Biomonitoring Data on Thyroid-Active Compounds: Database and Issues Regarding Variability and Interpretation

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"The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention and do not represent any agency determination or policy."

Biomonitoring

Human Biomonitoring for Environmental Chemicals (National Academies Press, 2006):

"method for assessing human exposure to chemicals by measuring the chemicals or their metabolites in human tissues or specimens, such as blood and urine."

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Biomonitoring Questions

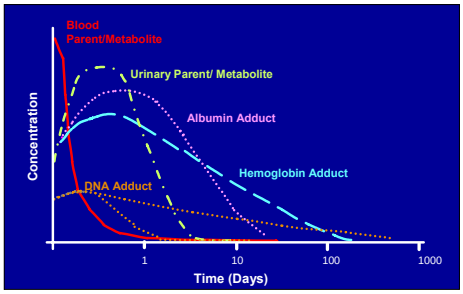
- What exposure has occurred (or is occurring)?
- Who has been exposed (or is being exposed)?
- How much has each person been exposed?
- Does exposure correlate with a health effect?
- Do interventions reduce exposure?

Development of Biomonitoring Methods

- What is the best chemical measure?
  - Parent, metabolite, adduct
  - Measurement time windows
- What is the best specimen?
  - Blood, urine
  - Breath, saliva, feces, hair, breast milk, fat, sweat, nails, semen, cavity and bronchial fluids
  - Chemically stable
  - Interferences
  - Uncontaminated

Concentration Time Course

Single Exposure: Non-persistent chemical



Modified from Needham and Sexton, JEAEE 10:611-629 (2000)

Analytical Methods for Biomonitoring

- Selecting definitive techniques
- Optimizing conditions
- Multi-analyte
- Define and validate
- Calibration-response
- Accuracy and precision
- QC, PT, contamination control
- Throughput and ruggedness
- Safety and security

National Health and Nutrition Examination Survey (NHANES)

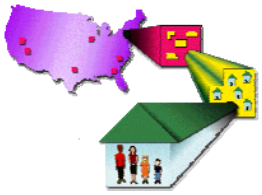
Blount BC, Valentin-Blasini L, Osterloh JD, Mauldin JP, and Pirkle JL (2006). Perchlorate Exposure of the U.S. Population, 2001- 2002. *J Expo Sci Environ Epidemiol*. advance online publication, October 18, 2006; doi:10.1038/sj.jes.7500535.

Blount BC, Pirkle JL, Osterloh JD, Valentin-Blasini L and Caldwell KL (2006). Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. *Environ Health Perspect* 114(12):1867-1871. doi:10.1289/ehp.9466

Steinmaus C, Miller M, and Howd R (2007). Impact of Smoking and Thiocyanate on Perchlorate and Thyroid Hormone Associations in the 2001-2002 National Health and Nutrition Examination Survey. Environ Health Perspect in press. doi:10.1289/ehp.10300

NHANES

- **Ongoing CDC cross-sectional survey designed to collect data on the health and nutritional status of the U.S. population (~5000/yr)**
- **Conducted by National Center for Health Statistics**
- **Complex, multistage, area probability design: samples the U.S. population based on age, sex, race/ethnicity, income**
- **NHANES surveys: I (71-75), II (76-80), III (88-94), 99-00, 01-02, 03-04, 05-06, 07-08, …**



NHANES Mobile Exam Centers

Data collected in 15 localities per year via mobile trailers

- Extensive questionnaire on demographics and health behaviors
- Physical exam
- Medical and nutritional lab tests
- Biomarkers of environmental exposure

Thyroid data collected as part of NHANES

- Study participants ages 12 +
- Full sample 1988-1994: TSH, T4, anti-TPO, anti-Tg
- 1/3 sample 1999-2000: TSH, T4
- 1/3 sample 2001-2002: TSH, T4
- Full sample 2007-2008: TSH, T4, fT4, T3, fT3, Tg, anti-TPO, anti-Tg
- 1/3 sample 2009-2010: TSH, T4, fT4, T3, fT3, Tg, anti-TPO, anti-Tg

Relevant covariates related to thyroid

- Age, sex, race/ethnicity
- BMI, total caloric intake, hours since last meal, MEC session
- Pregnancy, premenarche, post-menopausal status
- Medication categories: beta-blockers, estrogen formulations, glucocorticoids, androgens, and other drugs
- C-reactive protein, serum albumin
- Tobacco smoke exposure (serum cotinine)

Thyroid-active chemicals assessed in NHANES

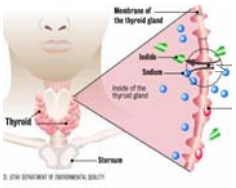
- **Phytoestrogens**
- **Iodine, selenium**
- **Medications:**
  - **Betablockers**
  - **Estrogens**
  - **Furosemide**
  - **Gabapentin**
  - **Steroids**
  - **Thyroid drugs**
- **Triclosan**
- **PBDEs**
- **PCBs**
- **PFOS**
- **Pesticides**
  - **Ethylenethiourea**
  - **Dacthal**
- **Nitrate/Thiocyanate**
- **Perchlorate**

Inhibition of Iodide Uptake at Sodium-Iodide Symporter (NIS)

NIS function
- Active transport of iodide across cell membrane using sodium ion gradient

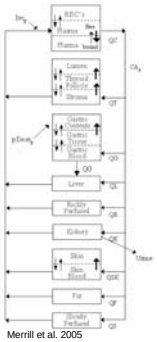
Tissues with NIS expression
- Thyroid
- Placenta
- Mammary gland

NIS Inhibitors
- ClO_4^-, SCN^-, NO_3^-



Matrix Selection: Perchlorate Distribution and Excretion

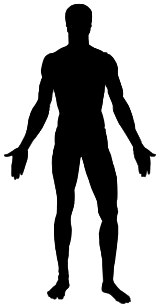
- Perchlorate is not metabolized in humans and unlikely to bioaccumulate significantly
- Perchlorate absorbed by body is excreted in urine (and milk)
- In non-lactating people, perchlorate in 24-hr urine approximates daily dose



Merrill et al. 2005

Validation of Method for Multiple Matrices

- Urine
- Amniotic Fluid
- Cell Lysates
- Breast Milk
- Infant Formula and food extracts
- Serum
- Whole Blood
- Dried Blood Spots



Perchlorate NHANES Objectives

1. What is the prevalence and magnitude of exposure to perchlorate in the US population?
2. Are environmental urinary perchlorate levels associated with changes in serum TSH and total T4 (thyroid function) in the general U.S. population?
3. Which exposure sources are associated with increased urinary perchlorate?
4. Are exposure levels changing over time in multiple NHANES study periods?

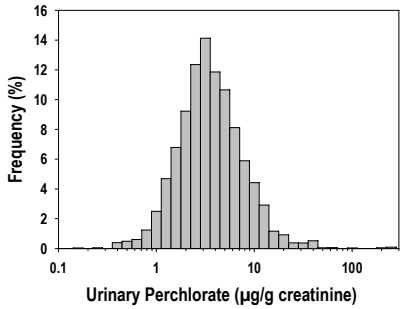
NHANES 2001 – 2002

- 2820 study participants
- Urinary perchlorate, nitrate, thiocyanate, iodine
- Serum thyroid stimulating hormone and thyroxine
- Additional measurements such as urine creatinine, serum cotinine
- Demographic information

NHANES 2001 – 2002: Characteristics of study population

Category	(n)	(%)
Age		
6 years and over	2820	100.0
6 to 11 years	374	13.3
12 to 19 years	828	29.4
20 years and over	1618	57.4
Sex		
Female	1485	52.7
Male	1335	47.3
Race/ethnic groups		
Non-Hispanic White	1228	43.5
Non-Hispanic Black	681	24.1
Mexican American	708	25.1
Other race/ethnic groups	203	7.2

Urinary Perchlorate Distribution NHANES 2001 – 2002



Distribution of urinary perchlorate (µg/g of creatinine) in the U.S. population ages 6+, NHANES 2001 - 2002

Age	N	Geometric mean	5th pctile	50th pctile	95th pctile
All	2818	3.56	1.10	3.38	12.7
6-11 yrs	374	5.71*	1.91	5.79	17.4
12-19 yrs	827	2.95	0.92	2.89	9.87
20+ yrs	1617	3.46	1.09	3.25	12.3

Estimating dose based on spot urine perchlorate

study participant assumptions:

- Uniform urinary excretion of perchlorate and creatinine
- measured body weight and height
- Daily creatinine excretion estimated from lean body mass:

$$k \times (140 - age[yr]) \times Wt(kg)^{1.5} \times Ht(cm)^{0.5}$$

Where k = 1.93 for men, 1.64 for women)

- Perchlorate dose estimated assuming spot urine representative of daily exposure per unit creatinine

Daily dose/bw = (ClO_4 µg/g Cre) × daily Cre g ÷ bw kg

Mage et al (2004) J Expo Anal Environ Epidemiol 2004:14: 457–465.

Estimated perchlorate dose in U.S. females, NHANES 2001 – 2002

Percentile	Urine perchlorate (µg/g of creatinine)	Estimated perchlorate dose (µg/kg/day)
5th	1.13	0.019
10th	1.48	0.026
25th	2.25	0.038
50th	3.59	0.062
75th	5.99	0.099
90th	10.0	0.176
95th	13.4	0.236

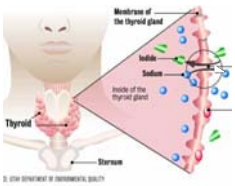
EPA RfD = 0.7 µg/kg/day

Study Objectives

1. What is the prevalence and magnitude of exposure to perchlorate, nitrate and thiocyanate in the US population?
2. Are environmental urinary perchlorate levels associated with changes in serum TSH and total T4 (thyroid function) in the general U.S. population?
3. Which exposure sources are associated with increased urinary perchlorate?
4. Are exposure levels changing over time in multiple NHANES study periods?

Perchlorate can inhibit the thyroid

- Perchlorate mode of action
  - Perchlorate competes with iodide for active transport into the thyroid
  - Perchlorate at pharmacological doses inhibits thyroxine production, leading to decreased serum thyroxine and increased serum TSH
- Key question
  - Does exposure to relatively low levels of perchlorate in the environment alter thyroid hormone levels?



Design and Methods

- Cross-sectional multiple regression analysis
- Random one-third subsample of NHANES 2001 - 2002
- Perchlorate, TSH and T4 measured in 2299 study participants, with 1111 women in final regression analysis

Multiple Regression Analysis

- Separate regression analyses for TSH and total T4 with urine perchlorate
  - Adjusted for complex survey design and population weighting
- Models included covariates known or suspected to affect thyroid function:
  - Age, sex, race/ethnicity,
  - BMI, total caloric intake, hours since last meal
  - Pregnancy, premenarche, post-menopausal status
  - Medication categories: beta-blockers, estrogen formulations, glucocorticoids, androgens, and other drugs
  - C-reactive protein (CRP), serum albumin, urinary creatinine, serum cotinine, urine nitrate, and urine thiocyanate
- Exclusions: <12 years old, thyroid disease, or taking thyroid medications

Results

Associations of urine perchlorate with serum TSH or T4:

- Men:
  - » Not significant for either TSH or T4
- Women
  - » Significant for both TSH and T4
- Women with urinary iodine < 100 µg/L (susceptible group)
  - » Significant for both TSH and T4
- Women with urinary iodine ≥ 100 µg/L
  - » Significant only for TSH

Results (cont'd)

- Significant covariates
  - Estrogen-related states (mainly on T4): estrogen meds, pre-menarche, pregnancy, post-menopause
  - Previously reported associations: age, race/ethnicity, BMI, caloric intake, CRP, smoking (thiocyanate)
- Predicted effect size of perchlorate on TSH and T4 in females with urinary iodine < 100 µg/L
  - Predicted effect is small to moderate
  - Beta coefficients predict mg/kg/day doses required to move median TSH or T4 to out of the normal range.

Regression analysis of log(perchlorate) for women by iodine level

	Urine iodine <100		Urine iodine ≥100	
	Beta for perchlorate	p-value	Beta for perchlorate	p-value
Log(TSH)	0.123	0.0010	0.114	0.0249
n	356		697	
R^2	0.061		0.145	
Total T4	-0.892	<0.0001	0.220	0.5591
n	348		724	
R^2	0.240		0.149	

Regression analysis of perchlorate and thyroid function for women by iodine level and smoking

		Smoke exposure category		
		High (cotinine > 10 ng/mL)	Medium (0.015 ≤ cotinine ≤ 10ng/mL)	Low (cotinine < 0.015 ng/mL)
		beta (p-value)	beta (p-value)	beta (p-value)
Total T4	All women	-1.2242 (.0131)	-0.5761 (.0236)	NS
	women with urinary iodine < 100 µg/L	-1.4761 (.0014)	-0.8955 (.0028)	NS
	women with urinary iodine ≥ 100 µg/L	-0.8423 (.1084)	NS	NS
TSH	All women	0.2171 (.0037)	0.1454 (.0035)	0.1317 (.0139)
	women with urinary iodine < 100 µg/L	0.2035 (.0242)	0.1295 (.0310)	0.1162 (.0232)
	women with urinary iodine ≥ 100 µg/L	0.2274 (.0035)	0.1535 (.0091)	0.1402 (.0280)

Steinmaus, et al July 2007 EHP

Predicted effect of perchlorate on TSH or T4: Females 12 + with urinary iodine < 100 µg/L

Change in urinary perchlorate	Change in Total T4 (µg/dL)	Change in TSH Starting at 2.97 (IU/L)
min → max (0.19-100 µg/L)	-2.43	3.45
5th → 95th percentile (0.65-12.0 µg/L)	-1.13	1.49
25th → 75th percentile (1.6-5.2 µg/L)	-0.45	0.60
Medical Normal Ranges	T4 5-12	TSH 0.3-4.5

Limitations

- Free T4, Anti-TPO not available
- Weak iodine assessment
- Cross-sectional association; perchlorate could be a surrogate for an unknown variable

Strengths

- Large number of women
- Targets a susceptible group
- Assesses chronic exposure
- Largest study of women with perchlorate exposure and low iodine status

NHANES Conclusions: Exposure

- Perchlorate detected in 100% of urine samples tested
- Log normal distribution
- Children (6 – 11 yrs) have higher urine perchlorate compared with older age groups (12 + yrs)
- 95th percentile of dose estimates for adults is approximately 1/3 the EPA reference dose

NHANES Conclusions: Thyroid Models

- For women, urinary perchlorate associated with biologically coherent changes in thyroid hormone levels:
  - Increased TSH and decreased T4
- Driven by susceptible groups:
  - Urine iodine < 100 µg/L
  - High thiocyanate (smokers)
- Model consistent with other known effectors of thyroid function
  - Estrogen, age, BMI, race/ethnicity, sex

Significance

- Perchlorate exposure is more prevalent than expected
- The predicted effect on T4 and TSH is at lower levels of perchlorate than previously determined experimentally in humans or in observational studies.
- Data provides additional information on perchlorate dose-response in the U.S. population

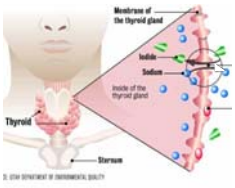
Case study:
Perinatal Perchlorate Exposure

Inhibition of Iodide Uptake at Sodium-Iodide Symporter (NIS)

NIS function
- Active transport of iodide across cell membrane using sodium ion gradient

Tissues with NIS expression
- Thyroid
- Placenta
- Mammary gland

NIS Inhibitors
- ClO_4^-, SCN^-, NO_3^-



Most Sensitive Life Stage: Developing Fetus

National Academies of Sciences Perchlorate Report (2005):
- Developing fetus is the life stage most sensitive to potential health effects from perchlorate exposure
- Individual biomonitoring data is best for assessing perchlorate exposure and potential health effects

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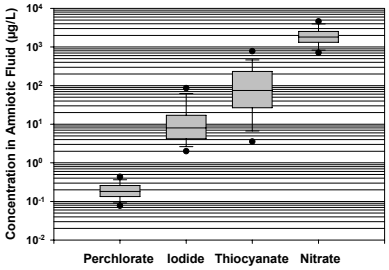
Collaborative Study of Perinatal Perchlorate Exposure

- 150 pregnant women
- Residing in New Jersey
- Elective C-section delivery
- Collect maternal urine and blood
- Collect fetal cord blood and amniotic fluid
- Analyze for perchlorate, nitrate, thiocyanate and iodide in all matrices

Fetal Matrices

- Cord blood: blood on fetal side of the placenta
- Amniotic fluid as "fetal urine"

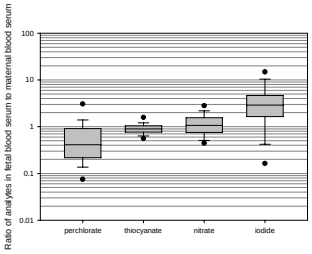
Analyte Concentrations in Amniotic Fluid



Distribution of Perchlorate (µg/L) in Various Maternal and Fetal Matrices

Matrix	N	% Detects	Geometric Mean	50th pctile	95th pctile
Amniotic Fluid	130	97%	0.144	0.145	0.380
Cord Blood	126	67%	0.133	0.139	0.480
Maternal Blood	132	94%	0.246	0.223	0.893
Maternal Urine	34	100%	2.14	2.10	12.0

Ratio of Anion Levels in Cord Blood to Maternal Blood



Pearson Correlation Analysis Perchlorate in Different Matrices (p-values)

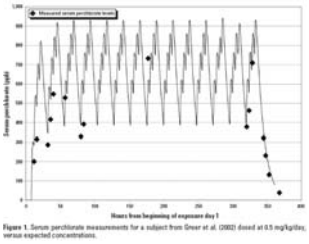
Matrix	Amniotic Fluid	Cord Blood	Maternal Blood	Maternal Urine
Amniotic Fluid	1.0000			
Cord Blood	<0.0001	1.0000		
Maternal Blood	0.0848	0.5999	1.0000	
Maternal Urine	0.0034	0.0462	0.8138	1.0000

Conclusions

- All women in study had perchlorate exposure
- Most fetal blood and amniotic fluid samples contained perchlorate, albeit at levels less than perchlorate in corresponding maternal fluids
- No evidence of preferential perchlorate accumulation in fetal blood or amniotic fluid
- No evidence of perchlorate-induced decreases in iodide transport to the fetus

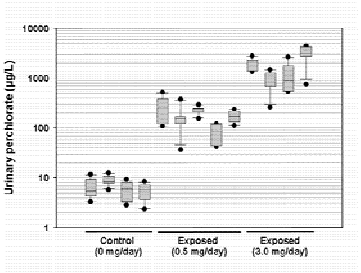
Variability in Serum Perchlorate Levels

Non-persistent compound: Serum levels change in response to varied and episodic exposure, and rapid clearance



Gibbs et al. 2005, based on Greer et al. 2002

Variability in 24-hr urine perchlorate



Based on Braverman et al. 2007

Future Directions

- Perchlorate exposure/iodine status and 6 additional thyroid-related markers in NHANES 2007-2008, 2001-2002
  - Free T4, free T3, total T3, Tg
  - Anti-TPO, anti-Tg
- Perchlorate source apportionment (food vs water)
- Perchlorate exposure and thyroid hormone levels in infants
- Track trends in US perchlorate exposure
- Study active transport of perchlorate in vitro and in vivo

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