethnicity	1 = white 2 = black 3 = Hispanic 4 = other	Nominal
Education	1 = No high school 2 = Some high school 3 = High school graduate 4 = Some college 5 = College graduate 6 = Grad./Prof. Degree 7 = Unknown	ordinal
Mother's Occupation	1 = Factory 2 = House wife 3 = Other	Nominal
Father's Occupation	1 = Landscaper/gardener 2 = Other outdoor work	Nominal
Number children		Continuous
How often breast feed	1 = < 25% 2 = 25-50% 3 = 51-75% 4 = 75-99% 5 = 100%	Ordinal
Smoked while pregnant	1 = yes 2 = no	Categorical
Smoked while breast feeding	1 = yes 2 = no	Categorical
Medical illnesses	1 = yes, has controlled medical illness 2 = no, does not have medical illness	Nominal
Primary drinking water (at home and away from home)	1 = Bottled water 2 = Well water, unfiltered 3 = Well water, filtered 4 = Municipal tap, unfiltered 5 = Municipal tap, filtered 6 = Other 7 = Don't know	Categorical
Food products	Eaten in past 24-hours: vegetables, fruits, grains, dairy, and iodine intake 0 = yes 1 = no	Nominal
Perchlorate	ng/mL Concentration detected in urine, water, breast milk	Continuous

Thiocyanate	ng/mL Concentration detected in urine, breast milk	Continuous
Nitrate	ng/mL Concentration detected in urine, water, breast milk	Continuous
Creatinine	mg/dL Concentration detected in urine	Continuous
Iodide	ng/mL Concentration detected in urine, water, breast milk	Continuous

In order to answer the primary research question, the following will be done:

1) a) Estimate total perchlorate exposure for lactating women based on perchlorate levels in maternal milk and urine;

   b) Estimate total perchlorate exposure for breast-fed infants based on perchlorate levels in maternal milk.

2) a) Determine the concentration of perchlorate present in the subject's primary source of drinking water;

   b) Based on the perchlorate levels found in drinking water determine the amount of perchlorate present in the breast milk of new mothers as a result of the contaminated water.

3) Correlate total perchlorate exposure with consumption of certain foods.

4) a) Estimate total iodide present in maternal milk;

   b) Estimate total iodide intake for breast-fed infants based on iodide levels in maternal milk;

   c) Correlate perchlorate levels in breast milk with iodide levels in breast milk.

5) Calculate mother and infant's total perchlorate daily dose and compare to the EPA reference dose.

Chapter 4

Results

Preliminary Results

This study consisted of 106 breast-feeding mothers. Eighty-three subjects provided all three sets of samples, 4 provided two sets of samples, and 19 provided only 1 sample set. No significant differences were found between the group of subjects who provided all three sample sets and those who did not. Initially a one-way analysis of variance (ANOVA) was conducted to determine the differences in groups who provided all three sets of samples from those who only provided one or two sets of samples. Subjects were divided into three groups based on how many sets of samples they provided (Group 1: provided only one sample set; Group 2: provided 2 sample sets; Group 3: provided all 3 sample sets). These groups were run against the demographic information that was collected from the survey. No significant differences were found among the groups in the ANOVA (Table 1, Appendix B). However, the number of subjects in Groups 1 (n = 18) and 2 (n = 4) were too small to run an ANOVA and obtain significant results. Therefore, Groups 1 and 2 were combined (Group 1: provided less than 3 sample sets; Group 2: provided all 3 sample sets). With only two groups to compare, chi-square and *t*-test analyses were performed. No significant differences were found between the groups ($p > 0.05$) after running the *t*-test analysis. The chi-square test violated the assumption of 'minimum expected cell frequency'. Tables 2-4 (Appendix B) show that two or more cells had expected frequencies < 5. Therefore, a Mann-Whitney U

Test, a non-parametric alternative, was used to test for differences between samples provided and race, mother's occupation, and father's occupation listed in Table 5, Appendix B. The significance value for race (p = 0.62), mother's occupation (p = 0.27), and father's race (p = 0.40) were all < 0.05. Therefore, there was not a significant difference between race, mother's occupation, and father's occupation and the number of samples that were provided.

Demographics

Subjects ranged in age from 18 to 38 years (mean 25.89). Hispanics made up 97% of the subjects and over 50% had no high school education (Table 6). Only two subjects (1.9%) smoked while pregnant and three (2.8%) were smoking during sample collection. One of these subjects smoked while pregnant and breast feeding.

Table 6. Characteristics of subjects

	Frequency	%
Race (n = 105)		
White	1	0.9
Black	4	3.8
Hispanic	97	91.5
Other	3	99.1
Education (n = 96)		
No high school	58	54.7
Some high school	11	10.4
High school graduate	10	9.4
Some college	8	9.4
College graduate	3	2.8
Graduate or professional degree	4	3.8
Unknown	2	1.9
Mother's Occupation (n = 40)		
Factory worker	13	12.3
Housewife	17	16.0
Other	10	9.4
Father's Occupation (n = 65)		
Landscaper/gardener	4	3.8
Other outdoor work	36	34.0
Other/indoor work	25	23.6
Number of Children (n = 105)		
One	32	30.2
Two	37	34.9
Three	23	21.7
Four	13	12.3

Note that one subject was given the wrong questionnaire during the first sample collection so no demographic data was obtained. Other missing data was due to the subject not answering the question.

24-Hour Dietary Recall

Perchlorate exposure through diet is highly likely due to the contamination of food crops irrigated with perchlorate contaminated water or fertilized with nitrate based fertilizers. In this study, food samples were not collected and analyzed for perchlorate. As previously mentioned, subjects were given a 24-hour dietary recall survey and asked to indicate what fruits, vegetables, grains, dairy products, and foods high in iodine they consumed in the 24-hours prior to sample collection. The subjects were asked to complete the survey at each sample collection.

Descriptive statistics were run on all 24-hour dietary surveys and can be seen in table form in Appendix B (Tables 6-10). Tomatoes, lettuce, carrots, and greens were the most eaten vegetables. Other vegetables subjects consumed that were not included on the survey were onions, chayote (gourd family), peppers, peas, potatoes, corn, cassava, cabbage, squash, beets, cauliflower, green beans, celery, chilies, leeks, chickpeas, and mixed vegetables from a can. Oranges, apples, and grapes were the most eaten fruits. Subjects also indicated consuming other fruits such as avocados, bananas, mangos, pineapple, pears, cherries, watermelon, peaches, strawberries, papaya, tangerines, and mandarins. Twenty-four hours prior to sample collection most subjects consumed whole grain wheat bread, whole grain cereal, and whole grain tortillas. A few subjects indicated eating raw cooked oatmeal as well. Greater than 75% of the subjects drank milk and over 30% ate yogurt. Since a sufficient amount of iodine may counteract the effects of perchlorate in healthy adults, subjects were also asked to report their iodine intake as well. Of the items listed on the survey; cheese, milk, and iodized salt were the most consumed. Vitamins containing iodine refer to prenatal vitamins the subjects were taking.

Drinking Water Contaminant Levels

Filtered municipal tap water was the primary source of drinking water of 57% of the subjects surveyed. This was followed by bottled water (31%) and only three subjects consumed well water (Table 7).

Table 7. Subject drinking water preferences

	Frequency	%
Primary source of drinking water at home (n = 104)		
Bottled water	31	29.2
Well water, unfiltered	1	0.9
Well water, filtered	2	1.9
Municipal tap water, unfiltered	8	7.5
Municipal tap water, filtered	57	53.8
Other	4	3.8
Don't know	1	0.9
Use filtered or store bought water at home (n = 105)		
Yes	81	76.4
No	24	22.6
Reason for using filtered water at home		
Worried about a chemical	38	35.8
Taste	22	20.8
No specific reason	17	16.0
Other	13	12.3
Don't know	4	3.8
Type of filter used at home (n = 101)		
Water filter attached to tap	68	64.2
Pitcher with filter	3	2.8
Filter in the refrigerator	2	1.9
Water filled from a store purifier	2	1.9
Other	2	1.9
Don't know	0	0
N/A	24	22.6

Descriptive statistics were run on each of the three sample sets provided by the subjects (Appendix B, Tables 11-18). Perchlorate was detected in almost all water samples (n = 253). The range, mean ± SD, and median for all drinking water perchlorate samples is 0.001-1.04 ng/mL, 0.168 ± 0.132 ng/mL, and 0.152 ng/mL. As previously mentioned, the NJDEP does not recommend that water with perchlorate levels above 5 ppb (5 ng/mL) be consumed. Perchlorate levels in drinking water samples collected from our subjects were all below 5 ppb.

Iodide levels in drinking water samples (n = 213) ranged from 0.008-1.95 ng/mL with a mean of 0.400 ± 0.332 ng/mL and a median of 0.308 ng/mL. Iodide seems to be rarely measured in water so a comparison to other studies was difficult to make. However, in the analyses of well water samples by NJDEP in Warren County, New Jersey iodide levels ranged from 20-300 ng/mL, which is much higher than the levels detected in this study's water samples. (S. Spayd, personal communication, February 20, 2009)

For nitrate in water, there are two datasets. The first dataset includes the nitrate values that were detected below the Limit of Detection (LOD), 700 ng/mL. Because these low values can be unreliable, imputed values have been used in place of the values below LOD in the second dataset. Both sets of data will be compared. The following equation was used to determine the imputed value of nitrate in water:

LOD/2 = nitrate in water (ng/mL)

Where;

LOD of nitrate = 700 ng/mL

In drinking water (n = 263) the nitrate range, mean ± SD, and median is 57–1.46×10^{-4} ng/mL, $3.22\times10^{-3} \pm 1.84\times10^{-3}$ ng/mL, and 3.01×10^{-3}, respectively. For the dataset with imputed nitrate (n = 271) values the range, mean ± SD, and median is 350-1.46×10^{-4} ng/mL, $3.14\times10^{-3} \pm 1.88\times10^{-3}$ ng/mL, and 2.97×10^{-3} ng/mL.

A paired-samples *t*-test was conducted to compare the actual nitrate values detected in drinking water to the dataset with the imputed nitrate values. There was not a significant difference in mean scores among sample sets 1, 2, and 3; t = 0.14, 0.21, and 0.11 respectively, $p > 0.05$. The EPA drinking water standard for nitrate is 44.3 mg/L (44300 ng/mL). Nitrate levels in our drinking water samples all fell below the nitrate standard for drinking water. Nitrate based fertilizers are one of the primary sources of nitrate contamination in drinking water. Unfortunately, no public information was available through the NJDEP or the New Jersey Department of Agriculture on nitrate based fertilizer use within the state.

Outliers were present in each of the three water sample sets for all analytes, which resulted in a positive skewness. To create a closer to normal distribution, the *log*10 of the analyte data were taken. These values will be used in later analyses (i.e. regression, etc.) The *log*10 values are presented in Appendix B, Tables11-18.

Breast Milk Contaminant Levels

Only 19.8% of the subjects breastfed 100% of the time (Table 8). Over 80% of the subjects used formula along with breastfeeding. This number may not be accurate as two of the subjects who reported breastfeeding 100% of the time also stated the use of formula. Thirty percent of the subjects who used formula mixed the formula with bottled water and over 80% used well or tap water to prepare formula (Table 8).

Table 8. Breast feeding and formula data

	Frequency	%
Breast feeding, % of time (n = 105)		
< 25%	5	4.7
25 – 50%	32	30.2
50 – 75%	24	22.6
75 – 99%	23	21.7
100%	21	19.8
Use formula (n = 105)		
Yes	86	81.1
No	19	17.6
Type of water used to mix formula (n = 105)		
Bottled water	30	28.3
Well water, unfiltered	87	82.1
Well water, filtered	1	0.9
Municipal tap water, unfiltered	87	82.1
Municipal tap water, filtered	20	18.9
Other	37	34.9
Don’t know	87	82.1

Perchlorate was detected in all breast milk samples provided (n = 276). The range, mean ± SD, and median for all breast milk perchlorate samples is 0.30-99.5 ng/mL, 6.80 ± 8.76 ng/mL, and 4.38 ng/mL. These levels are comparable to perchlorate levels in breast milk detected in other studies. In a study done by Pearce *et al*. (2007) with lactating Boston area women, perchlorate was detected in all breast milk samples (49) and levels ranged from 1.3-411 ng/mL (mean 33 ± 77). Kirk *et al*. (2005) obtained 36 breast milk samples from women in 18 different states. Perchlorate was detected in all 36 samples, ranging from 0.60-92.2 ng/mL (mean 10.5). These results included three samples from New Jersey, 50.7 ± 2.2, 92.2 ± 5.8, and 3.2 ± 0.3 ng/mL. In another study ten lactating women provided breast milk samples and perchlorate levels ranging from

0.5-39.5 ng/mL (mean 5.8 ± 6.2) (Kirk *et al*. 2007). A consistent level of perchlorate was not detected among the three sample sets provided by each subject. Perchlorate levels for sample set 2 were higher than sample sets 1 and 3. Of the 83 subjects who provided all three sample sets; 30 subjects had high perchlorate levels detected in the second sample set and 22 subjects had lower levels of perchlorate detected in sample set 2. In Appendix B, Figures 1-8 illustrates the increase in perchlorate levels in sample set 2. To determine if there was a significant difference between sample 2 and the other sample sets regression analysis and t-tests were run. Dummy variables (1 = perchlorate detected in sample; 0 = no perchlorate) were entered for breast milk perchlorate sample sets S1, S2, and S3 to help determine the difference between sample sets. The dependent variable used in the regression analysis was mean breast milk perchlorate and the independent variables were diet, demographics, and drinking water perchlorate. Regression analysis showed that sample set 2 was the greatest predictor (beta = 0.325; t = 1.754, $p > 0.05$). Since there is no variance in sample set 1 because everyone provided a sample, a t-test was run between mean breast milk perchlorate for sample sets 2 and 3 and showed there was a significant difference, t = 1.450 ($p > 0.05$). Almost all of the subjects were recruited within a week or two of delivery. Breast milk goes through different stages. Immediately after pregnancy mothers produce colostrum, which is high in proteins, vitamins, and immunoglobulins. Eventually, within a few weeks, possibly at the time of first sample collection, the colostrum is replaced with transitional milk, which contains high levels of fat. Perchlorate is not lypophilic and prefers more aqueous environments, so perchlorate levels may be lower during the first sample collection. The final stage of breast milk production is mature milk, which is 90% water and ideal for perchlorate which may have caused an increase in perchlorate levels by the second sample collection. Plus, there is an increase in breast milk production. (Green 2001)

Iodide was also detected in all breast milk samples provided (n = 276). The range, mean ± SD, and median for all breast milk iodide samples is 3.91-918 ng/mL, 181 ± 134 ng/mL, and 152 ng/mL. Kirk *et al*. (2005) obtained 36 breast milk samples from women in 18 different states. Iodide was detected in 23 of the samples, ranging from 4.5-162 ng/mL (mean 63.3). Iodide was detected in one of the three samples collected from New Jersey, 162 ± 2.1 ng/mL. In another study by Kirk *et al*. (2007), iodide levels in breast milk from ten lactating mothers ranged from 3.1-334 ng/mL (mean 87.9 ± 80.9 ng/mL, n = 108). Iodide was also measured in 57 lactating women in Boston and levels ranged from 2.7-1968 ng/mL (mean 205 ± 271 ng/mL) (Pearce *et al*. 2007).

Thiocyanate was detected in all breast milk samples provided (n = 276). The range, mean ± SD, and median for all breast milk thiocyanate samples is $5.32\text{-}2.9\times10^{-3}$ ng/mL, 425 ± 475 ng/mL, and 274 ng/mL. Kirk *et al*. (2007) collected breast milk samples from ten lactating women. Thiocyanate ranged from 0.4-228.3 ng/mL (mean 35.6 ± 57.9 ng/mL). Nitrate was detected in almost all breast milk samples provided (n = 275). The range, mean ± SD, and median for all breast milk nitrate samples is $100\text{-}1.07\times10^{-5}$ ng/mL, $2.45\times10^{-3} \pm 1.33\times10^{-3}$ ng/mL, and 1.99×10^{-3} ng/mL.

Descriptive statistics can be seen in table form for each of the three sample sets for breast milk perchlorate, iodide, thiocyanate, and nitrate in Appendix B, Tables 19-27. Outliers were present in each of the three breast milk sample sets for all analytes, which resulted in a positive skewness. To create a closer to normal distribution, the *log*10 of the analyte data were taken. These values will be used in later analyses (i.e. regression, etc.). The *log*10 values are presented in Appendix B, Tables 19-27.

Urine Contaminant Levels

Detectable levels of perchlorate were found in all urine samples provided (n = 273). Two subjects, however, were unable to provide urine samples at the first and third sample collection. The range, mean ± SD, and median for all urine perchlorate samples is 0.178-18.3 ng/mL, 3.19 ± 3.64 ng/mL, and 2.14 ng/mL. Blount *et al*. (2006) also found perchlorate in all 2820 samples tested during 2001 and 2002 as part of the National Health and Nutrition Examination Survey (NHANES) ranging from 0.19-160 ng/mL. The urine perchlorate levels in this study was lower than the NHANES study since subjects in this study are all lactating women who are also secreting perchlorate in breast milk.

Detectable levels of iodide were also present in the provided urine samples (n = 273). The range, mean ± SD, and median for all urine iodide samples is 2.20-603 ng/mL, 147 ± 103 ng/mL, and 117 ng/mL. In 2003-2004 Caldwell *et al*. (2008) selected about 5,000 individuals per year to participate in an NHANES study that measured urinary iodide levels in a random subsample of 2,526 individuals 6 years of age and older. For the general U.S. population, the median urinary iodide level was 160 ng/mL and women of reproductive age, pregnant and non-pregnant, had a median urinary iodide level of 139 ng/mL. The median values from our study are lower than the NHANES values, which may be a result of all the subjects in our study lactating and excreting iodide through breast milk.

Thiocyanate was also detected in all urine samples (n = 273). The range, mean ± SD, and median for all urine thiocyanate samples is 33.8-1.04×10^{-4} ng/mL, 898 ± 1.14×10^{-3} ng/mL, and 598 ng/mL. Nitrate was detected in all urine samples provided. The range, mean ± SD, and median for all urine thiocyanate samples is 2.85×10^{-3}-3.83×10^{-5} ng/mL, 5.00×10^{-3} ± 4.41×10^{-3} ng/mL, and 3.96×10^{-5} ng/mL.

Descriptive statistics of each of the three sample sets can be seen in table form in Appendix B, Tables28-35. Outliers were present in each of the three urine sample sets for all analytes, which resulted in a positive skewness. To create a closer to normal distribution, the *log*10 of the analyte data were taken. These values will be used in later analyses (i.e. regression, etc.). The *log*10 values are presented in Appendix B.

Urine Creatinine

Creatinine is a breakdown product of muscle during metabolism and is usually filtered through the kidney and excreted in the urine. Creatinine is usually measured in spot urine samples as a marker of the effect of hydration on other analytes being measured in the urine. Low creatinine levels usually result when there is high urine output because of increased fluid intake, and high creatinine levels may be due to dehydration.

Creatinine is used to correct for dilution and also to check the validity of spot urine samples. According to the World Health Organizations (WHO) guidelines for occupational monitoring, urine creatinine concentrations < 30 mg/dL are too dilute and concentrations > 300 mg/mL are too concentrated. It is sometimes recommended that values <30 and >300 mg/dL be excluded and another urine sample be collected. (Barr *et al*. 2006; WHO 1996)

In this study 16 subjects had creatinine levels < 30 mg/dL (0.30 – 29.5 mg/dL) and 4 subjects had creatinine levels > 300 mg/dL (320 – 358 mg/dL). The exposure data is still meaningful but the resulting dose estimates may be less precise compared to the rest of the data. Data analyses will be run with these values excluded and included to check for any variations.

Creatinine levels were measured in all urine samples, n = 272. The range, mean ± SD, and median for urine creatinine is 0.30 – 358 mg/dL, 116 ± 61.1 mg/dL, and 108 mg/dL. Descriptive statistics for each of the three sample sets for urine creatinine can be seen in table form in Appendix B, Tables 36-39. Barr *et al*. (2005) analyzed urinary creatinine concentrations in 22,245 subjects through NHANES (1988-1994). The ranges in the NHANES participants (25.8-270 mg/dL) are comparable to the ranges found in this study.

Outliers were present in each of the three urine sample sets for all analytes, which resulted in a positive skewness. Only one of the outliers was a result of the creatinine level being >300 mg/dL. To create a closer to normal distribution, the *log*10 of the creatinine data were taken. Urine creatinine levels for sample set 2 were normally distributed so the *log*10 was not taken for sample set 2. The *log*10 values are presented in Appendix B.

Descriptive statistics were then run excluding creatinine values < 30 mg/dL and > 300 mg/dL. Removing these values resulted in a more normal distribution among the sample sets. The range, mean ± SD, and median for urine creatinine excluding these values in sample set 1 (n = 100) were 30.7-283 ng/mL, 115 ± 49.9 ng/mL, and 107.90 ng/mL. For sample set 2 (n = 79), the range, mean ± SD, and median were 38.7-236 ng/mL, 124 ± 50.4 ng/mL, and 120 ng/mL. The range, mean ± SD, and median for sample set 3 (n = 74) were 30.7-241 ng/mL, 116 ± 54.7 ng/mL, and 108 ng/mL.

To correct for dilution among spot urine samples urinary biomonitoring data is usually adjusted to a constant creatinine concentration by dividing the weight of the analyte by the amount of creatinine in urine to get µg of perchlorate / g of creatinine. (Barr *et al*. 2005)

The range, mean ± SD, and median for perchlorate corrected for creatinine in urine samples (n = 272) were 0.151-93.3 µg/g, 3.51 ± 6.79 µg/g, and 2.19 µg/g. The range, mean ± SD, and median for iodide corrected for creatinine in urine samples (n = 272) were 22.1-733 µg/g, 141 ± 87.6 µg/g, and 124 µg/g. The range, mean ± SD, and median for nitrate corrected for creatinine in urine samples (n = 272) were 6.82×10^{-4} – 9.50×10^{-3} µg/g, $4.86\times10^{-4} \pm 6.28\times10^{-4}$ µg/g, and 3.52×10^{-4} µg/g. The range, mean ± SD, and median for thiocyanate corrected for creatinine in urine samples (n = 272) were 46.5-1.13×10^{-4} µg/g, 892 ± 1.06×10^{-4} µg/g, and 616 µg/g. Descriptive statistics for each sample set for perchlorate, iodide, nitrate, and thiocyanate can be found in table form in Appendix B, Tables 40-47.

Estimate of Daily Analyte Intake through Drinking Water and Breast Milk

Mother's daily intake of perchlorate, iodide, and nitrate through drinking water was calculated. According to the Exposure Factors Handbook, lactating women consume and average of 2.24 L of water per day (US EPA 1997). The following equation was used to estimate mother's daily analyte intake through drinking water:

Mother's Intake = $(C_{i,w})\times(V_{w,24})$

Where;

$C_{i,w}$ = total concentration of analyte *i* (perchlorate, iodide, nitrate) detected in drinking water (ng/mL)

$V_{w,24}$ = 2.24 L/day = average amount of water lactating women consume in a day (US EPA 1997)

The range, mean ± SD, and median for perchlorate intake through drinking water in sample set 1 (*n* = 96) were 0.02-2.01 µg/day, 0.37 ± 0.30 µg/day, and 0.33 µg/day. For sample set 2 (*n* = 81), the range, mean ± SD, and median were 0.03-1.03 µg/day, 0.35 ± 0.24 µg/day, and 0.31 µg/day. The range, mean ± SD, and median for sample set 3 (*n* = 76) were 0.003-2.33 µg/day, 0.41 ± 0.34 µg/day, and 0.38 µg/day.

For mother's iodine intake through drinking water, the range, mean ± SD, and median for sample set 1 (*n* = 77) were 0.02-4.23 µg/day, 0.81 ± 0.69 µg/day, and 0.62 µg/day. For sample set 2 (*n* = 72), the range, mean ± SD, and median were 0.05-4.03 µg/day, 0.92 ± 0.78 µg/day, and 0.73 µg/day. The range, mean ± SD, and median for sample set 3 (*n* = 64) were 0.02-4.37 µg/day, 0.95 ± 0.77 µg/day, and 0.76 µg/day.

The range, mean ± SD, and median for nitrate intake through drinking water in sample set 1 (*n* = 104) were 127-32704 µg/day, 7332 ± 4739 µg/day, and 7212 µg/day. For sample set 2 (*n* = 84), the range, mean ± SD, and median were 949-23744 µg/day, 7139 ± 3671 µg/day, and 6552 µg/day. The range, mean ± SD, and median for sample set 3 (*n* = 75) were 970-22.2 µg/day, 7148 ± 3689 µg/day, and 6429 µg/day. The range, mean ± SD, and median for nitrate intake through drinking water with imputed values in sample set 1 (*n* = 106) were 784-32704 µg/day, 7219 ± 4763 µg/day, and 7123.20 µg/day. For sample set 2 (*n* = 86), the range, mean ± SD, and median were 784-23744 µg/day, 6982 ± 3769 µg/day, and 6507 µg/day. The range, mean ± SD, and median for sample set 3 (*n* = 79) were 784-22176 µg/day, 6806 ± 3887 µg/day, and 63602 µg/day.

Infant's daily intake of perchlorate, iodide, nitrate, and thiocyanate through breast milk was also estimated. The average amount of breast milk consumed by an infant less than six months old was estimated using EPA's Child-Specific Exposure Factors Handbook (2008). The Handbook pooled human milk intake rates from numerous studies and the mean intake of 0-6 month olds was averaged to come up with and intake rate of 0.73 L/day of breast milk. The following equation was used to estimate infant's daily intake through breast milk:

$$\text{Baby's Intake} = (C_{i,milk})\times(V_{milk})$$

Where;

$C_{i,milk}$ = total concentration of analyte *i* (perchlorate, iodide, nitrate) detected in breast milk (ng/mL)

V_{milk} = 0.73 L/day = average amount of breast milk infants < 6 months consume in a day (US EPA 2008)

The range, mean ± SD, and median for perchlorate intake through breast milk in sample set 1 (*n* = 106) were 0.31-33.9 µg/day, 4.58 ± 5.09 µg/day, and 3.18 µg/day. For sample set 2 (*n* = 87), the range, mean ± SD, and median were 0.22-72.6 µg/day, 8.95 ± 9.10 µg/day, and 3.53 µg/day. The range, mean ± SD, and median for sample set 3 (*n* = 83) were 0.24-21.7 µg/day, 4.43 ± 3.93 µg/day, and 3.06 µg/day.

For infant's iodine intake through breast milk (Figure 4), the range, mean ± SD, and median for set 1 (*n* = 106) were 2.85-420 µg/day, 130 ± 90.7 µg/day, and 112 µg/day. For sample set 2 (*n* = 87), the range, mean ± SD, and median were 5.24-670 µg/day, 135 ± 105 µg/day, and 112 µg/day. The range, mean ± SD, and median for sample set 3 (*n* = 83) were 4.24-603 µg/day, 131 ± 101 µg/day, and 105 µg/day. As previously mentioned, infants 0-6 months require between 110-130 µg/day of iodine. Nearly 25% of the breast fed infants may be receiving inadequate amounts of iodine through mother's breast milk.

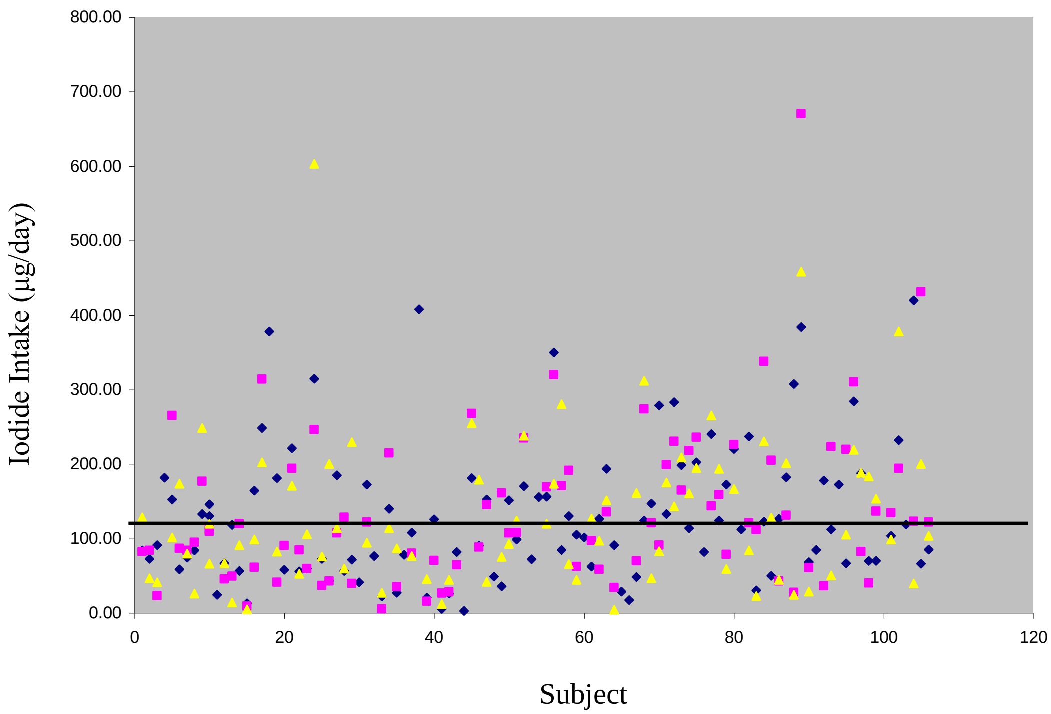


Figure 4. Infant iodide intake through breast milk. Line corresponds to approximate breast milk iodine content required to achieve adequate intake (110 µg/day) for an infant age 0-6 months (IOM 2004). ◆ Sample Set 1; ■ Sample Set 2; ▲ Sample Set 3.

The range, mean ± SD, and median for nitrate intake through breast milk in sample set 1 (n = 105) were 521-7811 µg/day, 2030 ± 1161 µg/day, and 1803 µg/day. For sample set 2 (n = 87), the range, mean ± SD, and median were 588-4205 µg/day, 1704 ± 794 µg/day, and 1453 µg/day. The range, mean ± SD, and median for sample set 3 (n = 83) were 73.0-4394 µg/day, 1564 ± 812 µg/day, and 1343 µg/day.

Estimation of Mother and Infant's Daily Perchlorate Dose

Subject weight was not measured at time of sample collection. Therefore, to estimate the mother's perchlorate dose through drinking water average body weight (BW) and intake rate (IR) was used (Table 9). (Ogden *et al*. 2004; US EPA 1997). Information of infant weight and intake rate were obtained from the EPA's Child-Specific Exposure Factors Handbook (2008). Infant dose was calculated at three different weights. Table xx below lists the average intake rates and body weights for the mothers and infants. An exposure factor (EF) of 1 was used to represent daily exposure to the contaminant for both mother and infant. Mother's perchlorate dose through drinking water and infant's perchlorate dose through breast milk was calculated as follows:

Dose calculation (ATSDR 1992):

$$D = \frac{(C \times IR \times EF)}{BW}$$

Where;

D = exposure dose (mg/kg/day)

C = concentration of perchlorate in drinking water / breast milk (mg/L)

IR = intake rate of contaminated water / breast milk (L/day)

EF = exposure factor (unitless)

BW = body weight (kg)

Table 9. Average intake rates and body weights for mother and infant

	Average Intake Rate* (L/day)	Average Body Weight (kg)
Infant		
0-1 month	0.51	4.8
1-3 months	0.72	5.6
3-6 months	0.76	7.4
Mother		
Hispanic woman, 20-39 years of age	2.24	72.0

*Intake rate for infant is the average amount of breast milk consumed and intake rate for mother is the average amount of drinking water consumed.

Descriptive statistics of mother and infant's dose are presented in table form in Appendix B, Tables 52-55. The range, mean ± SD, and median for mother's perchlorate dose through drinking water (figure 5) in sample set 1 (n = 96) were 2.511×10^{-7} - 2.797×10^{-5} mg/kg/day, $5.190 \times 10^{-6} \pm 4.216 \times 10^{-6}$ mg/kg/day, and 4.570×10^{-6} mg/kg/day. For sample set 2 (n = 81), the range, mean ± SD, and median were 3.702×10^{-7} – 1.428×10^{-5} mg/kg/day, $4.891 \times 10^{-6} \pm 3.272 \times 10^{-6}$ mg/kg/day, and 4.356×10^{-6} mg/kg/day. The range, mean ± SD, and median for sample set 3 (n = 76) were 3.671×10^{-8} – 3.236×10^{-5} mg/kg/day, $5.664 \times 10^{-6} \pm 4.729 \times 10^{-6}$ mg/kg/day, and 5.227×10^{-6} mg/kg/day. The estimated dose through drinking water is below the EPA reference dose (RfD) of 0.0007 mg/kg/day. This is expected since perchlorate is being detected well below New Jersey's suggested MCL of 5 ppb, as previously discussed.

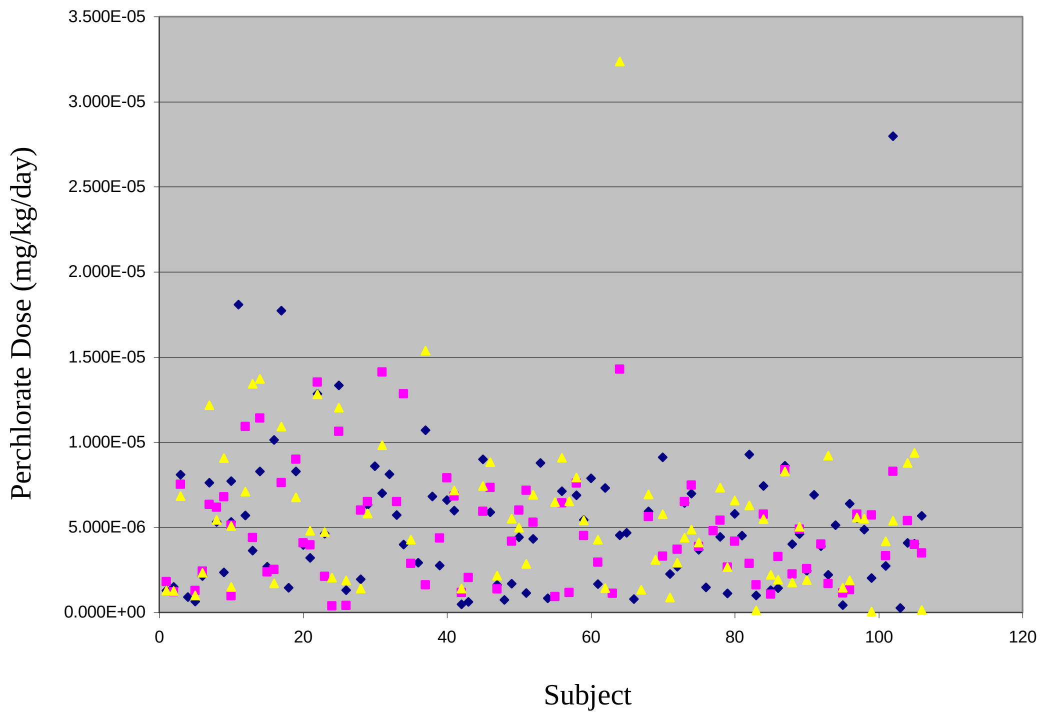


Figure 5. Mother's estimated perchlorate dose through drinking water. ◆ Samples Set 1; ■ Sample Set 2; ▲ Sample Set 3.

For infant's 0-1 month, the range, mean ± SD, and median for perchlorate dose through breast milk in sample set 1 (n = 106) were 6.403×10^{-5} – 7.057×10^{-3} mg/kg/day, 9.539×10^{-4} ± 1.061×10^{-3} mg/kg/day, and 6.616×10^{-4} mg/kg/day. For sample set 2 (n = 87), the range, mean ± SD, and median were 4.563×10^{-5} – 1.513×10^{-2} mg/kg/day, 1.239×10^{-3} ± 1.896×10^{-3} mg/kg/day, and 7.361×10^{-5} mg/kg/day. The range, mean ± SD, and median for sample set 3 (n = 83) were 4.912×10^{-5} – 4.517×10^{-3} mg/kg/day, 9.224×10^{-4} ± 8.171×10^{-4} mg/kg/day, and 6.372×10^{-4} mg/kg/day.

The range, mean ± SD, and median for perchlorate dose through breast milk for infants 1-3 months in sample set 1 (n = 106) were 5.488×10^{-5} – 6.049×10^{-3} mg/kg/day, 8.176×10^{-4} ± 9.097×10^{-4} mg/kg/day, and 5.671×10^{-4} mg/kg/day. For sample set 2 (n =

87), the range, mean ± SD, and median were 3.911×10^{-5} – 1.297×10^{-2} mg/kg/day, $1.062\times10^{-3} \pm 1.625\times10^{-3}$ mg/kg/day, and 6.309×10^{-4} mg/kg/day. The range, mean ± SD, and median for sample set 3 (n = 83) were 4.211×10^{-5} – 3.872×10^{-3} mg/kg/day, $7.906\times10^{-4} \pm 7.004\times10^{-4}$ mg/kg/day, and 5.462×10^{-4} mg/kg/day.

For infant's 3-6 month, the range, mean ± SD, and median for perchlorate dose through breast milk in sample set 1 (n = 106) were 4.153×10^{-5} – 4.577×10^{-3} mg/kg/day, $6.187\times10^{-4} \pm 6.884\times10^{-4}$ mg/kg/day, and 4.291×10^{-4} mg/kg/day. For sample set 2 (n = 87), the range, mean ± SD, and median were 2.295×10^{-5} – 9.816×10^{-2} mg/kg/day, $8.039\times10^{-4} \pm 1.230\times10^{-3}$ mg/kg/day, and 4.775×10^{-4} mg/kg/day. The range, mean ± SD, and median for sample set 3 (n = 83) were 3.186×10^{-5} – 2.930×10^{-3} mg/kg/day, $5.983\times10^{-4} \pm 5.300\times10^{-4}$ mg/kg/day, and 4.133×10^{-4} mg/kg/day. Dose calculations show that 25% of infants 0-6 months of age ingest perchlorate through breast milk above the EPA RfD. This will be further discussed in the Discussion section.

Previously mother's dose from water alone was calculated. However, water is not the only source of perchlorate. Therefore, mother's total dose will be estimated based on the amount of perchlorate excreted through urine and through breast milk. Before calculating total dose perchlorate mass in breast milk and urine were determined by multiplying estimated total amount of breast milk and urine produced per day by the concentration of perchlorate in breast milk and urine. About 700 mL of breast milk is produced per day and (Valentin 2003; Kent *et al*. 2006) about 2 L/day of urine is produced in an adult. To calculate mother's total perchlorate exposure:

$$\frac{total\ breast\ milk\ perchlorate\ (mg/day) + total\ urine\ perchlorate\ (mg/day)}{body\ weight\ (kg)}$$

= mother's total perchlorate exposure (mg/kg/day)

Only four subjects had perchlorate levels above the EPA RfD of 0.0007 mg/kg/day (Figure 6) and one subject had two samples above the RfD. The range, mean ± SD, and median for mother's total perchlorate dose in sample set 1 (n = 106) were 1.800×10^{-5} – 9.250×10^{-4} mg/kg/day, $1.459\times10^{-4} \pm 1.517\times10^{-4}$ mg/kg/day, and 1.017×10^{-4} mg/kg/day. For sample set 2 (n = 87), the range, mean ± SD, and median were 1.800×10^{-5} – 1.753×10^{-6} mg/kg/day, $1.705\times10^{-4} \pm 2.219\times10^{-4}$ mg/kg/day, and 1.026×10^{-4} mg/kg/day. The range, mean ± SD, and median for sample set 3 (n = 83) were 2.400×10^{-5} – 8.050×10^{-4} mg/kg/day, $1.461\times10^{-4} \pm 1.169\times10^{-4}$ mg/kg/day, and 1.110×10^{-4} mg/kg/day.

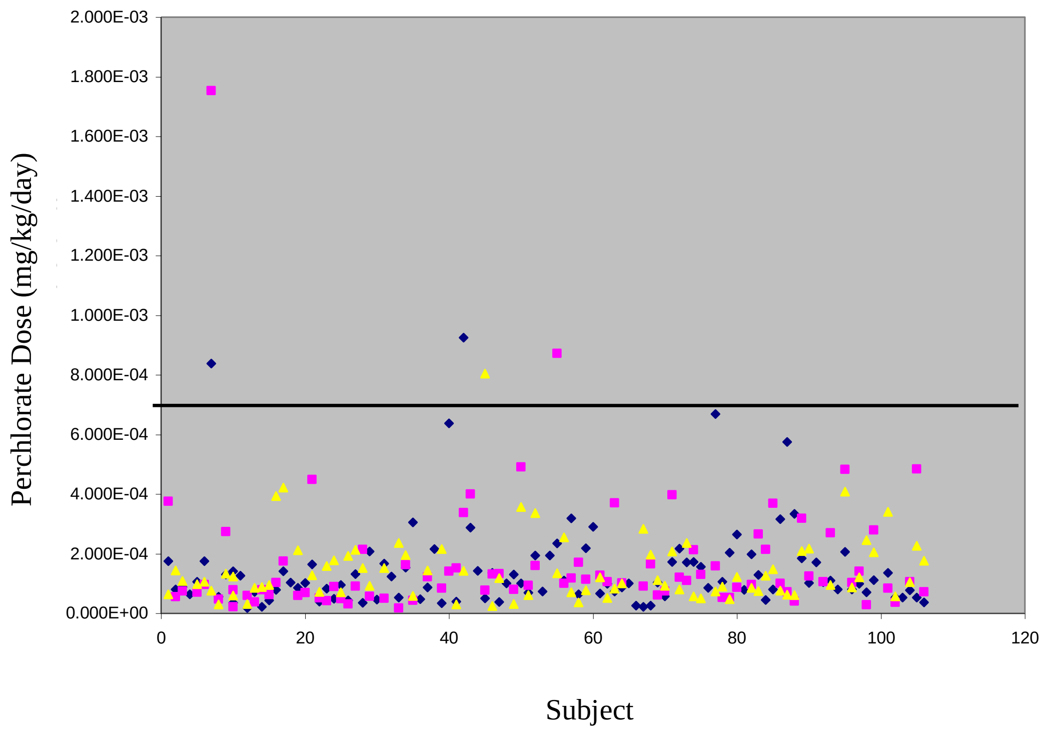


Figure 6. Mother's total daily perchlorate dose. Line corresponds to EPA's perchlorate reference dose of 0.0007 mg/kg/day. ◆ Sample Set 1; ■ Sample Set 2; ▲ Sample Set 3.

Statistical Model: Multiple Regression Analysis

Pearson product-moment correlation coefficient was used to determine whether there was a linear association between variables. Multivariate linear regression and stepwise regression were used to determine significant predictors of breast milk and urine perchlorate, thiocyanate, and nitrate levels.

Perchlorate levels in breast milk, perchlorate levels in urine, and total perchlorate excreted are the dependent variables. Pearson product-moment correlation coefficient was used to determine the relationship between urine and breast milk perchlorate levels.

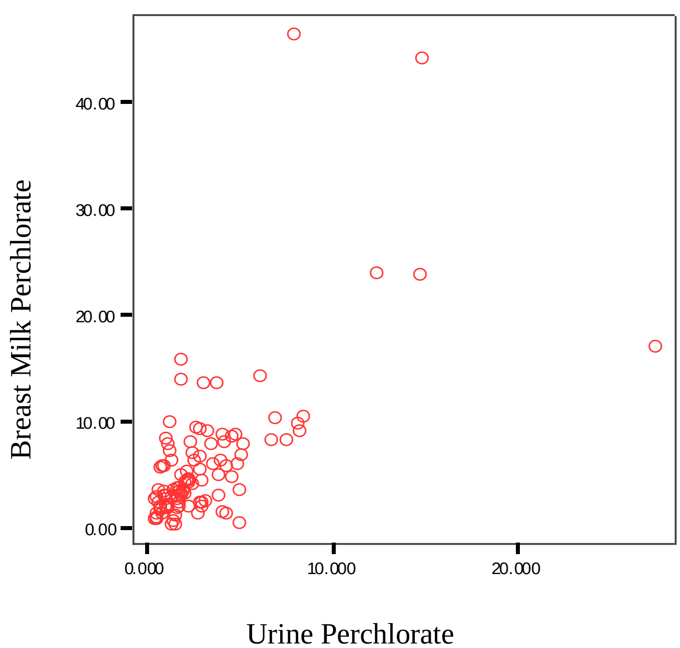
Log10 values of breast milk and urine perchlorate were used in these analyses. There was a strong positive correlation between urine and breast milk perchlorate levels in each sample set (Table 10, Figure 7a-c). Since perchlorate levels from these two excretion routes are correlated, total perchlorate excreted (breast milk and urine combined) will be run as the dependent variable in this model.

Table 10. Pearson product-moment correlation coefficient for urine and breast milk perchlorate

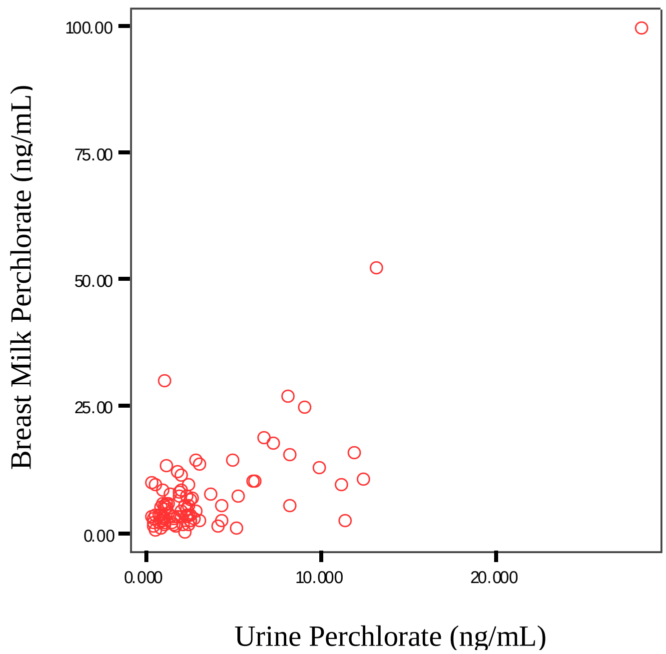
	Sample Set 1	Sample Set 2	Sample Set 3
r	0.578*	0.480*	0.337*
Significance (1-tailed)	0.000	0.000	0.001
N	104	87	82

* Correlation is significant at the 0.01 level (1-tailed).

a)



b)



c)

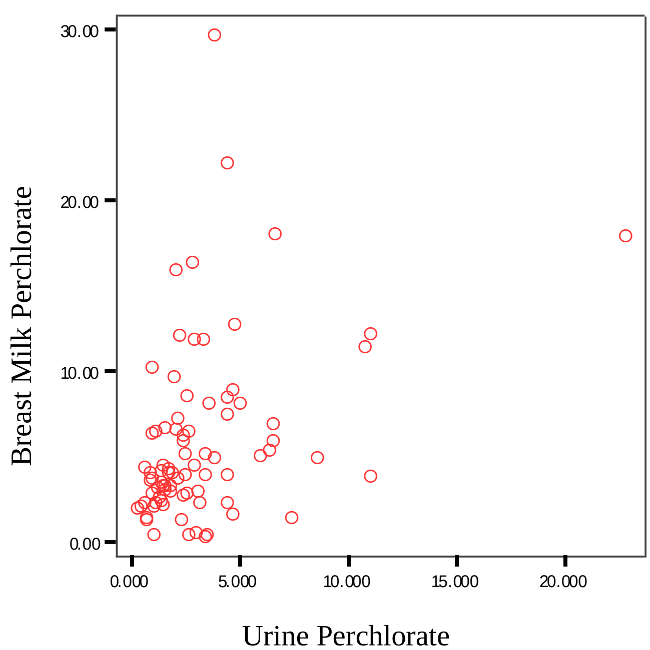


Figure 7. Paired perchlorate milk and urine samples from sample set 1 (a), 2 (b), and 3 (c)

First, three separate regression analyses were run to see how well the independent variables predict perchlorate levels. *Log10* values were used in these analyses. The dependent variable total perchlorate excreted was run against the independent variables from the dietary recall, drinking water perchlorate levels, and then demographics. Descriptive statistics showed that there were 66 missing cases for mother's occupation and 41 missing cases for father's occupation. Since the regression model deleted variables with missing cases pairwise, only 20 of the 106 cases were entered in the model. Since only about 10,000 people in the U.S. work with perchlorate, occupation is unlikely to be an important variable. Therefore, occupation was removed and was not run in the regression model. In addition, since over 90% of the subjects were Hispanic, race was re-entered as Hispanic or non-Hispanic.

After the individual regressions were ran, a stepwise analysis was run on all the independent variables to pull out the most significant variables. Stepwise regression is a systematic method for adding and removing terms from a multi-linear model based on their statistical significance in a regression. In stepwise regression, the independent variables are entered into the model if the significance level if it is F-value is less than the entry value and is removed if the significance level is greater than the removal value. As the entry and removal values are increased more variables are entered into the model.

Multiple regression analyses showed that perchlorate in drinking water was not a strong predictor of total perchlorate excreted. This can be seen by the small R values and the beta values show a weak negative association (Tables 11-13). This is expected since low levels of perchlorate were detected in the drinking water samples, and perchlorate can be found in many common food items.

Since drinking water is not a major source of perchlorate, it is assumed that greater perchlorate exposure occurs through food consumed by the subjects. A larger R value is seen in the regression analyses run with the food products consumed 24 hours prior to sample collection. The strongest food predictors of perchlorate varied among all three sample sets. Other vegetables, which included onions, chayote (gourd family), peppers, peas, potatoes, corn, cassava, cabbage, squash, beets, cauliflower, green beans, celery, chilies, and leeks was one of the strongest predictors for sample set 1 ($t = 2.457$, $p < 0.05$). Looking at the beta value in the table below, for every one unit increase in 'other vegetable', perchlorate would increase by 0.334 units. Leafy vegetables, such as lettuce and spinach showed a weak association. Lettuce showed a weak, non-significant, positive association ($t = 0.721$, $p > 0.05$). However, spinach showed a weak negative association. According to the beta value produced from the regression analysis, for every one unit increase in spinach consumed, perchlorate would decrease by 0.006 units.

For sample set 2, other dairy was the strongest predictor ($t = 2.185$, $p<0.05$). The estimated beta values shows that for every one unit increase in 'other dairy' consumed, perchlorate would increase by 0.274 units. Unlike for sample set 1, lettuce ($t = 1.302$, $p > 0.05$) and spinach ($t = 1.814$, $p > 0.05$) showed a strong positive association. Stepwise analyses showed that for sample set 2, education ($t = -1.664$, $p > 0.05$) and number of children ($t = 1.350$, $p>0.05$) were predictors of perchlorate. The beta values show that for every one unit increase in education level, perchlorate would decrease by 0.204 units and for every one unit increase in number of children, perchlorate would increase by 0.172 units.

Iodized salt was the greatest predictor of perchlorate for sample set 3, $t = 2.384$, $p < 0.05$. The estimated beta value shows for everyone unit increase in iodized salt, perchlorate will increase by 0.364 units. Theoretically, iodine levels should decrease as a result of increased perchlorate levels. This association was also seen when Pearson product-moment correlation was run on perchlorate and iodide levels and showed a positive association between the two: $r = 0.539$, 0.708, and 0.677 for sample sets 1, 2, and 3 respectively. Pearce *et al*. (2007) also found that there was no correlation between iodide and perchlorate in breast milk. It is suggested by Pearce *et al*. (2007) that this lack of correlation may be a result of diurnal and daily differences of iodide and perchlorate concentrations in breast milk. For sample set 3, lettuce ($t = -2.380$, $p < 0.05$) showed a strong negative association and spinach ($t = 0.961$, $p > 0.05$) showed a negative association. Again, lettuce is thought to contain high levels of perchlorate but for every one unit of lettuce consumed, perchlorate decreases by 0.349 units.

As previously mentioned, perchlorate has been found in drinking water, leafy green vegetables, fruits, milk, and grains. Perchlorate has also been detected in ground water and irrigation water and there is concern that fruits and vegetables irrigated with perchlorate-contaminated water may pose a public health risk. Perchlorate is highly soluble and mobile in water allowing it to follow the movement of water within plants and eventually accumulates in the leaves. It has been shown that leafy green vegetables such as lettuce accumulate perchlorate in its leaves. (Gu 2006; Sanchez 2005a) It was assumed that vegetables such as lettuce, spinach, etc. would be the strongest predictors for perchlorate exposure. However, other vegetables (onions, chayote, peppers, peas, potatoes, corn, etc.) normally not known to accumulate perchlorate and dairy products were more of a predictor for perchlorate. This may be due to the fact that information on where the food products came from, the type of soil it was grown in, and if the crops were irrigated with perchlorate contaminated water is not known. In addition, perchlorate levels in the produce may change by season depending on where the produce was grown. For example, the subjects may eat more locally grown food during the summer, which may have less perchlorate than produce grown with contaminated water out west. According to Sanchez *et al*. (2005a), during the winter months, greater than 90% of the lettuce eaten by people in the U.S. is grown in the Lower Colorado River region. Depending on the type of lettuce, Sanchez *et al*. (2005a) estimated that consumption of lettuce from this region would result in exposure ranging from 0.45-1.8 μg/day. Timing of urine and breast milk sample collection varied among subjects as well. Dairy products have been shown to contain perchlorate, often from cows grazing on contaminated pastures. Kirk *et al*. (2005) analyzed dairy milk randomly purchased from grocery stores in Lubbock, TX. Perchlorate was detected in 81 of 82 samples ranging from 2.0-10.5 μg/L. Foods high in iodine also showed to be strong predictors in all three sample sets. Foods high in iodine showed a negative association to total perchlorate excreted. Possibly showing as perchlorate levels increase, iodine levels may decrease.

Table 11. Regression analysis for total perchlorate excreted in sample set 1

Variables	Demographics R = 0.233	Diet R = 0.626	Drinking Water R = 0.109	Stepwise*		
				Run 1	Run 2	Run 3
Age	0.105					
Latin	-0.171					
Education	0.001					
Number of Children	-0.182					-0.145
Breast Feeding	-0.056					
Smoke	0.053					
Medical Illnesses	-0.047					
Lettuce		0.090				
Spinach		-0.006				
Other vegetables		0.334		0.278	0.278	0.268
Oranges		0.062				
Grapefruit		-0.060				
Lemons		0.109				
Milk		-0.364				
Cheese		-0.204		-0.227	-0.227	-0.245
Whole grain cereal		-0.246				-0.154
Drinking Water			-0.088			

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Table 12. Regression analysis for total perchlorate excreted in sample Set 2

Variables	Demographics R = 0.371	Diet R = 0.751	Water R = 0.104	Stepwise*		
				Run 1	Run 2	Run 3
Age	0.038					
Latin	0.093					
Education	-0.204					
Number of	0.172				0.202	0.225
Breast Feeding	-0.149					
Smoke	0.045					
Medical Illnesses	-0.164					
Lettuce		0.196				
Spinach		0.28				
Greens		0.011				0.224
Asparagus		-0.277				-0.18
Other vegetables		0.244				
Oranges		-0.003				
Grapefruit		-0.075				
Lemons		0.161		0.255	0.283	0.268
Yogurt						-0.143
Other dairy		0.274		0.306	0.267	0.291
Iodized salt		0.185		0.296	0.175	0.133
Hot dogs		-0.255			-0.217	-0.203
Whole grain cereal		-0.112			-0.226	-0.183
Drinking Water			-0.11			-0.157

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Table 13. Regression analysis for total perchlorate excreted in sample set 3

Variables	Demographics R = 0.377	Diet R = 0.758	Drinking Water R = 0.133	Stepwise*		
				Run 1	Run 2	Run 3
Age	-0.11					
Latin	0.252				0.294	0.358
Education	-0.096					
Number of Children	0.199					
Breast Feeding	-0.136					
Smoke	0.064					
Medical Illnesses	-0.232					
Lettuce		-0.349			-0.237	-0.351
Spinach		-0.205				
Other vegetables		-0.015				
Oranges		0.007				
Grapefruit		-0.155				
Lemons		0.096				
Grapes		0.262		0.312	0.388	0.325
Cantaloupe		0.17				0.172
Cheese		-0.268		-0.33	-0.267	-0.208
Yogurt		-0.271				-0.307
Sour cream		0.08				0.166
Other dairy		0.196			0.224	0.216
Iodized salt		0.364			0.279	0.355
Beans		-0.237			-0.287	-0.266
Hot dogs		0.018				
Ham		0.052				0.178
Butter		-0.13				-0.139
Cooked oatmeal		0.296				0.22
Whole grain cereal		-0.247		-0.271	-0.188	-0.194
Vitamins		-0.216				-0.173
Drinking Water			-0.123			

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Thiocyanate and Nitrate

Pearson product-moment correlation coefficient was used to determine whether there was a linear association between variables and multivariate linear regression and stepwise regression were used to determine significant predictors of breast milk and urine thiocyanate and nitrate levels also. *Log10* values of breast milk and urine thiocyanate and nitrate were used in these analyses. Regression analysis can be seen in table form in Appendix B, Tables 56-64. No significant results were found for nitrate. However, regression analysis showed that smoking was a strong predictor of total thiocyanate excretion. However, in the regression analysis there was a strong negative association between smoking and thiocyanate for sample sets 1 ($\beta = -0.305$, $t = -2.975$, $p < 0.05$), 2 ($\beta = -0.269$, $t = -2.184$, $p < 0.05$), and 3 ($\beta = -0.478$, $t = -4.342$, $p < 0.05$). Thiocyanate is a metabolite of cyanide in tobacco smoke and is also an iodine uptake inhibitor and will be discussed in more detail later.

Missing Data

One subject was given the wrong survey during the first scheduled sample collection so no demographic information was collected on that subject. Some drinking water data is missing because a few of the subjects (n = 5) forgot to bring their drinking water sample to the health center during the scheduled sample collection. Three subjects were unable to provide urine at time of sample collection. In addition, some of the mothers were either unable to provide a breast milk sample or were unable to provide an adequate amount of breast milk, which often resulted in the subject not returning for the next sample collection. Subjects may stop breast-feeding due to the baby not latching on or the mother had to return to work. Once the mother stops breast-feeding, milk is no longer produced.

Summary of Results by Research Questions and Hypothesis

How much perchlorate is found in the breast milk of lactating women and therefore is passed to the infant?

In sample set 1 (n = 106) perchlorate range, mean ± SD, and median were 0.42-46.4 ng/mL, 6.27 ± 6.98 ng/mL, and 4.35 ng/mL. For sample set 2 (n = 87) the range, mean ± SD, and median were 0.30-99.5 ng/mL, 8.15 ± 12.5 ng/mL, and 4.84 ng/mL. The range, mean ± SD, and median for sample set 3 (n = 83) were 0.32-29.7 ng/mL, 6.07 ± 5.37 ng/mL, and 4.19 ng/mL. As previously discussed, these levels are comparable to perchlorate levels in breast milk detected in other studies.

The perchlorate levels in breast milk was then used to determine infant's daily intake which was calculated by multiplying the concentration of perchlorate in the breast milk by the volume of breast milk consumed by an infant 0-6 months (0.73 L/day). For sample sets one, two, and three infants were consuming an average of 4.58 µg/dy, 5.95 µg/dy, and 4.48 µg/dy respectively.

Table 14. Perchlorate in breast milk (ng/mL)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	106	87	83
	Missing	0	19	23
Mean		6.27	8.15	6.07
Median		4.35	4.84	4.19
Std. Dev.		6.98	12.5	5.37
Minimum		0.42	0.30	0.32
Maximum		46.4	99.5	29.7
Percentiles	25	2.51	2.51	2.73
	50	4.35	4.84	4.19
	75	8.01	9.64	7.48

[a] Multiple modes exist. The smallest value is shown

Table 15. Infant's intake of perchlorate through breast milk (µg/day)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	106	87	83
	Missing	0	19	23
Mean		4.578	5.949	4.478
Median		3.175	3.530	3.060
Std. Dev.		5.094	9.103	3.923
Minimum		0.310	0.220	0.240
Maximum		33.870	72.640	21.680
Percentiles	25	1.833	1.830	1.990
	50	3.175	3.530	3.060
	75	5.848	7.040	5.460

[a] Multiple modes exist. The smallest value is shown

What factors influence the variation in maternal perchlorate dose?

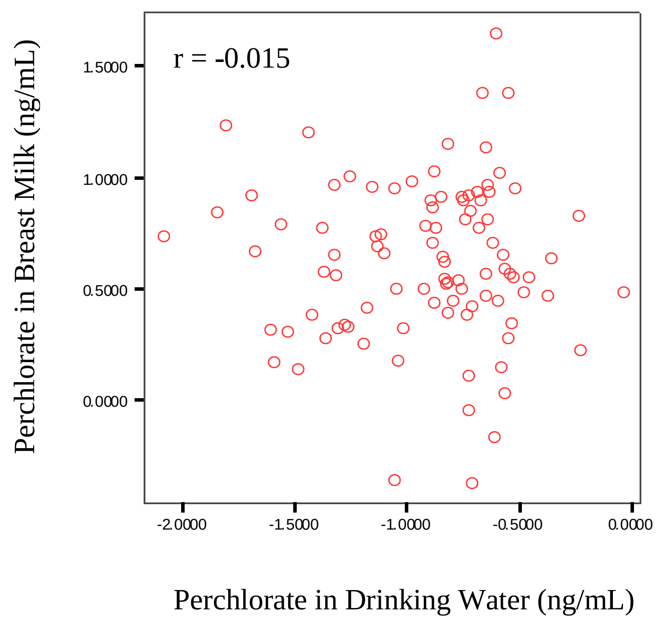
Variation was seen in the large perchlorate concentration ranges in breast milk and urine among the 106 subjects. Differences in perchlorate dose is seen between each sample set. According to the regression analysis previously discussed, diet was the greatest predictor of perchlorate. Drinking water was not a major source of perchlorate. Diets and timing of food intake may vary among subjects which can cause variations in maternal perchlorate dose. In addition, subject diets in the 24-hours prior to sample collection may not be representative of the subject's usual diet. Also, perchlorate levels in produce consumed may have varied by season. For example, subjects who provided samples during the summer may have purchased food locally that may have lower levels of perchlorate while subjects who provided samples during the winter may have consumed food from out west that may have contained more perchlorate. According to Sanchez *et al*. (2005a), during the winter months, greater than 90% of the lettuce eaten by people in the U.S. is grown in the Lower Colorado River region. Depending on the type of lettuce, Sanchez *et al*. (2005a) estimated that consumption of lettuce from this region would result in exposure ranging from 0.45-1.8 μg/day. Timing of urine and breast milk sample collection varied among subjects as well.

Study Hypothesis

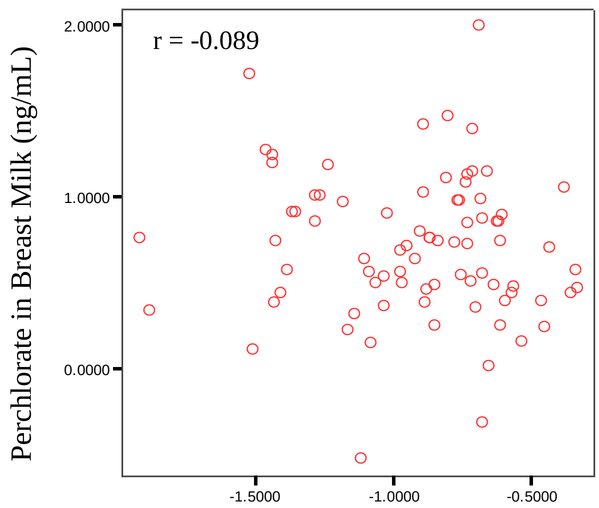
Perchlorate levels in breast milk will be positively associated with drinking water levels of perchlorate.

The relationship between perchlorate levels in breast milk and perchlorate levels in drinking water was investigated using Pearson product-moment correlation coefficient. *Log10* values of breast milk and drinking water perchlorate were used in these analyses. Perchlorate levels in breast milk are not positively associated with drinking water levels of perchlorate. There is a weak negative correlation (not statistically significant) between perchlorate in drinking water and breast milk (Figures a-c). Furthermore, multivariate analysis indicated that drinking water perchlorate levels was not associated with increased breast milk perchlorate levels in this population. Diet was found to be significantly associated with perchlorate levels.

(a)

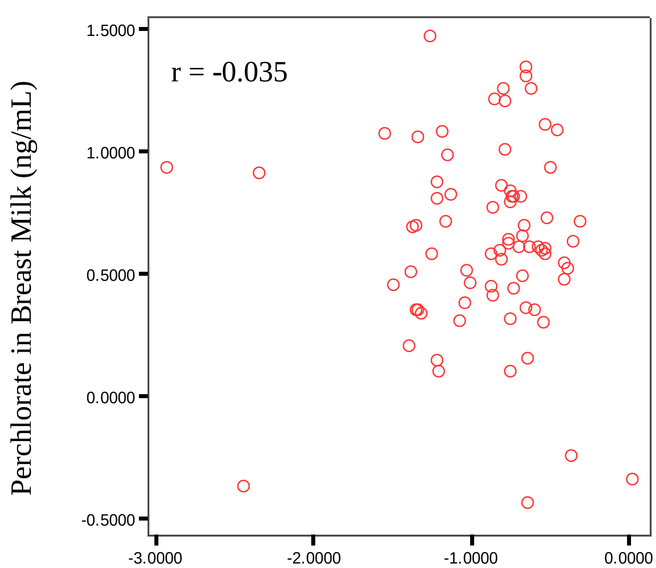


(b)



Perchlorate in Drinking Water (ng/mL)

(c)



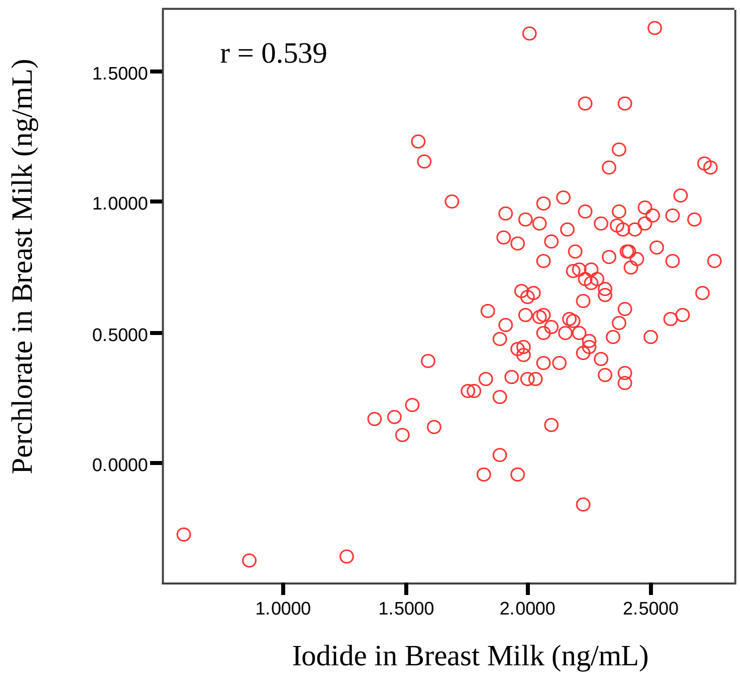
Perchlorate in Drinking Water (ng/mL)

Figure 8. Correlation of drinking water perchlorate with breast milk perchlorate: (a) Sample set 1; (b) Sample set 2; (c) Sample set 3.

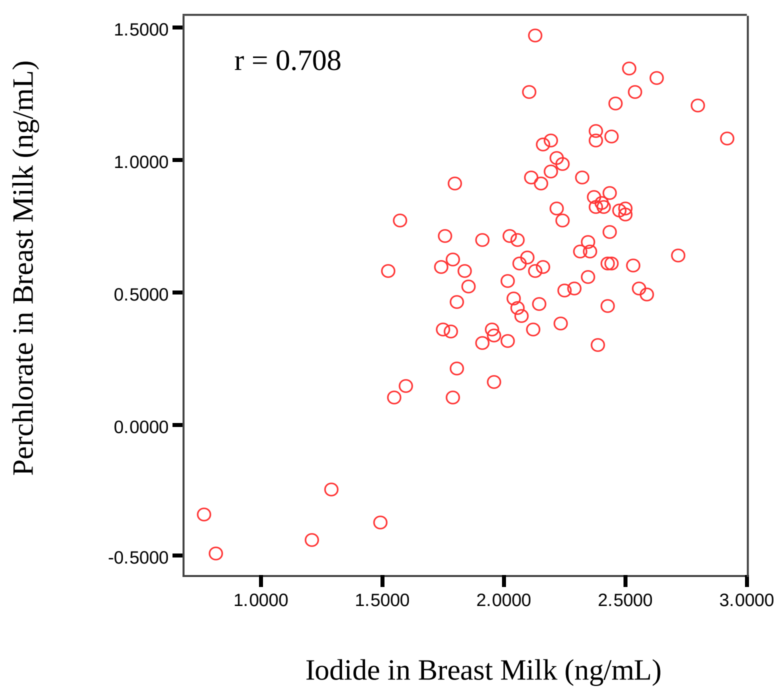
Perchlorate levels in breast milk will be negatively associated with iodide levels in breast milk.

The relationship between perchlorate and iodide levels in breast milk was investigated using Pearson product-moment correlation coefficient. *Log10* values of breast milk perchlorate and iodide were used in these analyses. Breast milk perchlorate levels were not inversely related to breast milk iodide levels. Perchlorate and iodide levels in breast milk were positively associated (Figures a-c). There was also a slight positive association between nitrate and iodide in breast milk ($r = 0.383$, 0.449, 0.264 for sample set 1, 2, and 3 respectively, correlation was significant at the 0.05 level). A weak association was shown between thiocyanate and iodide in breast milk ($r = 0.169$, 0.070, and -0.141). Pearce *et al*. (2007) also found that there was no correlation between iodide and perchlorate in breast milk. It is suggested by Pearce *et al*. (2007) that this lack of correlation may be a result of diurnal and daily differences of iodide and perchlorate concentrations in breast milk. Regression analyses showed that there was a positive association with total iodide excreted and consuming iodized salt, which may contribute to a positive association between perchlorate and iodide levels. In sample sets 2 and 3 iodized salt was a strong predictor of iodide levels. In sample set 2, for every one unit increase in iodide excreted, iodized salt would increase by 0.185 units. For sample set 3, for every one unit increase in iodide excreted, iodized salt would increase by 0.364 units.

(a)


r = 0.539
1.5000
1.0000
0.5000
0.0000
1.0000
1.5000
2.0000
2.5000
Perchlorate in Breast Milk (ng/mL)
Iodide in Breast Milk (ng/mL)

(b)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0.5000
0.0000
-0.5000
1.0000
1.5000
2.0000
2.5000
3.0000
Perchlorate in Breast Milk (ng/mL)
Iodide in Breast Milk (ng/mL)

(c)

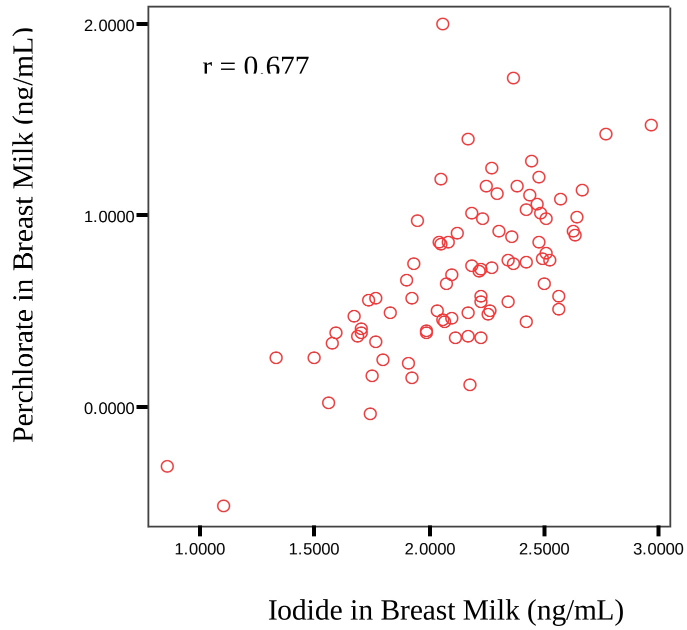


Figure 9. Correlation of breast milk perchlorate and breast milk iodide: (a) Sample set 1; (b) Sample set 2; (c) Sample set 3.

Will the mass of perchlorate excreted through breast milk be greater than the mass excreted through urine in the subject?

Higher levels of perchlorate were excreted in urine compared to breast milk (Tables 34 and 35). A matched paired *t*-test was conducted to evaluate the difference in means between breast milk and urine samples. *Log10* values of breast milk and urine perchlorate were used in these analyses. The amount of perchlorate excreted in urine was significantly higher than the amount excreted in breast milk for samples set 1 ($t = -4.140$, $p < 0.0005$) and sample set 3 ($t = -3.981$, $p < 0.0005$). No significant difference was seen in sample set 2 (t = -1.311, p > 0.05). Intuitively, both urinary perchlorate and milk perchlorate reflect serum perchlorate at different points in time. Thus, depending on the amount of time each sample integrates and whether serum perchlorate levels are increasing or decreasing, the relative level of perchlorate cleared by each pathway will vary, but still be related. As discussed in Capuco *et al*. (2005), blood serum is often use when investigating biomarkers since it is the matrix closest to the tissue targeted by the chemical of concern. Therefore, serum perchlorate would be considered the best measure of perchlorate delivered to the thyroid. However, urinary levels of perchlorate are often much higher than blood levels due to efficient renal clearance. As a result, the kidney is able to handle increasing loads of perchlorate while keeping a the partitioning coefficient between blood and urine consistent. On the other hand, the mammary gland not capable of sustaining the portioning coefficient between blood and milk. This could be because the mammary gland involves more of a passive transport and may be restricted by membrane transport mechanisms [Wolff-Chaikoff effect]. (Capuco *et al*. 2005) As previously discussed, Pearson product-moment correlation showed there was a strong positive correlation at the 0.01 level between urine and breast milk perchlorate levels in each sample set; r = 0.578, 0.480, and 0.337 for sample sets 1, 2, and 3 respectively.

Table 16. Descriptive statistics of perchlorate excretion in breast milk (mg/day)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	87	83
	Missing	0	19	23
Mean		4.39×10^{-3}	5.70×10^{-3}	4.25×10^{-3}
Median		3.05×10^{-3}	3.39×10^{-3}	2.93×10^{-3}
Std. Dev.		4.89×10^{-3}	8.73×10^{-3}	3.76×10^{-3}
Minimum		2.95×10^{-4}	2.10×10^{-4}	2.26×10^{-4}
Maximum		3.25×10^{-2}	6.97×10^{-2}	2.08×10^{-2}
Percentiles	5	6.28×10^{-4}	7.98×10^{-4}	3.35×10^{-4}
	10	1.01×10^{-3}	1.23×10^{-3}	9.88×10^{-4}
	25	1.76×10^{-3}	1.76×10^{-3}	1.91×10^{-3}
	50	3.05×10^{-3}	3.39×10^{-3}	2.93×10^{-3}
	75	5.61×10^{-3}	6.75×10^{-3}	5.24×10^{-3}
	90	8.05×10^{-3}	1.09×10^{-2}	8.79×10^{-3}
	95	1.17×10^{-2}	1.82×10^{-2}	1.26×10^{-2}

[a] Multiple modes exist. The smallest value is shown

Table 17. Descriptive statistics of perchlorate excretion in urine (mg/day)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	82
	Missing	2	19	24
Mean		6.23×10^{-3}	6.57×10^{-3}	6.35×10^{-3}
Median		4.11×10^{-3}	3.92×10^{-3}	4.71×10^{-3}
Std. Dev.		7.20×10^{-3}	8.20×10^{-3}	6.38×10^{-3}
Minimum		5.70×10^{-4}	3.92×10^{-4}	3.56×10^{-4}
Maximum		5.46×10^{-2}	5.66×10^{-2}	4.54×10^{-2}
Percentiles	5	8.85×10^{-4}	7.30×10^{-4}	1.10×10^{-3}
	10	1.29×10^{-3}	1.29×10^{-3}	1.67×10^{-3}
	25	2.37×10^{-3}	1.89×10^{-3}	2.66×10^{-3}
	50	4.11×10^{-3}	3.92×10^{-3}	4.71×10^{-3}
	75	7.74×10^{-3}	7.14×10^{-3}	7.79×10^{-3}
	90	1.34×10^{-2}	1.67×10^{-2}	1.29×10^{-2}
	95	1.66×10^{-2}	2.32×10^{-2}	2.07×10^{-2}

[a] Multiple modes exist. The smallest value is shown

If the major excretion route of perchlorate is breast milk, will iodide be excreted at a lower level in breast milk compared to urine?

As previously mentioned, perchlorate was excreted at a higher level in urine compared with breast milk. Iodide is also excreted at a higher level in urine compared to breast milk (Tables 36 and 37). A matched paired *t*-test was conducted to evaluate the difference in means between breast milk and urine samples. *Log10* values of breast milk and urine iodide were used in these analyses. A statistically significant difference was seen between urine and breast milk samples in samples set 1 ($t = 10.35$, $p < 0.0005$), 2 ($t = 6.48$, $p < 0.0005$), and 3 ($t = 8.86$, $p > 0.0005$). Iodide may also be excreted at a higher rate in urine than breast milk because of the difference in clearance between the mammary gland and kidneys as was discussed above. Renal clearance may be more of an active transport while the mammary gland may involve more of a passive transport where there may be more limitations with the membrane transport system and possible down-regulation of the NIS when iodide, or perchlorate, levels are too high.

Table 18. Descriptive statistics of iodide excretion in breast milk (mg/day)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	106	87	83
	Missing	0	19	23
Mean		0.125	0.130	0.126
Median		0.108	0.107	0.101
Std. Dev.		0.087	0.100	0.097
Minimum		0.003	0.005	0.004
Maximum		0.403	0.643	0.578
Percentiles	5	0.021	0.023	0.015
	10	0.029	0.034	0.027
	25	0.064	0.059	0.057
	50	0.108	0.107	0.101
	75	0.167	0.186	0.176
	90	0.247	0.255	0.234
	95	0.324	0.304	0.293

[a] Multiple modes exist. The smallest value is shown

Table 19. Descriptive statistics of iodide excretion in urine (mg/day)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	105	87	82
	Missing	1	19	24
Mean		0.285	0.284	0.312
Median		0.274	0.214	0.245
Std. Dev.		0.177	0.219	0.225
Minimum		0.039	0.004	0.033
Maximum		0.928	1.004	1.206
Percentiles	5	0.064	0.051	0.062
	10	0.090	0.086	0.087
	25	0.149	0.123	0.177
	50	0.274	0.214	0.245
	75	0.376	0.372	0.418
	90	0.528	0.598	0.662
	95	0.653	0.846	0.792

a Multiple modes exist. The smallest value is shown

Will the consumption of certain food products such as vegetables, fruits, and grains be positively associated with higher perchlorate levels in breast milk and urine?

Compared to drinking water perchlorate and demographics, diet was the strongest predictor of perchlorate levels in breast milk and urine. Certain food products were more positively associated with perchlorate levels in breast milk and urine than others and some were negatively associated. The strongest food predictors of perchlorate varied among all three sample sets. No consistent patterns with specific foods were seen. It was assumed that green leafy vegetables such as lettuce and spinach would be the strongest predictors for perchlorate exposure based on previous literature reports. Depending on the type of lettuce, Sanchez *et al*. (2005a) estimated that consumption of lettuce from the Lower Colorado River area would result in exposure ranging from 0.45-1.8 μg/day. However, other vegetables (onions, chayote, peppers, peas, potatoes, corn, etc.) normally not known to accumulate perchlorate and dairy products were stronger predictors for increased perchlorate levels in breast milk and urine. Murray *et al*. (2008) looked at the perchlorate levels recorded by the US FDA's Total Diet Study and estimated daily intake of perchlorate through food products for 2005-2006 and showed that other vegetables and food products were associated with perchlorate intake. It was estimated that from 46% to 59% of the total estimated perchlorate intake of teenagers and adults comes from dairy and vegetable products combined (Murray *et al*. 2008).

If perchlorate is detected in breast milk samples, will the infant be ingesting perchlorate at a level that exceeds the EPA reference dose?

As previously discussed, perchlorate was detected in all breast milk samples provided. Dose calculations estimate that 25% of infants 0-6 months of age ingest perchlorate through breast milk above the recommended EPA RfD of 0.0007 mg/kg/day (Table 38). Since infants weigh much less than adults, the same amount (in mg/day) to an infant will give a much higher dose in mg/kg/day than it will give to an adult in mg/kg/day. Infants consume more food per body weight and have different food consumption patterns than adults, which may result in a daily perchlorate dose greater than the EPA RfD. At some point above this RfD, inhibition of iodine uptake would be expected to occur, depending on a number of modifying conditions, which include, but are not limited to, the amount of milk or formula actually ingested, the concentration of perchlorate in each meal, iodine intake and other nutritional factors, and numerous individual physiological factors.

Table 20. Estimated breast milk perchlorate doses (mg/kg/dy) for infants 0-6 months of age

Sample Set	N	Mean	Percentiles						
			5th	10th	25th	50th	75th	90th	95th
0-1 month									
1	106	9.539×10^{-4}	1.364×10^{-4}	2.204×10^{-4}	3.818×10^{-4}	6.616×10^{-4}	1.218×10^{-3}	1.749×10^{-3}	2.537×10^{-3}
2	87	1.239×10^{-3}	1.734×10^{-4}	2.665×10^{-4}	3.818×10^{-4}	7.361×10^{-4}	1.466×10^{-3}	2.357×10^{-3}	3.963×10^{-3}
3	83	9.224×10^{-4}	7.282×10^{-5}	2.147×10^{-4}	4.152×10^{-4}	6.372×10^{-4}	1.138×10^{-3}	1.910×10^{-3}	2.735×10^{-3}
1-3 months									
1	106	8.176×10^{-4}	1.169×10^{-4}	1.889×10^{-4}	3.272×10^{-4}	5.671×10^{-4}	1.044×10^{-3}	1.499×10^{-3}	2.174×10^{-3}
2	87	1.062×10^{-3}	1.486×10^{-4}	2.284×10^{-4}	3.272×10^{-4}	6.309×10^{-4}	1.257×10^{-3}	2.021×10^{-3}	3.397×10^{-3}
3	83	7.906×10^{-4}	6.241×10^{-5}	1.841×10^{-4}	3.559×10^{-4}	5.462×10^{-4}	9.751×10^{-4}	1.637×10^{-3}	2.343×10^{-3}
3-6 months									
1	106	6.187×10^{-4}	8.846×10^{-5}	1.429×10^{-4}	2.476×10^{-4}	4.291×10^{-4}	7.899×10^{-4}	1.134×10^{-3}	1.645×10^{-3}
2	87	8.039×10^{-4}	1.125×10^{-4}	1.728×10^{-4}	2.476×10^{-4}	4.775×10^{-4}	9.510×10^{-4}	1.529×10^{-3}	2.571×10^{-3}
3	83	5.983×10^{-4}	4.723×10^{-5}	1.393×10^{-4}	2.693×10^{-4}	4.133×10^{-4}	7.379×10^{-4}	1.239×10^{-3}	1.774×10^{-3}

Additional Findings

Thiocyanate is a metabolite of cyanide, which is found in tobacco smoke. Originally, it was proposed that the effects of thiocyanate on iodide levels in breast milk and urine be investigated among smokers and non-smokers. However, it was difficult to make this comparison since only four subjects indicated they smoked. Initially an Independent-samples t-test was run to determine the difference between smokers and non-smokers. There was no significant difference between groups as $p > 0.05$. However, sample set 3 showed a significant difference in mean scores, $t = -3.610$, $p < 0.05$. Since only four of the subjects smoked; 3 during sample collection, 2 while pregnant, one of these subjects smoked while pregnant and during sample collection, a Mann-Whitney U Test, a non-parametric alternative, was used to test the differences between smokers versus non-smokers. Sample set 3, again, was the only group that showed a statistically significant difference, $z = -2.255$, $p < 0.05$. Pearson product-moment correlation did show a weak association between thiocyanate and iodide in breast milk; for sample sets 1, 2, and 3 r = 0.169, 0.070, and -0.141, respectively. A weak positive association was also shown between urine thiocyanate and urine iodide; for sample sets 1, 2, and 3 r = 0.356, 0.457, and 0.135, respectively.

Even though iodide levels were not affected by perchlorate levels in breast milk, it is still important to determine if a population is iodine deficient. Breast milk iodide levels ranging from 150 – 180 ng/mL is considered sufficient while breast milk iodide levels < 50 ng/mL are considered to be "consistent with iodine deficiency" (Bazrafshan *et al*. 2005; Kirk *et al*. 2007). Breast milk iodide levels showed that the population is likely to be iodide sufficient (Table 21). In American women, the median iodine concentration in breast milk for mothers who consumed iodized and noniodized salt was 146 ng/mL, 14 days to 3.5 years postpartum (Food and Nutrient Board/IOM 2000). This is similar to the median breast milk iodide level for this study, which is 141 ng/mL. For infants 0-6 months, adequate iodine intake is 110-130 µg/day. Nearly 25% of the breast fed infants are receiving 70 µg/day or lower. It should also be taken into consideration that iodide is excreted in urine and that breast milk sampling times varied.

Table 21. Descriptive statistics of breast milk iodide levels (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	87	83
	Missing	0	19	23
Mean		178.049	185.466	179.613
Median		154.000	153.000	144.000
Std. Dev.		124.226	143.230	138.330
Minimum		3.910	7.180	5.810
Maximum		575.000	918.000	826.000
Percentiles	5	29.300	33.440	21.740
	10	41.110	47.940	38.220
	25	91.575	84.100	81.100
	50	154.000	153.000	144.000
	75	238.000	266.000	251.000
	90	352.600	363.800	334.400
	95	462.200	434.800	418.400

a Multiple modes exist. The smallest value is shown

According to the World Health Organization (1994), an iodine sufficient population should have urine iodide levels > 100 ng/mL, however, no more that 20% of the population should have urine iodide levels < 50 ng/mL. Despite the fact that iodide is also excreted in breast milk 50% of the subjects had urine iodide levels above 100 ng/mL.

Table 22. Descriptive statistics of urine iodide levels (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	82
	Missing	2	19	24
Mean		143.618	141.948	156.391
Median		138.000	107.000	122.500
Std. Dev.		88.018	109.597	112.281
Minimum		19.400	2.200	16.700
Maximum		464.000	502.000	603.000
Percentiles	5	37.175	25.600	30.850
	10	45.600	43.180	43.250
	25	75.550	61.300	88.450
	50	138.000	107.000	122.500
	75	190.500	186.000	209.000
	90	264.500	299.000	330.800
	95	328.250	422.800	395.900

a Multiple modes exist. The smallest value is shown

For creatinine adjusted iodide concentrations in urine, levels < 50 μg/g creatinine may also indicate possible iodine deficiency. Again, even though iodide is also excreted through breast milk, only 10% of the urine samples had creatinine corrected iodide levels below 50 μg/g creatinine. Therefore, this population is likely to be iodine sufficient.

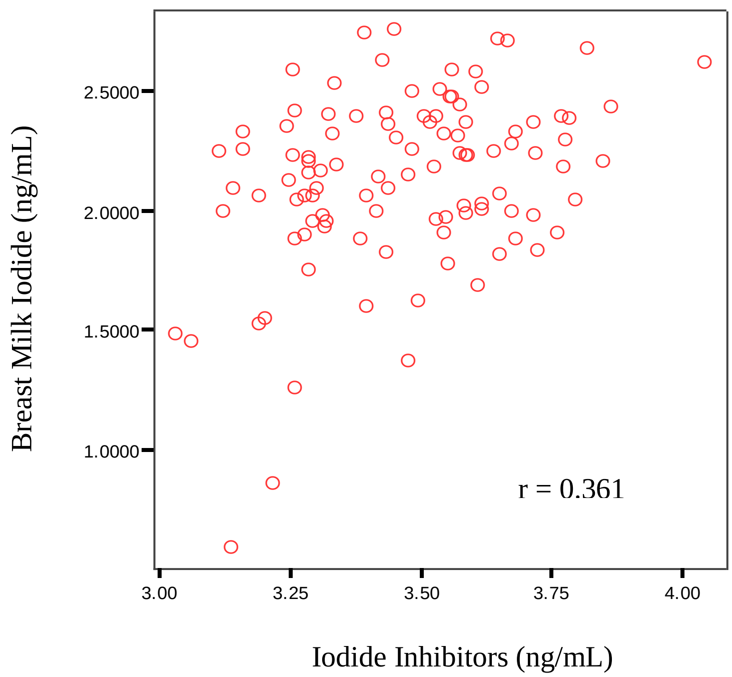
Table 23. Descriptive statistics of creatinine corrected urine iodide levels (μg/g creatinine)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	81
	Missing	2	19	25
Mean		137.820	139.898	145.012
Median		123.100	120.120	130.730
Std. Dev.		82.093	100.909	79.753
Minimum		22.610	22.110	23.400
Maximum		414.330	733.330	492.910
Percentiles	5	33.583	33.248	37.816
	10	50.750	42.844	56.956
	25	72.248	81.1900	84.200
	50	123.100	120.120	130.730
	75	175.953	173.970	188.800
	90	250.080	263.138	243.264
	95	322.298	314.814	280.042

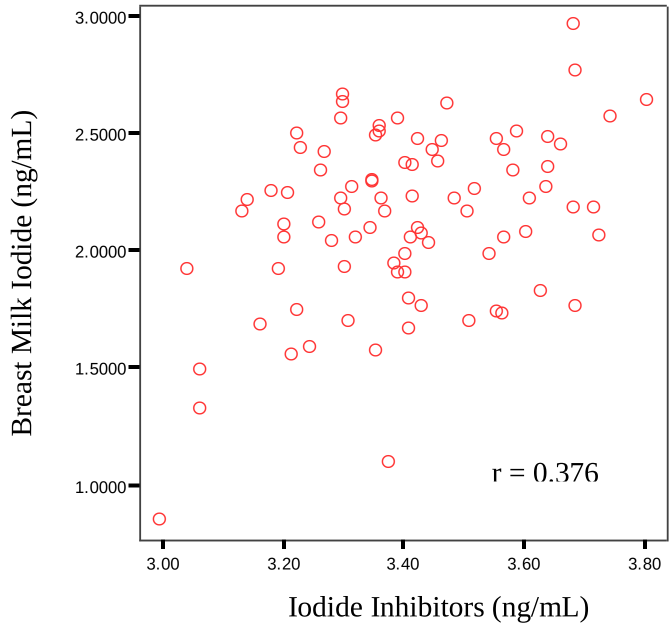
a Multiple modes exist. The smallest value is shown

Perchlorate, nitrate, and thiocyanate were grouped into "iodide inhibitors" in both breast milk and urine. Pearson product-moment correlation coefficient was used to see if the iodine inhibitors combined may have a cumulative effect on iodide levels in breast milk and urine. The l*og10* values were used in these analyses to see if "iodide inhibitors" in breast milk and urine were inversely related to iodide levels in breast milk and urine. The data show that the iodide inhibitors combined were positively associated with iodide levels in breast milk and urine. This association was also seen with perchlorate and iodide levels in breast milk being positively associated. As discussed previously, it is suggested by Pearce *et al*. (2007) that this lack of correlation may be a result of diurnal and daily differences of iodide and perchlorate concentrations in breast milk. Tonacchera *et al*. (2004) looked at the combined effect of perchlorate, nitrate, and thiocyanate. Humans are nutritionally and environmentally exposed to other competitive inhibitors of iodide uptake, including thiocyanate and nitrate. The relative potency of perchlorate to inhibit radioactive iodine uptake in the NIS was found to be 15, 30, and 240 times that of thiocyanate, iodine, and nitrate respectively on a molar concentration basis. Neither of these approaches found evidence of either synergism or antagonism. The data indicated that these RAIU inhibitors interact in a simple additive fashion. (Tonacchera *et al*. 2004)

(a)



(b)



(c)

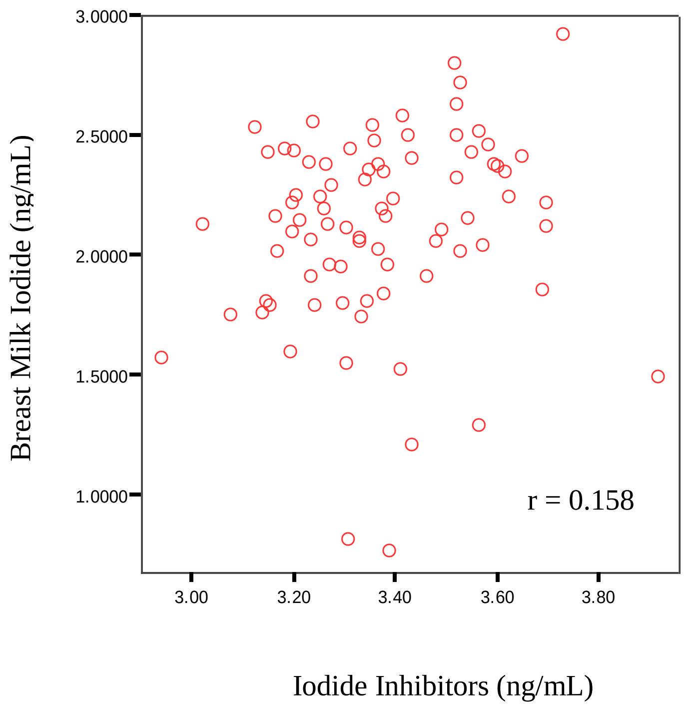
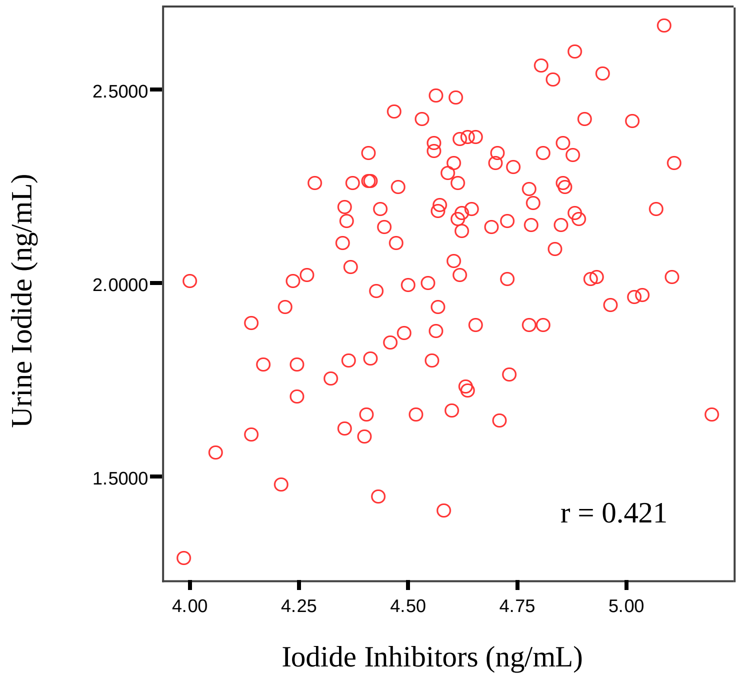


Figure 10. Correlation of breast milk iodide inhibitors and breast milk iodide: (a) Sample set 1; (b) Sample set 2; (c) Sample set 3.

(a)


2.5000
2.0000
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Urine Iodide (ng/mL)
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4.75
5.00
Iodide Inhibitors (ng/mL)
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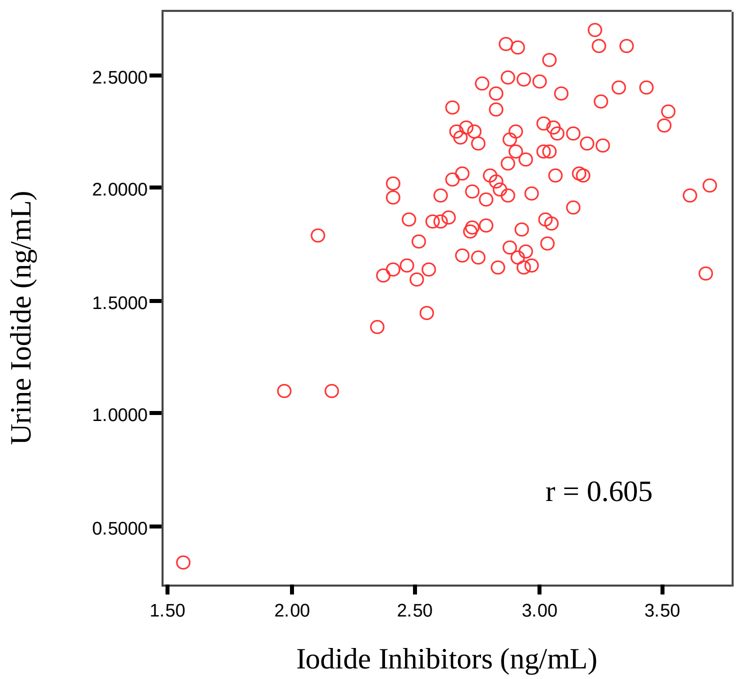
(b)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2.0000
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1.0000
0.5000
Urine Iodide (ng/mL)
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2.00
2.50
3.00
3.50
Iodide Inhibitors (ng/mL)
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(c)

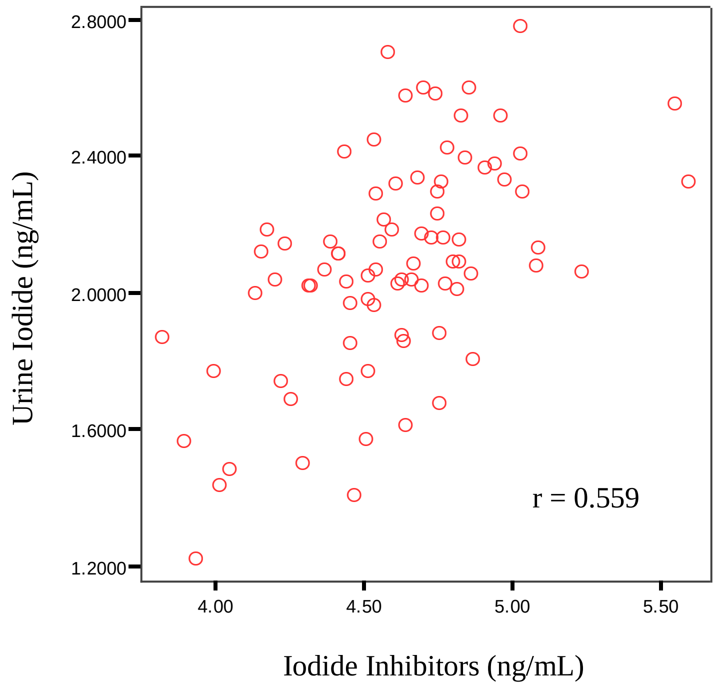

Figure 11. Correlation of iodide inhibitors and urine iodide: (a) Sample set 1; (b) Sample set 2; (c) Sample set 3.

Chapter 5

Discussion

Detectable levels of perchlorate were found in all urine and breast milk samples tested. This finding is consistent with widespread perchlorate exposure in lactating women and infants, and that breast milk is a viable exposure route for infants.

Variation in perchlorate levels were seen among individuals in both breast milk and urine samples. One reason for this variation may be varying levels of perchlorate consumed through drinking water. Subjects also often brought samples of drinking water that were different from the primary drinking water source that was indicated on the questionnaire. Diets and timing of food intake varied among subjects, which can cause differences in perchlorate levels. Dietary patterns in food consumption of certain food products can be diverse among different age and ethnicity groups. In addition, subject diets in the 24-hours prior to sample collection may not be representative of the subject's usual diet. It cannot be presumed that during lactation the mother's diet remains consistent. The levels of perchlorate in a given type of food can vary dramatically depending on when and how that food is grown/produced. Intake changes can result from cultural beliefs, recommendations from the subject's doctor, and mother's change in appetite and energy needs due to the changing needs of the breastfed infant. (Blount *et al*. 2006; IOM 1991)

Timing of urine and breast milk sample collection varied among subjects. The subjects ranged in age from 18-38. Pharmacokinetics and dose and exposure associations can vary with age. Other iodide inhibitors such as nitrate and thiocyanate, which also competitively bind to the sodium (Na^+)/iodide (I^-) symporter, may affect perchlorate levels as well. According to Steinmaus *et al*. (2007), there is evidence that proposes that thiocyanate, nitrate, and perchlorate may interact in effecting the thyroid and hormone production, a possible issue typical risk assessment methods not always account for. There is still a need for more research and information in the literature on perchlorate levels and variability.

Perchlorate levels in breast milk were not positively associated with perchlorate levels in drinking water. In an analyses of perchlorate in breast milk (n = 36) and dairy milk (n = 47), Kirk *et al*. (2005) also measured perchlorate exposure through drinking water and also found that perchlorate levels in drinking water were not well correlated with perchlorate levels in breast milk. As previously mentioned, the NJDEP does not recommend that water with perchlorate levels above 5 ppb (5 ng/mL) be consumed. Perchlorate levels in drinking water samples collected from our subjects were all well below 5 ppb indicating that drinking water is not the primary source of perchlorate. Subjects may be getting most of their perchlorate through food sources. Food such as vegetables, fruits, grains, and dairy products showed some correlation with perchlorate levels in breast milk and urine. It was assumed that green leafy vegetables, such as lettuce, would be the strongest dietary predictors of perchlorate since these foods tend to accumulate perchlorate in their leaves. However, there was a large variability between all three sample collections in regards to food products that were the strongest predictors. Again, this could be a result of inconsistent sample collection and the differing diets among subjects. On the other hand, studies have shown that perchlorate may accumulate more in the outer leaves of the vegetable than the edible head and perchlorate levels vary between certain types of lettuce. For example, iceberg and romaine lettuce are usually trimmed of their outer leaves resulting in a 40% decrease in perchlorate exposure. Naked romaine, green leaf, red leaf, and butter head lettuce have a more open leaf structure that does not require trimming resulting in perchlorate accumulating more in the edible portion. (Sanchez 2005a)

From previous literature, it was assumed that perchlorate would be excreted at higher levels in breast milk compared to urine and perchlorate levels in breast milk would reduce the amount of iodide present in breast milk thus reducing iodide intake by the infant. Both iodide and perchlorate were excreted at higher levels in urine and no reduction in iodide in breast milk was shown as a result of perchlorate in breast milk. In a study of 57 lactating Boston area women, breast milk iodide and perchlorate levels and urine iodide and perchlorate levels were analyzed. It was also shown that breast milk iodide concentrations and perchlorate exposure were not significantly correlated. The diurnal and daily differences in iodide and perchlorate levels in breast milk was suggested as a reason for levels not being significantly correlated. (Pearce *et al*. 2007) As previously mentioned there are variations in perchlorate and iodide levels in breast milk. Dietary patterns in food consumption differ among subjects and timing of urine and breast milk sample collection varied among subjects, which can also contribute to the lack of correlation. In goat studies performed by Kirk *et al*. (2005), it was shown that after exposure to perchlorate in food and drinking water, perchlorate was present within a short time in breast milk and was cleared rapidly after dosing suggesting variability in time which may mean immediate dietary history should be recorded. This may also suggest that breast milk samples should be collected at the beginning of feeding as perchlorate may be more concentrated in the watery fore milk compared to the fatty hind milk.

Dose calculations showed that 25% of infants 0-6 months of age ingest perchlorate through breast milk above the recommended EPA RfD of 0.0007 mg/kg/day. Since infants weigh much less than adults, the same amount (in mg/day) to an infant will give a much higher dose in mg/kg/day than it will give to an adult in mg/kg/day. Infants consume more food per body weight and have different food consumption patterns than adults, which may result in a daily perchlorate dose greater than the EPA RfD.

At some point above this RfD, inhibition of iodine uptake would be expected to occur, depending on a number of modifying conditions, which include, but are not limited to, the amount of milk or formula actually ingested, the concentration of perchlorate in each meal, iodine intake and other nutritional factors, and numerous individual physiological factors. Interpretation of these estimated perchlorate exposure levels must take into account the fact that the RfD is by definition an estimate spanning as much as an order of magnitude, and that the perchlorate RfD is based on a precursor to an adverse effect. Such data gaps could be filled by biomonitoring studies focused on infants. Although the results of this preliminary risk assessment suggest that the perchlorate RfD may be exceeded for certain breastfed infants, these data do not support changing the public health recommendation for breastfeeding. Perchlorate has a short half-life (6-8 hr) in the adult human body. Because the lack of information on changes in perchlorate levels in human milk over the course of one feeding, or over the course of lactation, it is not know whether breastfeeding represents a continuous exposure to infants, or a sporadic exposure, nor is the relationship between these exposures and infant health understood. The RfD is based on a NOEL for iodide uptake inhibition which is considered a precursor to a possible adverse health effect. While adults have a large T4 store in the colloid of the thyroid gland, neonates/infants do not. Therefore, a more critical relationship between iodide uptake inhibition and thyroid hormone synthesis is likely to be present in neonates and infants. (Baier-Anderson *et al*. 2006)

Additional Findings

Thiocyanate, which is a metabolite of cyanide in tobacco smoke, is also an iodine uptake inhibitor. Differences in urine and breast milk iodide levels in smokers versus non-smokers was difficult to determine since only a very small portion of the subjects smoked. Only three subjects were smoking at the time of sample collection and two subjects smoked while pregnant. One of these subjects smoked while pregnant and during sample collection. However, a weak association was shown between thiocyanate and iodide in breast milk; for sample sets 1, 2, and 3 r = 0.169, 0.070, and -0.141, respectively. A weak positive association was also shown between urine thiocyanate and urine iodide; for sample sets 1, 2, and 3 r = 0.356, 0.457, and 0.135, respectively.

Laurberg *et al*. (2004) studied women who had just given birth and classified them as smokers (n = 50) and non-smokers (n = 90) and showed that smoking was associated with reduced breast milk iodide. The study also showed that mothers who smoked had significantly higher serum levels of thiocyanate than mothers who did not smoke. Pearce *et al*. (2007) also showed that breast milk iodide levels was significantly lower in smokers compared to non-smokers. The study found that breast milk iodide was inversely correlated with cotinine concentrations in urine.

Iodine is essential for thyroid hormone synthesis along with normal development, growth, and metabolism. Inadequate iodine levels in the thyroid lumen can result in the decreased synthesis of the thyroid hormones. Thyroid hormones are important in the development of the skeletal system and the central nervous system in fetuses and infants. Starting from the second trimester to three years of age are the most critical periods for development. (NAS 2005; WHO/UNICEF/ICCIDD 2007) Iodine actively accumulates in the mammary gland during lactation that makes sure that the newborn is receiving an ample amount of iodine, and in non-lactating adults > 90% of iodine will be excreted in the urine making urinary iodide levels an ideal indicator of iodine intake. (IOM 1991; Semba 2001; Delange 2007) The data show that that only 10% of the infants may be iodide deficient, if they receive a majority of their nutrition through breast milk, and even though iodide is also excreted in breast milk 50% of the mothers were still in the iodide sufficient range. It should be taken into consideration that iodide is being excreted in both urine and breast milk and that breast milk and urine sampling times varied, which can affect iodide levels. Studies have also show that variability in iodine levels exists in individuals in iodine sufficient populations (Delange 2007). In addition, the nutritional needs of lactating and pregnant women are different from the general population. Perchlorate and iodine intake may be higher given their higher caloric needs. Also, nutrient needs may be greater among mothers who exclusively breast feed compared to those who only partially breast feed. (IOM 1991; Murray *et al*. 2008)

Study Limitations

The subjects in this study were selected out of convenience. Convenience sampling does not represent the entire population so this may be considered a bias, but still provides useful information for a pilot study. Recall bias may be an issue with the 24-hour dietary questionnaire. The dietary questionnaire may not be fully representative of the subject's usual diet. The questionnaires were not pre-tested so some of the subjects may have misunderstood some of the questions. This could be seen in some of the validity checks. For example, some mothers reported breast-feeding 100% of the time but also reported that they used formula. Also, subjects were asked to bring a sample of their primary source of drinking water at each visit. Subjects often brought samples from different water sources, which may not be representative of their usual source of drinking water. In addition, subject weight was not recorded at time of sample collection, which was needed for dose calculations. Average weight of a Hispanic adult female was used which my not be representative of our population of lactating women who currently gave birth.

The advantage of spot urine samples is that they are convenient and easy to collect; however, they may not accurately correspond to when the subject was exposed to perchlorate. Perchlorate is rapidly excreted from the body and has a half-life of about six hours in humans (Gu *et al*. 2006) and subjects may be exposed to varying amounts of perchlorate throughout the day. Another weakness of collecting spot urine samples is inconsistency in volume and the presence of endogenous and exogenous substances between voids (Barr 2005). Variation in diet at each sample collection could have affected other measures as well. For example, measures for other samples collected may not portray the mother's or infant's iodine intake if samples were collected at times when iodide intake was low. Another weak point of the study is that the weight of the subject and the weight and age of the breast-fed infant were not obtained. Therefore, perchlorate dose estimates may not be fully representative of the general population.

In addition, language barriers make it difficult for recruitment due to different dialects and patients who speak very little English and no Spanish. A majority of this population lacks transportation making it difficult to schedule appointments. Mothers may stop breast feeding, causing them to no longer produce milk, because they often must return to work or have not been properly educated on breast feeding, resulting in incomplete collection of the three sets of samples. Cultural factors were also a concern. As previously mentioned, over 90% of the subjects are Hispanic. Recruitment was sometimes difficult because the father of the subject's baby or the subject's mother often influenced the potential subject's participation in the study, usually not allowing potential subject to participate.

Conclusions

One of the main purposes of this study was to estimate total perchlorate exposure for lactating women based on perchlorate levels in maternal milk and urine. In addition, based on perchlorate levels in maternal milk total, perchlorate exposure for breast-fed infants was also estimated. Detectable levels of perchlorate were found in all urine and breast milk samples collected, which may indicate that the general population including infants is exposed to perchlorate and that breast milk is a viable exposure route. For this study population, drinking water was only a minor source of perchlorate exposure.

Infants who are breast fed only, rely on mother's milk as their main source of iodine, which is needed for production of thyroid hormones and establishing hormone stores incase there are insufficient levels of iodine (Kirk *et al*. 2007). In a study by Blount *et al*. (2006), the relationship between serum thyroid hormones and perchlorate levels in urine were evaluated in males and females who took part in NHANES 2001-2002. The study showed that in women, perchlorate was a significant predictor of thyroid hormones. Therefore, it is also important that the mother is consuming enough nutrients so the breast fed infant is getting the nutrients they require for proper development. A portion of the subjects in this study had low levels of iodine intake, which may have resulted in the low levels of iodine in the breast milk. Mental impairments in infants and children can develop in situations of iodine deficiency. To prevent iodine deficiency, The American Thyroid Association (2008) recommends that mothers who are breastfeeding should take a daily supplement containing 150 μg of iodine.

Breast-feeding is important in an infant's growth and development. Not only does breast milk provide immunologic protection, it also contains a high source of nutrients needed by the infant. Infants, and children, go through rapid transformations in physical development, language development, and cognitive and motor capabilities, which make them more at risk from the effects of environmental contaminants. Endocrine functions in many organs are changing and functional and structural changes in the digestive tract, reproductive system, lungs, and bones are occurring throughout infancy and childhood. Clearance rate, which is the rate of chemical eliminated from the body (mL/min), is slower in infants and infants consume more per unit of body weight than adults, which may result in infants being exposed to a higher level of perchlorate, which can lead to hypothyroidism or neurodevelopment problems. (Kirk 2006; Klaassen *et al*. 2003; Landrigan *et al.* 2002; Robson 2007) That is why it is important that breast milk is monitored for environmental contaminants. As explained by LaKind et al. (2002), it is important that research on environmental contaminants is done so researchers, those who provide healthcare, and parents can better understand the health status of infants who are breast fed compared to infants who are provided other forms of nourishment and to gain a better understanding of environmental contaminant concentrations and exposures in human breast milk and other sources of infant nourishment.

Recommendations for Future Research

1. Drinking water is not the only source of perchlorate. As previously mentioned, perchlorate has been detected in ground water and irrigation water and there is concern that fruits and vegetables irrigated with perchlorate-contaminated water may pose a public health risk. Perchlorate is highly soluble and mobile allowing it to move with water within plants and eventually begins to accumulate within the leaves. (Gu 2006) To determine perchlorate levels in food produced in New Jersey food specimens from agricultural areas should be collected with over-sampling of those areas thought to have a high perchlorate exposure based on prior evidence. Information on the type of water supply for each location should be obtained as well.

As with the US FDA (2007b) study, samples were selected based on high water content, high consumption, and if the plants were known to be irrigated with perchlorate-contaminated water or grown in soil containing perchlorate.

2. Collection and processing of food consumed 24-hours prior to sample collection may be useful in obtaining information on perchlorate levels from other sources among the subjects.
3. Recall bias may have been an issue with the 24-hour dietary recall; therefore, a journal to record food intake may be useful in future studies.
4. Estimating daily perchlorate dose from spot urine samples can be imprecise due to the variability in perchlorate exposure. If possible, 24-hour urine samples should be considered.
5. Besides breast feeding, some mothers also use formula. More detailed information on what powdered formula is mixed with should be obtained along with samples of infant formula. It has been shown that powdered infant formula is also contaminated with perchlorate and may be causing the infant to ingest perchlorate at levels above the RfD. Schier et al. (2009) found that perchlorate was a contaminant of powdered infant formulas that were commercially available. Bovine milk-based with lactose, soy-based, bovine milk-based but lactose-free, and elemental powdered infant formulas were mixed with perchlorate free water and found perchlorate ranging from 0.68-5.05 µg/L, 0.10-0.44 µg/L, 0.03-0.93 µg/L, and 0.08-0.4 µ/L, respectively. When evaluating the potential risk to infants, all sources of exposure need to be taken into consideration (Sonawane 1995).

6. Only a few of the subjects smoked during sample collection and while pregnant. Surveys for future studies should also ask if the mother does not smoke if she is exposed to second hand smoke.
7. Currently there is a lack of information on the difference in perchlorate levels in the fore milk versus the hind milk. Perchlorate is non-lipophilic so breast milk samples from one entire feeding should be sampled to see if perchlorate is more concentrated in the more watery fore milk than the fatty hind milk.
8. The cumulative effects of perchlorate, thiocyanate, and nitrate as iodide uptake inhibitors needs to be further investigated.
9. Epidemiological data on exposure levels is not enough to demonstrate adverse outcomes due to perchlorate exposure in vulnerable populations. More data is needed on the effects of perchlorate on sensitive populations such as infants and on the effects of perchlorate on thyroid function in these sensitive populations.

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Appendices

Appendix A

Demographic and 24-hour Dietary Recall Questionnaire

Pilot Study of Perchlorate in Breast Milk and Drinking Water Questionnaire

Name: ____

Age: ______

Date of Birth: ____________

Race: ☐ White ☐ Black ☐ Hispanic ☐ Other _________

Education:
- ☐ No high school
- ☐ Some high school
- ☐ High school graduate
- ☐ Some college
- ☐ College graduate
- ☐ Graduate or professional degree
- ☐ Unknown

Mother's Occupation: ______________________________

Father's Occupation:
- ☐ Landscaper / gardener
- ☐ Other outdoor work (e.g. construction) ___________________
- ☐ Unemployed
- ☐ Unknown

Number of Children: ________

How often do you breast feed?

☐ Less than 25% ☐ 25% - 50% ☐ 51% -75% ☐ 75% - 99% ☐ 100%

Did you smoke during your pregnancy? ☐ Yes ☐ No

Are you currently smoking? ☐ Yes ☐ No

Do you have any current medical illnesses? ____________________________________
____

Drinking Water Survey

1. What is your primary source of drinking water at home?

    - ☐ Bottled Water
    - ☐ Well water, unfiltered
    - ☐ Well water, Filtered
    - ☐ Municipal tap water, unfiltered
    - ☐ Municipal tap water, Filtered
    - ☐ Other (please specify):______________________
    - ☐ Don't know.

2. Do you use a filter or store bought water for your drinking water?

    ☐ Yes ☐ No (if no, go to Question 6 on the next page)

3. Why do you use filtered water?

    - ☐ Worried about a chemical (please explain):________________
    - ☐ Taste
    - ☐ No specific reason
    - ☐ Other (please explain):___________________
    - ☐ Don't know

4. What type of filter do you use?

    - ☐ A water filter attached to the tap (like Brita™ water filters)
    - ☐ A pitcher type of filter
    - ☐ A filter in the refrigerator
    - ☐ Water filled from a store purifier
    - ☐ Other (please specify):______________________ (for example, bottled water)
    - ☐ Don't know

5. Do you use a filter because you think it is more pure than unfiltered water?

    ☐ Yes ☐ No ☐ Don't know

6. Do you spend a lot of time away from home (e.g., have a job away from home)?

    ☐ Yes ☐ No

7. How much water do you drink away from home?

    ☐ Less than 25% ☐ 25% - 50% ☐ 51% -75% ☐ 75% - 99% ☐ 100%

8. What is the primary source of your drinking water away from home?

    - ☐ Bottled Water
    - ☐ Well water, unfiltered
    - ☐ Well water, Filtered
    - ☐ Municipal tap water, unfiltered
    - ☐ Municipal tap water, Filtered
    - ☐ Other (please specify):________________________
    - ☐ Don't know.

9. Do you give your baby (check all that apply)

    - ☐ Breast milk
    - ☐ Formula
    - ☐ Cow's milk
    - ☐ Juice
    - ☐ Other: ________________

10. If you feed your baby any formula, what type of water do you make the formula with?

    - ☐ Bottled Water
    - ☐ Well water, unfiltered
    - ☐ Well water, Filtered
    - ☐ Municipal tap water, unfiltered
    - ☐ Municipal tap water, Filtered
    - ☐ Other (please specify):________________________
    - ☐ Don't know.

24-Hour Dietary Recall

Have you eaten any of the items listed below in the past 24 hours? (check all that apply)

Vegetables:

- ☐ Lettuce
- ☐ Spinach
- ☐ Kale
- ☐ Broccoli
- ☐ Asparagus
- ☐ Tomatoes
- ☐ Other: ____

Fruits:

☐ Oranges
☐ Grapefruit
☐ Lemons
☐ Grapes
☐ Cantaloupe
☐ Apples
☐ Other: ____

Grains:

☐ Dark bread
☐ White bread
☐ Cooked oatmeal
☐ Rice
☐ Cereal
☐ Muffins
☐ Tortillas
☐ Cornmeal
☐ Other: _______________________________________

Foods High in Iodine:

☐ Cheese
☐ Sour Cream
☐ Eggs
☐ Beans
☐ Ham
☐ Bacon
☐ Sausage
☐ Hot dogs
☐ Butter
☐ Iodized salt
☐ Milk
☐ Vitamins containing iodine

Appendix B

Additional Data Tables and Figures

Preliminary Results

Table 1. Independent sample test

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% CI of the Difference	
	Equal Variances	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Age	Assumed	-1.57	103	0.12	-1.7	1.08	-3.84	0.45
	Not Assumed	-1.6	33.78	0.12	-1.7	1.06	-3.86	0.47
Education	Assumed	0.2	94	0.84	0.08	0.39	-0.7	0.85
	Not Assumed	0.21	36.74	0.84	0.08	0.37	-0.68	0.83
No. of Children	Assumed	-1.09	103	0.28	-0.26	0.24	-0.74	0.21
	Not Assumed	-1.1	33.06	0.28	-0.26	0.24	-0.75	0.23
Breast Feeding	Assumed	-1.76	103	0.08	-0.51	0.29	-1.08	0.07
	Not Assumed	-1.74	32.61	0.09	-0.51	0.29	-1.1	0.09
Smoke While Pregnant	Assumed	-1.01	103	0.31	-0.03	0.03	-0.1	0.03
	Not Assumed	-0.71	24.02	0.48	-0.03	0.047	-0.13	0.06
Currently Smoking	Assumed	-0.52	102	0.6	-0.02	0.04	-0.1	0.06
	Not Assumed	-0.43	27.25	0.67	-0.02	0.05	-0.12	0.08
Medical Illnesses	Assumed	-1.07	103	0.29	-0.06	0.05	-0.16	0.05
	Not Assumed	-0.83	25.07	0.42	-0.06	0.07	-0.19	0.08

Table 2. Race compared to the number of samples sets provided (chi-square)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.70[a]	3	0.44
Likelihood Ratio	2.48	3	0.48
Linear-by-Linear Association	0.08	1	0.78
N of Valid Cases	105		

[a] 6 cells (75.0%) have expected count < 5. The minimum expected count is 0.21.

Table 3. Mother's occupation compared to the number of samples sets provided (chi-square)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.88[a]	2	0.39
Likelihood Ratio	1.81	2	0.40
Linear-by-Linear Association	1.16	1	0.28
N of Valid Cases	40		

[a] 3 cells (50.0%) have expected count < 5. The minimum expected count is 2.50.

Table 4. Father's occupation compared to the number of samples sets provided (chi-square)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.36[a]	2	0.19
Likelihood Ratio	4.17	2	0.13
Linear-by-Linear Association	0.40	1	0.53
N of Valid Cases	65		

[a] 2 cells (33.3%) have expected count < 5. The minimum expected count is 0.80.

Table 5. Mann-Whitney U test

	Race	**Mother's Occupation**	**Father's Occupation**
Mann-Whitney U	884.000	117.000	293.000
Wilcoxon W	1137.000	172.000	384.000
Z	-0.496	-1.103	-0.839
Asymp. Sig. (2-tailed)	-0.620	0.270	0.401
Exact Sig. [2*(1-tailed Sig.)]		0.315[a]	

Grouping variables: Sample collection combined
[a] Not corrected for ties.

24-Hour Dietary Recall

Table 6. Vegetable consumption during the 24-hours prior to sample collection

	Sample Collection					
	1 (*n = 106*)		2 (*n = 88*)		3 (*n = 82*)	
	N	%	n	%	n	%
Lettuce	29	28	39	37	40	38
Spinach	2	2	3	3	5	5
Kale	12	11	17	16	13	12
Broccoli	16	17	25	24	23	22
Asparagus	2	2	3	3	1	1
Tomatoes	74	70	72	68	64	60
Greens	39	37	39	37	37	35
Carrots	41	39	41	39	35	33
Other Vegetables	33	31	39	37	27	26

Table 7. Fruit consumption during the 24-hours prior to sample collection

	Sample Collection					
	1 (*n = 106*)		2 (*n = 88*)		3 (*n = 82*)	
	n	%	n	%	N	%
Orange	43	41	48	45	40	38
Grapefruit	3	3	1	1	4	4
Lemon	19	18	29	28	32	30
Grapes	43	41	31	30	30	28
Cantaloupe	24	23	25	24	18	17
Apple	51	48	44	42	40	38
Other Fruit	46	43	44	42	36	34

Table 8. Whole grain consumption during the 24-hours prior to sample collection

	Sample Collection					
	1 (*n = 106*)		2 (*n = 88*)		3 (*n = 82*)	
	n	%	n	%	n	%
Whole grain wheat bread	42	40	32	30	31	30
Cooked oatmeal	25	24	19	18	17	16
Brown rice (not white)	21	20	18	17	19	18
Whole grain cereal	33	31	30	28	26	25
Whole grain muffins	5	5	3	3	4	4
Whole grain tortillas	27	26	24	23	24	23
Whole grain cornmeal	4	4	7	7	8	8
Other grains	0	0	1	1	2	2

Table 9. Milk and dairy product consumption during the 24-hours prior to sample collection

	Sample Collection					
	1 (*n = 106*)		2 (*n = 88*)		3 (*n = 82*)	
	n	%	N	%	n	%
Milk	94	89	80	76	79	75
Yogurt	47	44	32	30	31	29
Ice milk / frozen yogurt	32	30	32	30	27	26
Other dairy	1	1	1	1	1	1

Table 10. Consumption of foods high in iodine during the 24-hours prior to sample collection

	Sample Collection					
	1 (*n = 106*)		2 (*n = 88*)		3 (*n = 82*)	
	n	%	n	%	N	%
Cheese	74	70	64	60	64	60
Sour Cream	18	17	21	20	13	12
Eggs	61	58	55	52	64	60
Beans	50	47	51	48	54	51
Ham	26	25	20	19	28	26
Bacon	4	4	4	4	10	9
Sausage	14	13	21	20	19	18
Hot dogs	4	4	3	3	6	6
Butter	30	28	22	21	21	20
Iodized salt	86	81	75	71	72	68
Milk	92	92	78	74	77	73
Vitamins containing Iodine	39	37	38	36	34	32

Drinking Water Contaminant Levels

Table 11. Descriptive statistics of perchlorate in drinking water (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	96	81	76
	Missing	10	25	30
Mean		0.168	0.157	0.182
Median		0.147	0.140	0.168
Std. Dev.		0.135	0.105	0.152
Minimum		0.008	0.012	0.001
Maximum		0.899	0.459	1.040
Percentiles	25	0.065	0.074	0.062
	50	0.147	0.140	0.168
	75	0.225	0.209	0.235

[a] Multiple modes exist. The smallest value is shown.

Table 12. Descriptive statistics of perchlorate in drinking water (ng/mL) – *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	96	81	76
	Missing	10	25	30
Mean		-0.923	-0.917	-0.909
Median		-0.833	-0.854	-0.775
Std. Dev.		0.392	0.345	0.474
Minimum		-2.093	-1.920	-2.928
Maximum		-0.046	-0.338	0.017
Percentiles	25	-1.181	-1.130	-1.205
	50	-0.833	-0.854	-0.775
	75	-0.648	-0.680	-0.630

[a] Multiple modes exist. The smallest value is shown

Table 13. Descriptive statistics of iodide in drinking water (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	77	72	64
	Missing	29	34	42
Mean		0.362	0.417	0.426
Median		0.277	0.330	0.340
Std. Dev.		0.309	0.347	0.343
Minimum		0.009	0.021	0.008
Maximum		1.890	1.800	1.950
Percentiles	25	0.156	0.152	0.185
	50	0.278	0.330	0.340
	75	0.472	0.638	0.558

[a] Multiple modes exist. The smallest value is shown.

Table 14. Descriptive statistics of iodide in drinking water (ng/mL) – *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	77	72	64
	Missing	29	34	42
Mean		-0.609	-0.550	-0.536
Median		-0.558	-0.482	-0.469
Std. Dev.		0.441	0.432	0.443
Minimum		-2.066	-1.680	-2.12
Maximum		0.276	0.255	0.290
Percentiles	25	-0.809	-0.819	-0.732
	50	-0.558	-0.482	-0.469
	75	-0.327	-0.196	-0.254

[a] Multiple modes exist. The smallest value is shown.

Table 15. Descriptive statistics of nitrate in drinking water (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	84	75
	Missing	2	22	31
Mean		3273	3187	3191
Median		3220	2925	2870
Std. Dev.		2116	1639	1647
Minimum		57	424	433
Maximum		14600	10600	9900
Percentiles	25	2363	2235	2410
	50	3220	2925	2870
	75	3855	3535	3780

[a] Multiple modes exist. The smallest value is shown.

Table 16. Descriptive statistics of nitrate in drinking water (ng/mL) – *log*10

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	104	84	75
	Missing	2	22	31
Mean		3.397	3.455	3.453
Median		3.508	3.466	3.458
Std. Dev.		0.411	0.212	0.222
Minimum		1.760	2.630	2.640
Maximum		4.160	4.030	4.000
Percentiles	25	3.373	3.349	3.382
	50	3.508	3.466	3.458
	75	3.586	3.548	3.578

[a] Multiple modes exist. The smallest value is shown.

Table 17. Descriptive statistics of nitrate in drinking water with imputed values (ng/mL)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	106	86	79
	Missing	0	20	27
Mean		3223	3117	3039
Median		3180	2905	2840
Std. Dev.		2126	1683	1735
Minimum		350	350	350
Maximum		14600	10600	9900
Percentiles	25	2303	2178	2300
	50	3180	2905	2840
	75	3825	3525	3740

[a] Multiple modes exist. The smallest value is shown.

Table 18. Descriptive statistics of nitrate in drinking water with imputed values (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	86	79
	Missing	0	20	27
Mean		3.400	3.430	3.399
Median		3.502	3.463	3.453
Std. Dev.		0.356	0.263	0.315
Minimum		2.540	2.540	2.540
Maximum		4.160	4.030	4.000
Percentiles	25	3.362	3.338	3.362
	50	3.502	3.463	3.453
	75	3.583	3.547	3.573

Breast Milk Contaminant Levels

Table 19. Descriptive statistics of perchlorate in breast milk (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	87	83
	Missing	0	19	23
Mean		6.272	8.149	6.065
Median		4.350	4.840	4.190
Std. Dev.		6.978	12.469	5.373
Minimum		0.420	0.300	0.320
Maximum		46.400	99.500	29.700
Percentiles	25	2.510	2.510	2.730
	50	4.350	4.840	4.190
	75	8.008	9.640	7.480

[a] Multiple modes exist. The smallest value is shown

Table 20. Descriptive statistics of perchlorate in breast milk (ng/mL) – *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	87	83
	Missing	0	19	23
Mean		0.632	0.693	0.628
Median		0.638	0.685	0.622
Std. Dev.		0.380	0.416	0.396
Minimum		-0.376	-0.523	-0.491
Maximum		1.667	1.998	1.473
Percentiles	25	0.400	0.3400	0.436
	50	0.638	0.685	0.622
	75	0.903	0.984	0.874

[a] Multiple modes exist. The smallest value is shown

Table 21. Regression analysis of perchlorate in breast milk using dummy variables

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
	B	Std. Error	Beta	Zero-order	Partial	B	Std. Error
(Constant)	0.301	0.718		0.42	0.677		
Age	0	0.011	-0.005	-0.034	0.973	0.373	2.682
Education	-0.065	0.034	-0.316	-1.899	0.064	0.315	3.17
Number of Children	0.029	0.05	0.087	0.574	0.569	0.38	2.635
Breast Feeding	0.039	0.037	0.145	1.048	0.301	0.454	2.202
Smoke	0.001	0.25	0.001	0.006	0.995	0.405	2.467
Medical Illnesses	0.183	0.209	0.119	0.876	0.386	0.471	2.122
Latin	-0.029	0.191	-0.022	-0.152	0.88	0.409	2.443
Lettuce	-0.082	0.129	-0.091	-0.635	0.528	0.427	2.342
Spinach	0.223	0.33	0.098	0.676	0.502	0.413	2.42
Kale	0.053	0.198	0.049	0.265	0.792	0.257	3.896
Broccoli	-0.082	0.153	-0.081	-0.538	0.593	0.386	2.593
Asparagus	-0.026	0.365	-0.01	-0.072	0.943	0.501	1.995
Tomato	-0.098	0.138	-0.112	-0.712	0.481	0.351	2.845
Greens	0.307	0.329	0.365	0.933	0.356	0.057	17.548
Carrots	-0.133	0.119	-0.164	-1.122	0.268	0.409	2.445
Other vegetables	0.434	0.138	0.499	3.143	0.003	0.346	2.892
Oranges	0.05	0.128	0.062	0.392	0.697	0.349	2.867
Grapefruit	0.103	0.447	0.037	0.231	0.818	0.334	2.992
Lemons	0.04	0.137	0.04	0.291	0.772	0.454	2.203
Grapes	0.049	0.139	0.056	0.35	0.728	0.344	2.907
Cantaloupe	-0.049	0.153	-0.05	-0.319	0.752	0.348	2.871
Apples	0.156	0.125	0.193	1.245	0.22	0.362	2.764
Other fruit	-0.156	0.164	-0.168	-0.953	0.346	0.281	3.552
Whole wheat bread	-0.026	0.112	-0.03	-0.231	0.818	0.522	1.916
Cooked oatmeal	0.106	0.123	0.111	0.866	0.391	0.534	1.874
Brown rice	-0.143	0.175	-0.14	-0.819	0.417	0.297	3.362
Whole grain cereal	-0.118	0.172	-0.125	-0.685	0.497	0.26	3.846
Whole grain muffin	0.146	0.31	0.074	0.47	0.641	0.349	2.868
Whole grain tortillas	0.184	0.15	0.197	1.227	0.227	0.339	2.948
Whole grain cornmeal	0.1	0.276	0.06	0.363	0.718	0.325	3.08
Other grains	-0.022	0.786	-0.004	-0.028	0.978	0.503	1.987
Milk	-0.27	0.218	-0.203	-1.24	0.222	0.325	3.078
Yogurt	-0.073	0.117	-0.089	-0.624	0.536	0.425	2.35
Ice milk	-0.084	0.117	-0.099	-0.719	0.476	0.462	2.166
Other dairy	1.911	0.71	0.32	2.693	0.01	0.617	1.62
Cheese	0.001	0.162	0.001	0.007	0.995	0.31	3.222

Sour cream	-0.064	0.193	-0.066	-0.333	0.741	0.226	4.433
Eggs	-0.093	0.126	-0.11	-0.735	0.466	0.387	2.586
Beans	-0.087	0.113	-0.109	-0.775	0.443	0.441	2.269
Ham	0.114	0.147	0.114	0.774	0.443	0.4	2.503
Bacon	-0.103	0.335	-0.057	-0.308	0.76	0.255	3.927
Sausage	0.295	0.173	0.239	1.698	0.097	0.442	2.264
Hot dogs	-1.274	0.385	-0.565	-3.308	0.002	0.299	3.343
Butter	0.129	0.144	0.14	0.901	0.373	0.363	2.757
Iodized salt	0.236	0.167	0.218	1.416	0.164	0.367	2.726
Vitamins	0.031	0.111	0.041	0.285	0.777	0.429	2.331
Drinking water	0.076	0.13	0.086	0.586	0.561	0.409	2.447
S3b	-0.135	0.153	-0.17	-0.885	0.381	0.235	4.253
S2b	0.25	0.142	0.325	1.754	0.087	0.255	3.928

Table 22. Descriptive statistics of iodide in breast milk (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	87	83
	Missing	0	19	23
Mean		178.049	165.466	179.613
Median		154.000	153.000	144.000
Std. Dev.		124.226	143.230	138.330
Minimum		3.910	7.180	5.810
Maximum		575.000	918.000	826.000
Percentiles	25	91.575	84.100	81.100
	50	154.000	153.000	144.000
	75	238.000	266.000	251.000

a Multiple modes exist. The smallest value is shown

Table 23. Descriptive statistics of iodide in breast milk (ng/mL) – *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	87	83
	Missing	0	19	23
Mean		2.129	2.140	2.116
Median		2.188	2.185	2.158
Std. Dev.		0.370	0.367	0.384
Minimum		0.592	0.856	0.764
Maximum		2.760	2.963	2.917
Percentiles	25	1.962	1.925	1.909
	50	2.188	2.185	2.158
	75	2.377	2.425	2.400

a Multiple modes exist. The smallest value is shown

Table 24. Descriptive statistics of thiocyanate in breast milk (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	87	83
	Missing	0	19	23
Mean		471.1	409.6	381.0
Median		295.5	275.0	244.0
Std. Dev.		482.4	490.3	450.3
Minimum		5.3	17.4	37.7
Maximum		2670.0	2990.0	2370.0
Percentiles	25	156.8	129.0	132.0
	50	2295.5	275.0	244.0
	75	639.8	499.0	453.0

a Multiple modes exist. The smallest value is shown

Table 25. Descriptive statistics of thiocyanate in breast milk (ng/mL) – *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	87	83
	Missing	0	19	23
Mean		2.461	2.400	2.403
Median		2.471	2.439	2.387
Std. Dev.		0.470	0.451	0.377
Minimum		0.726	1.241	1.576
Maximum		3.427	3.476	3.375
Percentiles	25	2.195	2.111	2.121
	50	2.471	2.439	2.387
	75	2.806	2.698	2.656

a Multiple modes exist. The smallest value is shown

Table 26. Descriptive statistics of nitrate in breast milk (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	105	87	83
	Missing	1	19	23
Mean		2781.4	2335.2	2142.0
Median		2470.0	1990.0	1840.0
Std. Dev.		1590.8	1087.1	1112.2
Minimum		714.0	806.0	100.0
Maximum		10700	5760.0	6020.0
Percentiles	25	1645.0	1600.0	1380.0
	50	2470.0	1990.0	1840.0
	75	3590.0	2770.0	2770.0

[a] Multiple modes exist. The smallest value is shown

Table 27. Descriptive statistics of nitrate in breast milk (ng/mL) – *log*10

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	105	87	83
	Missing	1	19	23
Mean		3.382	3.325	3.265
Median		3.393	3.299	3.265
Std. Dev.		0.232	0.193	0.276
Minimum		2.854	2.906	2.000
Maximum		4.029	3.760	3.780
Percentiles	25	3.216	3.204	3.140
	50	3.393	3.299	3.265
	75	3.555	3.442	3.435

[a] Multiple modes exist. The smallest value is shown

Urine Contaminant Levels

Table 28. Descriptive statistics of perchlorate in urine (ng/mL)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	104	87	82
	Missing	2	19	24
Mean		3.117	3.286	3.174
Median		2.055	1.960	2.355
Std. Dev.		3.597	4.101	3.192
Minimum		0.285	0.196	0.178
Maximum		27.300	28.300	22.700
Percentiles	25	1.185	0.944	1.328
	50	2.055	1.960	2.355
	75	3.868	3.570	3.893

[a] Multiple modes exist. The smallest value is shown

Table 29. Descriptive statistics of perchlorate in urine (ng/mL) – *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	82
	Missing	2	19	24
Mean		0.320	0.297	0.354
Median		0.313	0.292	0.372
Std. Dev.		0.382	0.430	0.361
Minimum		-0.545	-0.708	-0.750
Maximum		1.436	1.452	1.356
Percentiles	25	0.073	-0.025	0.123
	50	0.313	0.292	0.372
	75	0.587	0.553	0.589

[a] Multiple modes exist. The smallest value is shown

Table 30. Descriptive statistics of iodide in urine (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	82
	Missing	2	19	24
Mean		143.62	141.95	156.39
Median		138.00	107.00	122.50
Std. Dev.		88.02	109.60	112.28
Minimum		19.40	2.20	16.70
Maximum		464.00	502.00	603.00
Percentiles	25	75.55	61.30	88.45
	50	138.00	107.00	122.50
	75	190.50	186.00	209.00

[a] Multiple modes exist. The smallest value is shown

Table 31. Descriptive statistics of iodide in urine (ng/mL) – *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	82
	Missing	2	19	24
Mean		2.071	2.017	2.090
Median		2.140	2.029	2.088
Std. Dev.		0.289	0.383	0.313
Minimum		1.288	0.342	1.223
Maximum		2.667	2.701	2.780
Percentiles	25	1.878	1.787	1.945
	50	2.140	2.029	2.088
	75	2.280	2.270	2.320

[a] Multiple modes exist. The smallest value is shown

Table 32. Descriptive statistics of thiocyanate in urine (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	82
	Missing	2	19	24
Mean		896.8	847.4	953.5
Median		614.0	591.0	594.0
Std. Dev.		1239.3	900.9	1247.7
Minimum		105.0	34.0	109.0
Maximum		10400.0	4810.0	9210.0
Percentiles	25	330.0	316.0	391.5
	50	614.0	591.0	594.0
	75	1025.0	977.0	1100.0

a Multiple modes exist. The smallest value is shown

Table 33. Descriptive statistics of thiocyanate in urine (ng/mL) - *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	82
	Missing	2	19	24
Mean		2.786	2.759	2.801
Median		2.788	2.772	2.774
Std. Dev.		0.349	0.388	0.367
Minimum		2.021	1.529	2.037
Maximum		4.017	3.682	3.964
Percentiles	25	2.518	2.500	2.593
	50	2.788	2.772	2.774
	75	3.011	2.990	3.041

a Multiple modes exist. The smallest value is shown

Table 34. Descriptive statistics of nitrate in urine (ng/mL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	82
	Missing	2	19	24
Mean		46692	48697	53955
Median		39400	38600	41775
Std. Dev.		29143	42561	57931
Minimum		9410	2850	6190
Maximum		157000	270000	382500
Percentiles	25	25613	26900	26162
	50	39400	38600	41775
	75	61100	57400	61700

a Multiple modes exist. The smallest value is shown

Table 35. Descriptive statistics of nitrate in urine (ng/mL) – *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	82
	Missing	2	19	24
Mean		4.593	4.558	4.596
Median		4.595	4.587	4.621
Std. Dev.		0.261	0.357	0.334
Minimum		3.974	3.485	3.792
Maximum		5.196	5.431	5.583
Percentiles	25	4.408	4.430	4.418
	50	4.595	4.587	4.621
	75	4.786	4.759	4.790

a Multiple modes exist. The smallest value is shown

Urine Creatinine Levels

Table 36. Descriptive statistics of urine creatinine (mg/dL)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	81
	Missing	2	19	25
Mean		114.77	113.93	118.69
Median		107.15	113.60	106.72
Std. Dev.		55.56	56.89	72.05
Minimum		24.20	0.30	20.00
Maximum		334.00	236.10	358.20
Percentiles	25	75.20	73.70	62.16
	50	107.15	113.60	106.72
	75	136.78	153.52	160.01

a Multiple modes exist. The smallest value is shown
Note: One subject provided urine for perchlorate analyses but not creatinine.

Table 37. Descriptive statistics of urine creatinine (mg/dL) – *log*10

		Sample Set 1	**Sample Set 3**
N	Valid	104	81
	Missing	2	25
Mean		2.010	1.990
Median		2.030	2.028
Std. Dev.		0.215	0.289
Minimum		1.384	1.302
Maximum		2.524	2.554
Percentiles	25	1.876	1.793
	50	2.030	2.028
	75	2.136	2.204

a Multiple modes exist. The smallest value is shown

Table 38. Descriptive statistics of urine creatinine (mg/dL), excluding values <30 mg/dL and >300 mg/dL

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	100	79	73
	Missing	6	27	33
Mean		115.24	123.57	116.03
Median		107.90	119.60	107.80
Std. Dev.		49.87	50.36	54.73
Minimum		30.70	38.67	30.69
Maximum		282.50	236.10	241.70
Percentiles	25	76.01	88.13	73.30
	50	107.90	119.60	107.80
	75	136.78	159.55	156.21

a Multiple modes exist. The smallest value is shown
Note: One subject provided urine for perchlorate analyses but not creatinine.

Table 39. Descriptive statistics of urine creatinine (mg/dL), excluding values <30 mg/dL and >300 mg/dL – after *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	100	79	74
	Missing	6	27	32
Mean		2.023	2.052	2.001
Median		2.033	2.078	2.030
Std. Dev.		0.186	0.195	0.239
Minimum		1.487	1.587	1.318
Maximum		2.451	2.373	2.383
Percentiles	25	1.881	1.945	1.822
	50	2.033	2.078	2.030
	75	2.136	2.203	2.191

a Multiple modes exist. The smallest value is shown
Note: One subject provided urine for perchlorate analyses but not creatinine.

Creatinine Corrected Levels

Table 40. Descriptive statistics for perchlorate corrected for creatinine (µg perchlorate/ g creatinine)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	81
	Missing	2	19	25
Mean		3.043	4.533	3.019
Median		2.306	2.029	2.248
Std. Dev.		3.338	11.178	2.432
Minimum		0.151	0.162	0.309
Maximum		25.926	93.333	11.400
Percentiles	25	1.098	1.146	1.432
	50	2.306	2.029	2.248
	75	3.678	3.520	3.738

a Multiple modes exist. The smallest value is shown

Table 41. Descriptive statistics perchlorate corrected for creatinine (µg perchlorate/ g creatinine) – *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	81
	Missing	2	19	25
Mean		0.310	0.335	0.356
Median		0.363	0.307	0.352
Std. Dev.		0.396	0.448	0.340
Minimum		-0.821	-0.790	-0.510
Maximum		1.414	1.970	1.057
Percentiles	25	0.041	0.059	0.156
	50	0.363	0.307	0.352
	75	0.566	0.547	0.573

a Multiple modes exist. The smallest value is shown

Table 42. Descriptive statistics for iodide corrected for creatinine (µg iodide/ g creatinine)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	81
	Missing	2	19	25
Mean		137.82	139.90	145.01
Median		123.10	120.12	130.73
Std. Dev.		82.09	100.91	79.75
Minimum		22.61	22.11	23.40
Maximum		414.33	7333.33	492.91
Percentiles	25	72.25	81.19	84.20
	50	123.10	120.12	130.73
	75	175.95	173.97	188.80

a Multiple modes exist. The smallest value is shown

Table 43. Descriptive statistics iodide corrected for creatinine (µg iodide/ g creatinine) – *log*10

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	104	87	81
	Missing	2	19	25
Mean		2.061	2.055	2.094
Median		2.090	2.080	2.116
Std. Dev.		0.273	0.288	0.255
Minimum		1.354	1.345	1.369
Maximum		2.617	2.865	2.693
Percentiles	25	1.859	1.910	1.925
	50	2.090	2.080	2.116
	75	2.245	2.240	2.276

a Multiple modes exist. The smallest value is shown

Table 44. Descriptive statistics for nitrate corrected for creatinine (µg nitrate/ g creatinine)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	104	87	81
	Missing	2	19	25
Mean		43520	54321	48909
Median		34595	33997	38416
Std. Dev.		26903	101487	36033
Minimum		15694	18284	6825
Maximum		177803	950000	226684
Percentiles	25	26309	28319	28901
	50	34595	33997	38416
	75	49548	50073	54355

a Multiple modes exist. The smallest value is shown

Table 45. Descriptive statistics for nitrate corrected for creatinine (µg nitrate/ g creatinine) – *log*10

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	104	87	81
	Missing	2	19	25
Mean		4.583	4.596	4.617
Median		4.539	4.531	4.585
Std. Dev.		0.207	0.261	0.235
Minimum		4.196	4.262	3.834
Maximum		5.250	5.978	5.355
Percentiles	25	4.420	4.452	4.461
	50	4.539	4.531	4.585
	75	4.695	4.700	4.735

a Multiple modes exist. The smallest value is shown

Table 46. Descriptive statistics for thiocyanate corrected for creatinine (µg thiocyanate/ g creatinine)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	104	87	81
	Missing	2	19	25
Mean		786.76	1010.86	898.40
Median		594.85	673.50	628.40
Std. Dev.		697.04	1531.87	812.90
Minimum		88.00	57.10	46.50
Maximum		5479.50	11266.70	4969.80
Percentiles	25	398.43	332.70	398.25
	50	594.85	673.50	628.40
	75	960.10	1086.30	1161.90

a Multiple modes exist. The smallest value is shown

Table 47. Descriptive statistics for thiocyanate corrected for creatinine (µg thiocyanate/ g creatinine) – *log*10

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	104	87	81
	Missing	2	19	25
Mean		2.776	2.797	2.814
Median		2.774	2.828	2.798
Std. Dev.		0.326	0.400	0.356
Minimum		1.944	1.757	1.667
Maximum		3.739	4.052	3.696
Percentiles	25	2.600	2.522	2.600
	50	2.774	2.828	2.798
	75	2.982	3.036	3.065

a Multiple modes exist. The smallest value is shown

Infant's Intake through breast Milk

Table 48. Infant's intake of perchlorate through breast milk (µg/day)

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	87	83
	Missing	0	19	23
Mean		4.578	5.949	4.478
Median		3.175	3.530	3.060
Std. Dev.		5.094	9.103	3.923
Minimum		0.310	0.220	0.240
Maximum		33.87	72.640	21.68
Percentiles	25	1.833	1.830	1.990
	50	3.175	3.530	3.060
	75	5.848	7.040	5.460

a Multiple modes exist. The smallest value is shown

Table 49. Infant's intake of iodide through breast milk (µg/day)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	106	87	83
	Missing	0	19	23
Mean		129.676	135.391	131.117
Median		112.420	111.690	105.120
Std. Dev.		90.685	104.558	100.981
Minimum		2.850	5.240	4.240
Maximum		419.750	670.140	602.980
Percentiles	25	66.853	61.390	59.200
	50	112.420	111.690	105.120
	75	173.740	194.180	183.230

a Multiple modes exist. The smallest value is shown

Table 50. Infant's intake of nitrate through breast milk (µg/day)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	105	87	83
	Missing	1	19	23
Mean		2030.422	1704.718	1563.686
Median		1803.100	1452.700	1343.200
Std. Dev.		1161.253	793.581	811.888
Minimum		521.220	588.380	73.000
Maximum		7811.000	4204.800	4394.600
Percentiles	25	1200.850	1168.000	1007.400
	50	1803.100	1452.700	1343.200
	75	2620.700	2022.100	1985.600

a Multiple modes exist. The smallest value is shown

Table 51. Infant's intake of thiocyanate through breast milk (µg/day)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	106	87	83
	Missing	0	19	23
Mean		343.868	299.022	278.121
Median		215.715	200.750	178.120
Std. Dev.		352.128	357.902	328.728
Minimum		3.880	12.700	27.520
Maximum		1949.100	2182.700	1730.100
Percentiles	25	114.428	94.170	96.360
	50	215.715	200.750	178.120
	75	467.018	364.270	330.270

a Multiple modes exist. The smallest value is shown

Pearson Product-Moment Correlation: Breast Milk and Urine Perchlorate and 24-hour Dietary Recall

Table 52. Mother's estimated dose of perchlorate through drinking water (mg/kg/day)

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	96	81	76
	Missing	10	25	30
Mean		5.190×10^{-6}	4.891×10^{-6}	5.664×10^{-6}
Median		4.570×10^{-6}	4.356×10^{-6}	5.227×10^{-6}
Std. Dev.		4.216×10^{-6}	3.272×10^{-6}	4.729×10^{-6}
Minimum		2.511×10^{-7}	3.702×10^{-7}	3.671×10^{-8}
Maximum		2.797×10^{-5}	1.428×10^{-5}	3.236×10^{-5}
Percentiles	25	2.050×10^{-6}	2.307×10^{-6}	1.943×10^{-6}
	50	4.570×10^{-6}	4.356×10^{-6}	5.227×10^{-6}
	75	6.990×10^{-6}	6.502×10^{-6}	7.296×10^{-6}

a Multiple modes exist. The smallest value is shown

Table 53. Infant's estimated dose of perchlorate through breast milk (mg/kg/day) – 0 to 1 month old

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	106	87	83
	Missing	0	19	23
Mean		9.539×10^{-4}	1.239×10^{-3}	9.224×10^{-4}
Median		6.616×10^{-4}	7.361×10^{-4}	6.372×10^{-4}
Std. Dev.		1.011×10^{-3}	1.896×10^{-3}	8.171×10^{-4}
Minimum		6.403×10^{-5}	4.563×10^{-5}	4.192×10^{-5}
Maximum		7.057×10^{-3}	1.513×10^{-2}	4.517×10^{-3}
Percentiles	25	3.8181×10^{-4}	3.817×10^{-4}	4.152×10^{-4}
	50	6.616×10^{-4}	7.361×10^{-4}	6.372×10^{-4}
	75	1.218×10^{-3}	1.466×10^{-3}	1.138×10^{-3}

a Multiple modes exist. The smallest value is shown

Table 54. Infant's estimated dose of perchlorate through breast milk (mg/kg/day) – 1 to 3 months old

		Sample Set 1	Sample Set 2	Sample Set 3
N	Valid	106	87	83
	Missing	0	19	23
Mean		8.176×10^{-4}	1.062×10^{-3}	7.906×10^{-4}
Median		5.671×10^{-4}	6.309×10^{-4}	5.462×10^{-4}
Std. Dev.		9.097×10^{-4}	1.625×10^{-3}	7.004×10^{-4}
Minimum		5.488×10^{-6}	3.911×10^{-5}	4.211×10^{-5}
Maximum		6.049×10^{-3}	1.297×10^{-2}	3.872×10^{-3}
Percentiles	25	3.272×10^{-4}	3.272×10^{-4}	3.559×10^{-4}
	50	5.671×10^{-4}	6.309×10^{-4}	5.462×10^{-4}
	75	1.044×10^{-3}	1.257×10^{-3}	9.751×10^{-4}

a Multiple modes exist. The smallest value is shown

Table 55. Infant's estimated dose of perchlorate through breast milk (mg/kg/day) – 3 to 6 month old

		Sample Set 1	**Sample Set 2**	**Sample Set 3**
N	Valid	106	87	83
	Missing	0	19	23
Mean		6.187×10^{-4}	8.039×10^{-4}	5.983×10^{-4}
Median		4.291×10^{-4}	4.775×10^{-4}	4.133×10^{-4}
Std. Dev.		6.884×10^{-4}	1.230×10^{-3}	5.300×10^{-4}
Minimum		4.153×10^{-5}	2.295×10^{-5}	3.186×10^{-5}
Maximum		4.577×10^{-3}	9.816×10^{-3}	2.930×10^{-3}
Percentiles	25	2.476×10^{-4}	2.476×10^{-4}	2.693×10^{-4}
	50	4.291×10^{-4}	4.775×10^{-4}	4.133×10^{-4}
	75	7.899×10^{-4}	9.510×10^{-4}	7.379×10^{-4}

a Multiple modes exist. The smallest value is shown

Table 56. Regression analyses, thiocyanate sample set 1 (estimated beta values)

Variables	Demographics R = 0.455	Diet R = 0.602	Stepwise*		
			Run 1	Run 2	Run 3
Age	-0.120				
Latin	-0.034				
Education	0.185				
Number of Children	0.227				
Breast feeding	-0.090				
Smoke	-0.305		-0.352	-0.387	-0.383
Medical Illnesses	-0.098				
Lettuce		-0.114			-0.125
Spinach		0.032			
Asparagus		-0.178		-0.168	-0.178
Other vegetables		0.113		0.155	0.147
Oranges		-0.224			
Grapefruit		0.053			
Lemons		-0.030			
Cheese		-0.160		-0.165	-0.160
Other dairy		-0.114			
Iodized salt		-0.070			
Brown rice		0.199		0.193	0.201
Whole grain tortillas		-0.196	-0.197	-0.229	-0.246
Vitamins		-0.389	-0.303	-0.299	-0.298

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Table 57. Regression analyses, thiocyanate sample set 2 (estimated beta values)

Variables	Demographics R = 0.321	Diet R = 0.659	Stepwise*		
			Run 1	Run 2	Run 3
Age	-0.108				0.104
Latin	0.005				-2.10
Education	0.162				0.513
Number of Children	0.036				-0.677
Breast feeding	-0.048				0.413
Smoke	-0.269			-0.310	-1.52
Medical Illnesses	-0.098				2.72
Lettuce		0.188			0.475
Spinach		-0.069			0.140
Greens		-0.132			-0.472
Kale		0.162			-0.074
Asparagus		-0.216			-0.222
Tomatoes		-0.214			0.189
Carrots		-0.171			0.352
Broccoli		0.098			0.109
Other vegetables		0.125			0.429
Oranges		-0.265			1.25
Grapefruit		0.071			-0.163
Lemons		-0.056			-0.094
Apples		-0.316	-0.297	-0.203	-0.121
Cantaloupe		-0.080			-0.856
Grapes		-0.247			-1.05
Other fruits		-0.010			0.285
Milk		-0.146			-1.81
Yogurt		0.150			0.472
Ice milk/frozen yogurt		0.010			-0.345
Cheese		-0.084		-0.225	0.410
Sour cream		0.031			0.572
Other dairy		-0.037			0.146
Iodized salt		0.034			1.63
Butter		0.127			0.031
Bacon		-0.266		-0.199	-1.26
Sausage		0.233			-0.160
Hot dogs		0.225			-1.27
Ham		0.002			-0.464
Eggs		-0.060			-0.238
Beans		0.275			-0.029
Brown rice		0.119			0.785
Whole wheat bread		0.010			0.311
Whole grain tortillas		-0.017			0.022
Cooked Oatmeal		0.135			0.176

Whole grain muffins	0.014	0.228
Whole grain cornmeal	-0.115	-0.139
Whole grain cereal	0.022	0.034
Vitamins	0.081	-0.706

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Table 58. Regression analyses, thiocyanate sample set 3 (estimated beta values)

Variables	Demographics R = 0.562	Diet R = 0.602	Stepwise*		
			Run 1	Run 2	Run 3
Age	-0.085				
Latin	0.048				
Education	0.342		0.365	0.325	0.361
Number of Children	0.082				
Breast feeding	0.005				
Smoke	-0.478		-0.464	-0.437	-0.476
Medical Illnesses	0.236		0.303	0.280	0.300
Lettuce		-0.114	-0.243	-0.297	-0.305
Spinach		0.032			
Asparagus		-0.178			
Carrots				0.163	0.144
Other vegetables		0.113			
Oranges		-0.224			0.131
Grapefruit		0.053		0.204	0.210
Lemons		-0.030			
Grapes				0.187	0.178
Yogurt			-0.372	-0.389	-0.423
Cheese		-0.160			
Other dairy		-0.114	-0.206	-0.175	
Iodized salt		-0.070			
Hot dogs			0.228	0.242	0.227
Eggs				-0.203	-0.215
Brown rice		0.199			
Whole wheat bread			0.193	0.178	0.175
Whole grain tortillas		-0.196			
Whole grain cornmeal				-0.262	-0.289
Other grains				0.138	0.141
Vitamins		-0.389			

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Table 59. Regression analyses, nitrate sample set 1 (estimated beta values)

Variables	Demographics R = 0.186	Diet R = 0.631	Drinking Water R = 0.085	Stepwise*		
				Run 1	Run 2	Run 3
Age	-0.087					
Latin	0.042					
Education	0.081					
Number of Children	0.097					
Breast Feeding	0.063					
Smoke	-0.036					
Medical Illnesses	-0.112					
Lettuce		0.101				
Spinach		0.093				
Carrots		0.171			0.198	0.167
Asparagus		-0.269		-0.257	-0.292	-0.27
Other vegetables		0.169				0.168
Oranges		0.038				
Grapefruit		0.116				
Lemons		-0.013				
Iodized salt		0.01				
Brown rice		0.181				0.18
Whole grain tortillas		-0.213				-0.293
Ham		-0.184				-0.14
Vitamins		0.059				-0.196
Drinking Water			-0.085			

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Table 60. Regression analyses, nitrate sample set 2 (estimated beta values)

Variables	Demographics R = 0.317	Diet R = 0.706	Drinking Water R = 0.093	Stepwise*		
				Run 1	Run 2	Run 3
Age	-0.059					
Latin	0.162				0.201	0.256
Education	-0.09					
Number of	0.154					
Breast Feeding	0.086					
Smoke	0.16					
Medical Illnesses	-0.134					
Lettuce		0.385				0.201
Spinach		-0.074				
Carrots		0.009				
Asparagus		-0.188				
Tomatoes		-0.219				-0.182
Other vegetables		0.098				
Oranges		0.012				
Grapefruit		0.041				
Lemons		-0.035				
Yogurt		0.4				0.213
Milk		-0.113				-0.226
Brown rice		0.181				
Whole grain		-0.213		0.23	0.18	0.185
Cheese		-0.05				-0.133
Sour cream		0.343			0.269	0.304
Butter		-0.001			-0.196	-0.143
Ham		-0.229				
Hot dogs		-0.2				-0.21
Iodized salt		0.105				
Vitamins		-0.433			-0.229	-0.254
Drinking Water			0.093			

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Table 61. Regression analyses, nitrate sample set 3 (estimated beta values)

Variables	Demographics R = 0.371	Diet R = 0.736	Drinking Water R = 0.070	Stepwise*		
				Run 1	Run 2	Run 3
Age	-0.037					
Latin	0.183					
Education	0.025					
Number of Children	0.048					
Breast Feeding	0.119					
Smoke	-0.367				-0.241	-0.265
Medical Illnesses	0.009					
Lettuce		-0.14				-0.15
Spinach		-0.393		-0.245	-0.273	-0.297
Other vegetables		-0.153				
Oranges		0.149				
Grapefruit		0.052				
Lemons		0.225		0.228	0.231	0.257
Ice milk/frozen yogurt		0.157			0.187	0.202
Whole grain tortillas		-0.197			-0.204	-0.221
Whole grain muffins		0.402		0.292	0.175	0.18
Iodized salt		-0.133				
Hot dogs		0.298		0.269	0.197	0.203
Vitamins		-0.15			-0.227	-0.227
Drinking Water			-0.07			

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Table 62. Regression analyses, nitrate imputed sample set 1 (estimated beta values)

Variables	Demographics R = 0.186	Diet R = 0.631	Drinking Water R = 0.040	Stepwise*		
				Run 1	Run 2	Run 3
Age	-0.087					
Latin	0.042					
Education	0.091					
Number of Children	0.097					
Breast Feeding	0.063					
Smoke	-0.036					
Medical Illnesses	-0.112					
Lettuce		0.101				
Spinach		0.093				
Asparagus		-0.269		-0.292	-0.292	-0.27
Carrots		0.171		0.198	0.198	0.167
Other vegetables		0.169				0.168
Oranges		0.038				
Grapefruit		0.116				
Lemons		-0.013				
Ice milk/frozen yogurt		-0.127				
Whole grain tortillas		-0.213				-0.293
Brown rice		0.181				0.18
Iodized salt		0.01				
Ham		-0.184				-0.14
Vitamins		0.059				-0.196
Drinking Water			0.04			

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Table 63. Regression analyses, nitrate imputed sample set 2 (estimated beta values)

Variables	Demographics R = 0.317	Diet R = 0.706	Drinking Water R = 0.092	Stepwise*		
				Run 1	Run 2	Run 3
Age	-0.059					
Latin	0.162				0.201	0.242
Education	-0.09					
Number of Children	0.154					
Breast Feeding	0.086					
Smoke	0.16					
Medical Illnesses	-0.134					
Lettuce		0.385				0.35
Spinach		-0.074				
Asparagus		-0.188				-0.248
Tomatoes		-0.219				-0.264
Other vegetables		0.098				
Oranges		0.012				
Grapefruit		0.041				
Lemons		-0.035				
Milk		-0.113				-0.228
Yogurt		0.4				0.306
Whole grain muffins		0.2		0.23	0.18	0.199
Iodized salt		0.105				
Butter		-0.001			-0.196	
Sour cream		0.343			0.269	0.33
Hot dogs		-0.2				-0.148
Ham		-0.229				-0.24
Bacon		-0.241				-0.19
Vitamins		-0.433			-0.229	-0.357
Drinking Water			0.092			

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Table 64. Regression analyses, nitrate imputed sample set 3 (estimated beta values)

Variables	Demographics R = 0.371	Diet R = 0.736	Drinking Water R = 0.002	Stepwise*		
				Run 1	Run 2	Run 3
Age	-0.037					
Latin	0.183					
Education	0.025					
Number of Children	0.048					
Breast Feeding	0.119					
Smoke	-0.367				-0.241	-0.263
Medical Illnesses	0.009					
Lettuce		-0.14				-0.155
Spinach		-0.393		-0.245	-0.273	-0.32
Other vegetables		-0.153				
Oranges		0.149				
Grapefruit		0.052				
Lemons		0.225		0.228	0.231	0.255
Ice milk/frozen yogurt		0.157			0.187	0.209
Whole grain muffins				0.292	0.175	0.195
Whole grain tortillas					-0.204	-0.167
Brown rice						-0.139
Iodized salt		-0.133				
Hot dogs				0.269	0.197	0.238
Vitamins		-0.15			-0.227	-0.242
Drinking Water			0.002			

*Run 1: 0.05 (enter), 0.10 (removal); Run 2: 0.10 (enter), 0.20 (removal); Run 3: 0.20 (enter); 0.30 (removal)

Figure 1. Breast Milk Perchlorate Levels, Subjects 1-25

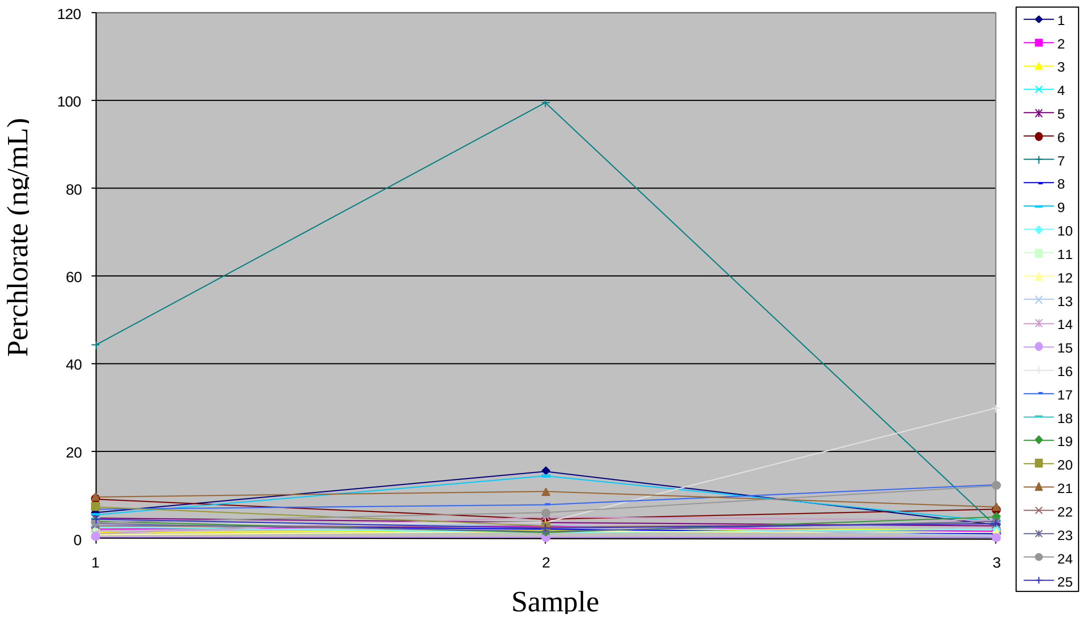


Figure 2. Breast Milk Perchlorate Levels, Subjects 26-51

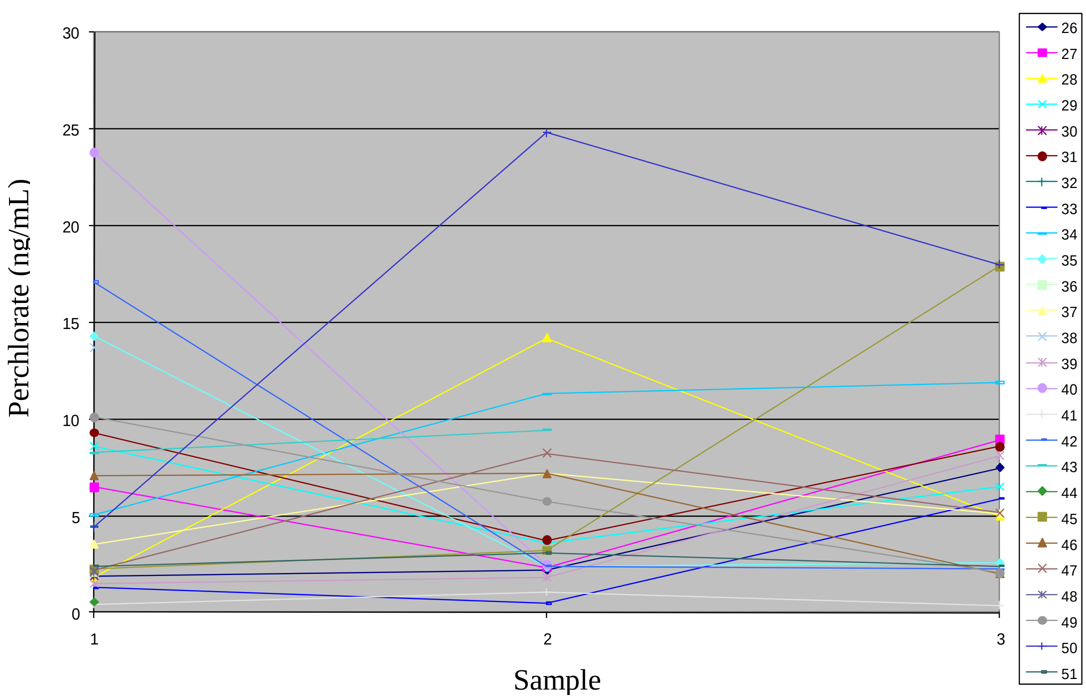


Figure 3. Breast Milk Perchlorate Levels, Subjects 52-80

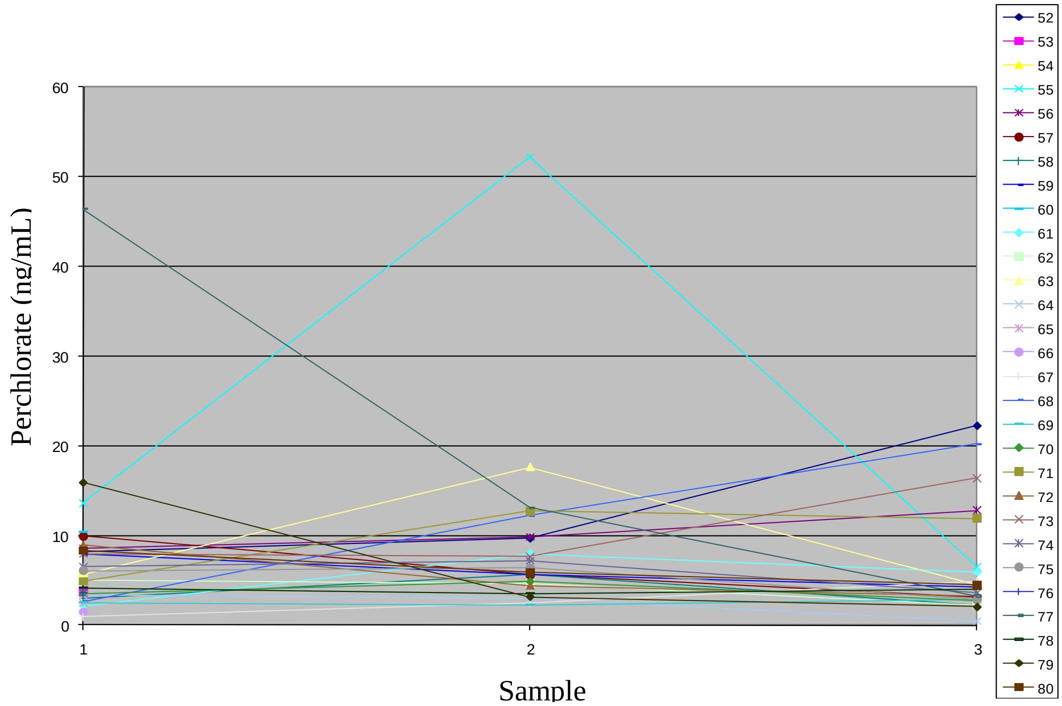


Figure 4. Breast Milk Perchlorate Levels, Subjects 81-106

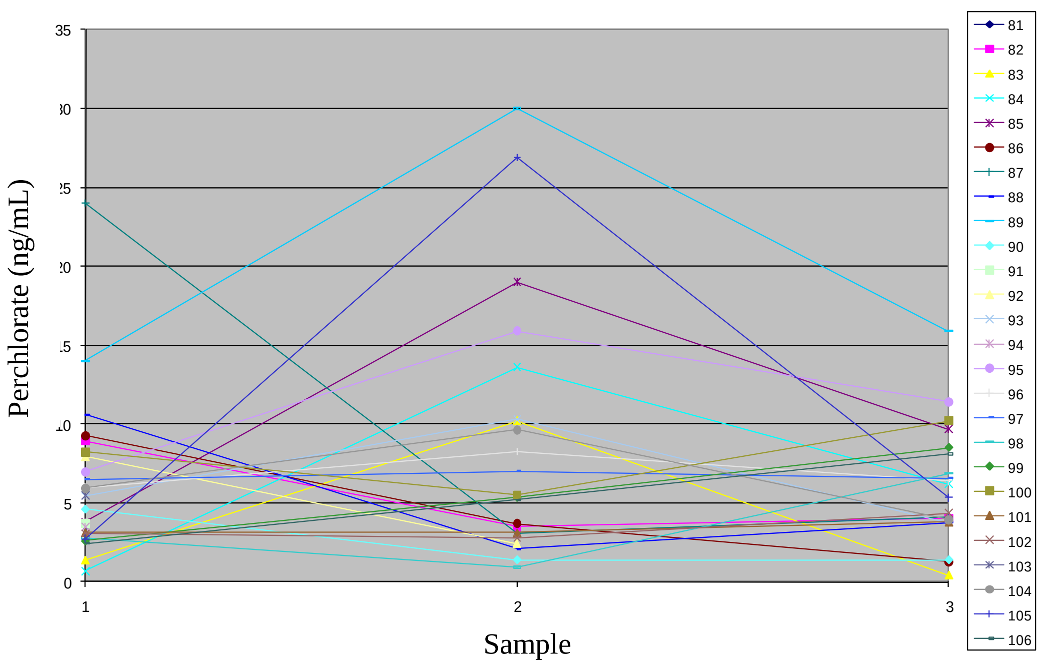


Figure 5. Urine Perchlorate Levels, Subjects 1-25

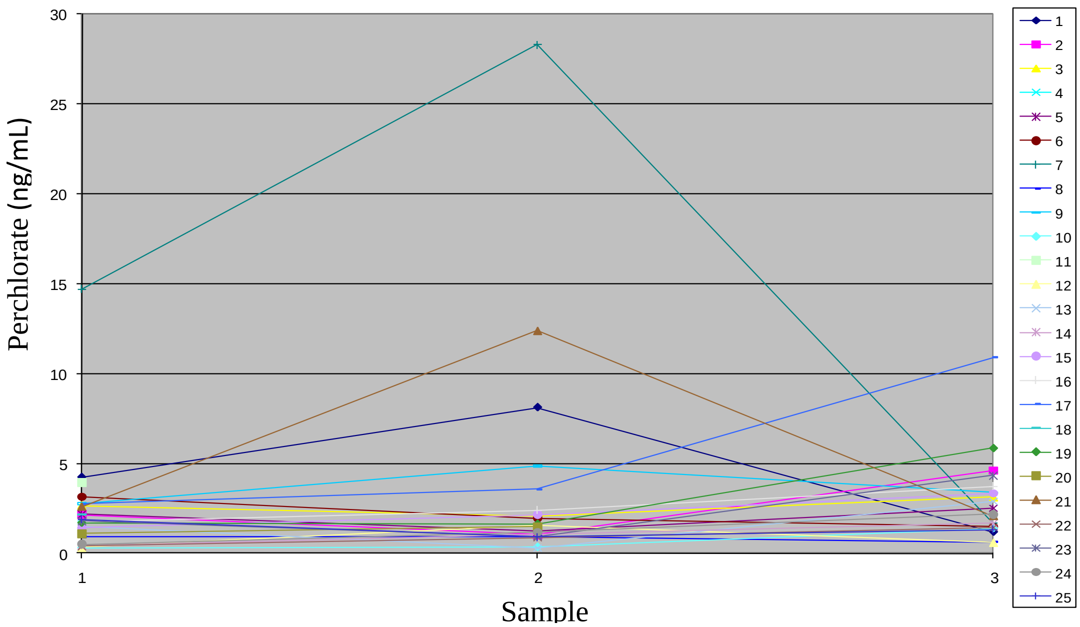


Figure 6. Urine Perchlorate Levels, Subjects 26-51

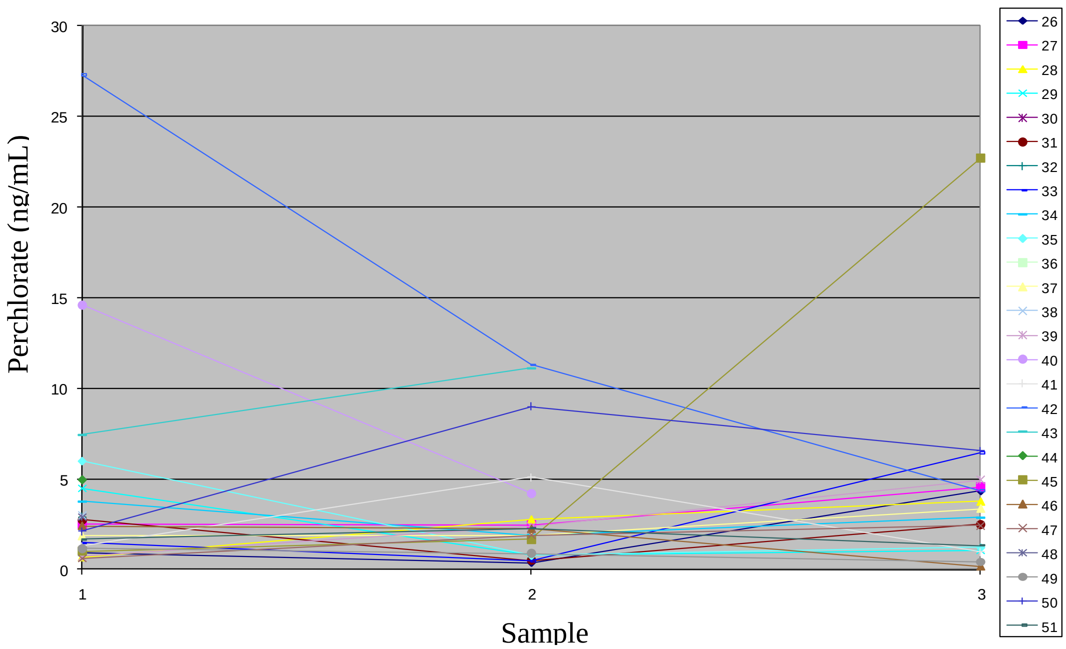


Figure 7. Urine Perchlorate Levels, Subjects 52-80

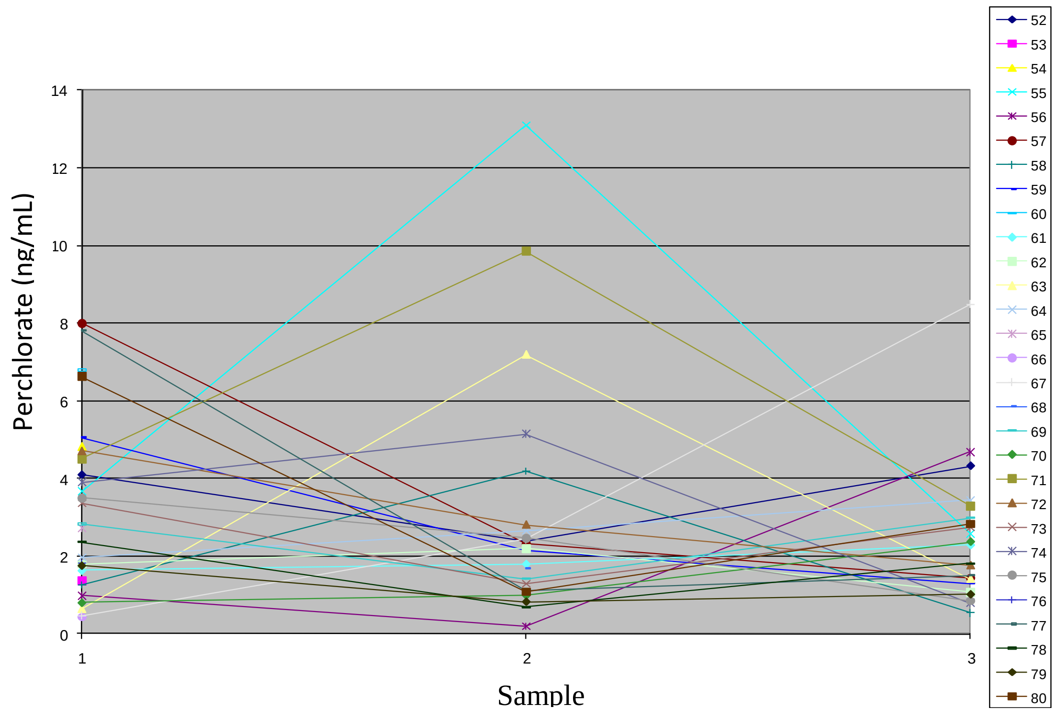
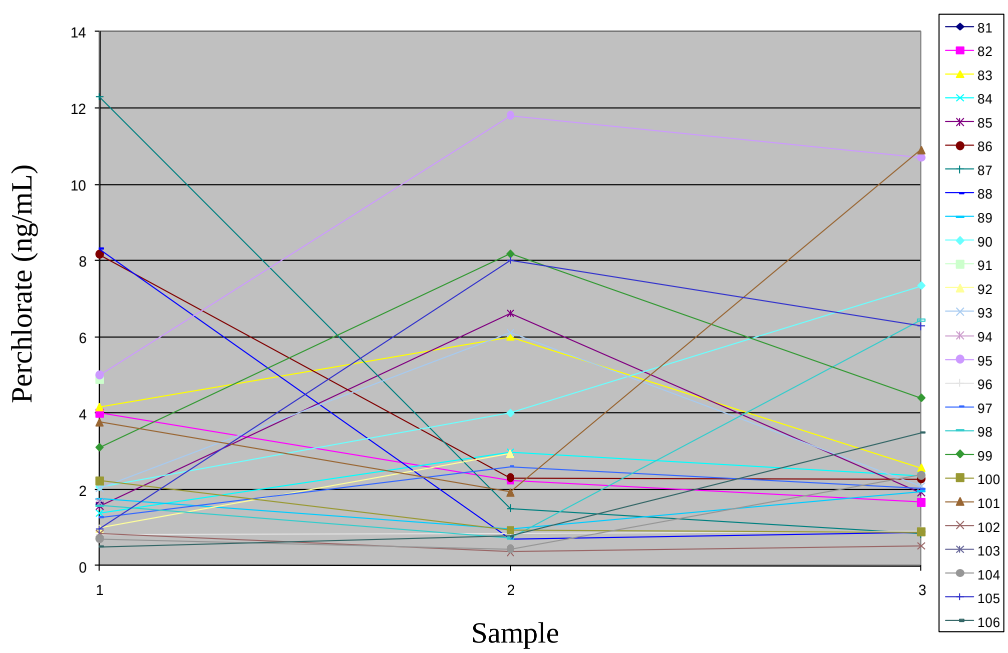


Figure 8. Urine Perchlorate Levels, Subjects 81-106



Curriculum Vita

Marija Borjan

Education

Ph.D., Environmental and Occupational Health
UMDNJ – School of Public Health, 2009

M.P.H., Environmental and Occupational Health
UMDNJ – School of Public Health, 2006

B.S., Biology
Indiana University, 2001

Professional Experience

2006 – Present Rutgers University, New Brunswick, NJ
Laboratory Researcher
- Concurrently with my academic work, assist Director of NJ Agricultural Experiment Station, and now Dean of Agricultural and Urban Programs, School of Environmental and Biological Science, in general administrative work
- Manage NIH funded grants and research projects on pesticides and endocrine disruptors as they relate to human health
- Coordinate projects with outside institutions, manage data, and prepare manuscripts for publication

2004 – 2006 UMDNJ – School of Public Health, Piscataway, NJ
Research Assistant
- Assist Chair of the Department of Environmental and Occupational Health in general administrative work
- Prepare manuscripts for publication

Summer 2005 Robert Wood Johnson Medical School, Piscataway, NJ
Science Enrichment Program Coordinator
- Report to Director of Multicultural Affairs
- Coordinated high school science program
- Designed and instructed Cultural Competency and Health Disparities course

2003 – 2004 New Mexico State University, Las Cruces, NM

Senior Research Assistant

- Supervised and trained undergraduates in lab techniques
- Optimized and developed efficient and rapid methods to detect pathogens in drinking water as part of grant in collaboration with the Department of Defense and the Centers for Disease Control and Prevention

2001 – 2002 The Community Hospital, Munster, IN

Histological Technician

- Histology Technologist (HTL), American Society for Clinical Pathology, eligible
- Performed hematoxylin and eosin stain, special stains, immunohistochemistry (automated), tissue embedding, and microtomy
- Assist pathologist during gross analysis

Publications

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