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Perchlorate: Concern About Environmental Exposures, Thyroid Homeostasis, and Developmental Impacts

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Dr. Mark Miller, University of California, San Francisco-Pediatric Environmental Health Specialty Unit (PEHSU)

Dr. Craig Steinmaus, California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA)

Srp Superfund Research Program

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National Institute of Environmental Health Services


Perchlorate: Environmental exposures, thyroid homeostasis, and development
Craig Steinmaus MD, MPH
University of California, Berkeley
Office of Environmental Health Hazard Assessment, CA EPA
Mark Miller MD, MPH
Director, Pediatric Environmental Health Specialty Unit, UCSF
Office of Environmental Health Hazard Assessment, CA EPA
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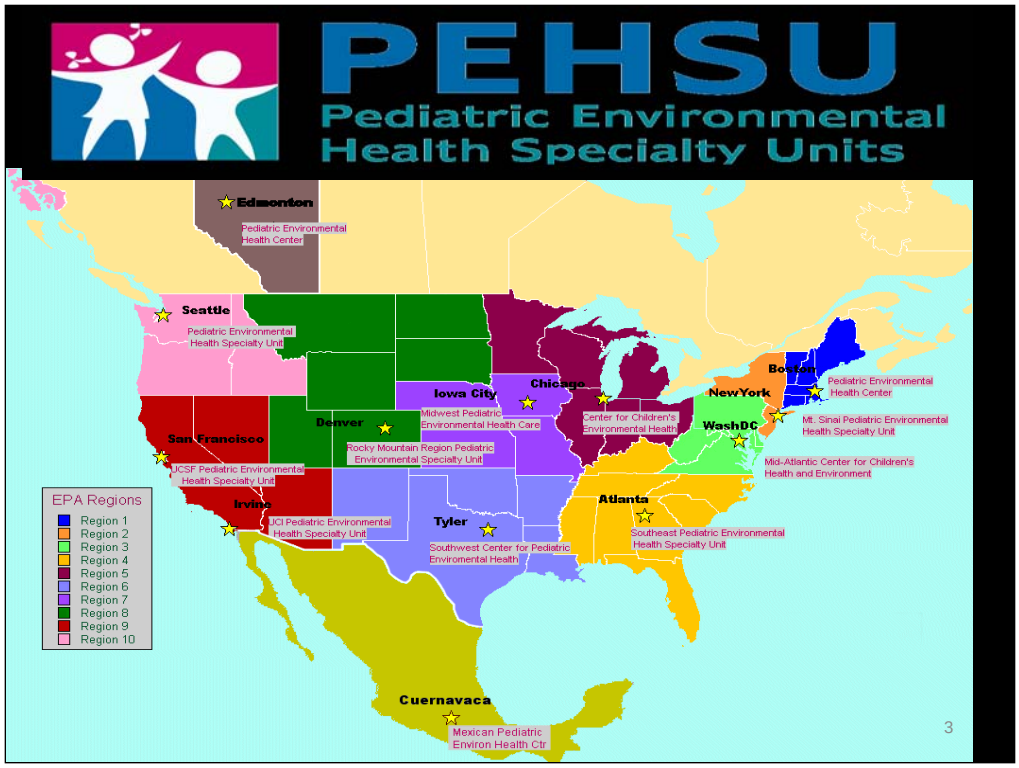
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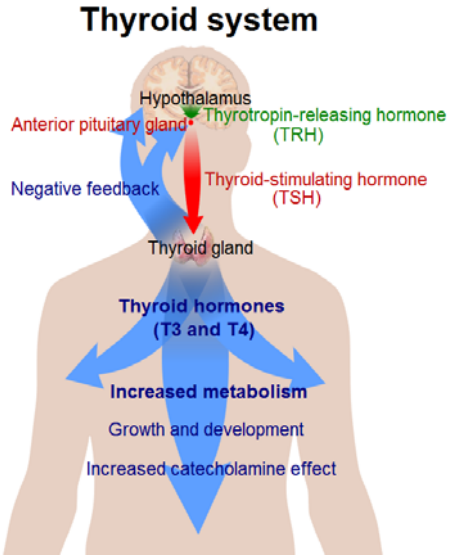
Link between Thyroid and Mental Retardation Recognized for 100 Years



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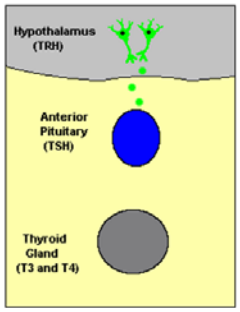
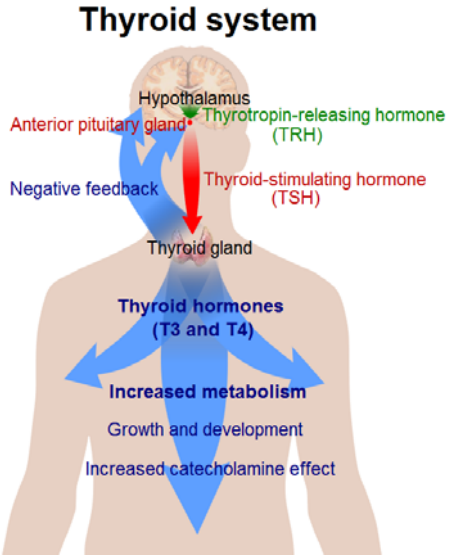
Neurons in the hypothalamus secrete thyroid releasing hormone (TRH), which stimulates cells in the anterior pituitary to secrete thyroid-stimulating hormone (TSH).
TSH binds to receptors on epithelial cells in the thyroid gland, stimulating synthesis and secretion of thyroid hormones, which affect probably all cells in the body.
When blood concentrations of thyroid hormones increase above a certain threshold, TRH-secreting neurons in the hypothalamus are inhibited and stop secreting TRH. *This is an example of "negative feedback".*

Link between Thyroid and Mental Retardation Recognized for 100 Years





During first trimester fetus is dependant on maternal thyroid

5

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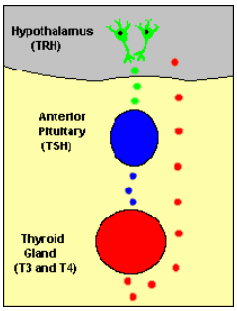

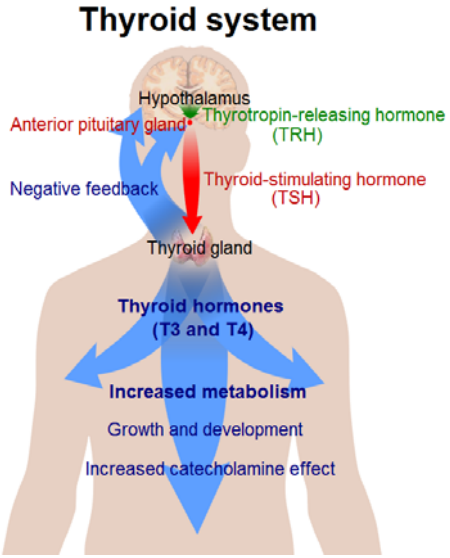

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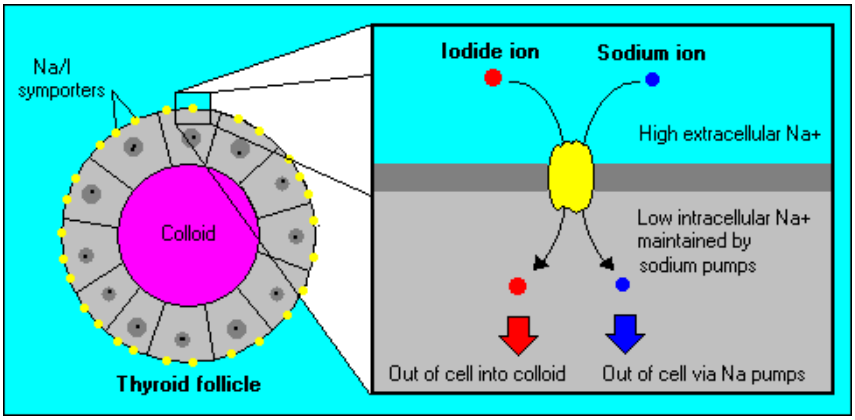
Thyroid gland ability to transport and concentrate iodide from blood is necessary for synthesis of thyroid hormones.
Inhibited by perchlorate, thiocyanate, nitrate.



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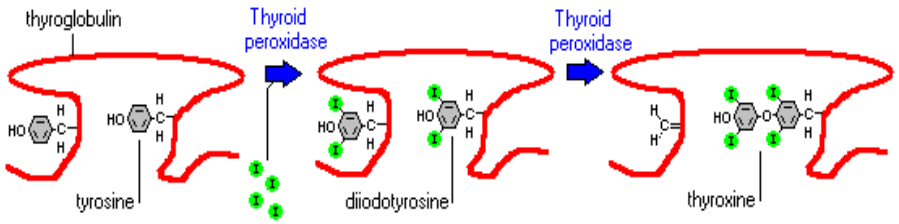
As its name indicates, **the sodium-iodide symporter simultaneously transports both Na^+ and I^- ions** from extracellular fluid (i.e. blood) into the thryoid epithelial cell. This process is an example of secondard active transport. Energy is provided by the electrochemical gradient of sodium across the cell membrane; the low intracellular concentration of sodium is maintained by sodium pumps.
The sodium-iodide symporter is most highly expressed in thyroid epithelial cells. Lower levels of expression can be detected in mammary gland, salivary gland, stomach and colon, but none of these tissues is known to organify iodide. The presence of the symporter in mammary gland leads to secretion of iodine in milk, which is probably important for thyroid function in neonatal animals.
The most important stimulator of symporter gene and protein expression is thyroid-stimulating hormone, similar to what is observed with other important thyroid proteins such as thyroglobulin and thyroid peroxidase.
The ability of the thyroid gland to transport and concentrate iodide from blood is absolutely necessary for the synthesis of thyroid hormones. The key player in this process is the sodium-iodide symporter, an integral membrane protein that resides in the basolateral membrane of thyroid epithelial cells.

Three or Four Iodines are Added to Tyrosine to Make T_3 or T_4



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•**Tyrosines** are provided from a large glycoprotein scaffold called **thyroglobulin**, which is synthesized by thyroid epithelial cells and secreted into the lumen of the follicle - colloid is essentially a pool of thyroglobulin. A molecule of thyroglobulin contains 134 tyrosines, although only a handful of these are actually used to synthesize T4 and T3.

•**Iodine**, or more accurately iodide (I^-), is avidly taken up from blood by thyroid epithelial cells, which have on their outer plasma membrane a sodium-iodide symporter or "*iodine trap*". Once inside the cell, iodide is transported into the lumen of the follicle along with thyroglobulin.

Fabrication of thyroid hormones is conducted by the enzyme thyroid peroxidase, an integral membrane protein present in the apical (colloid-facing) plasma membrane of thyroid epithelial cells. Thyroid peroxidase catalyzes two sequential reactions:

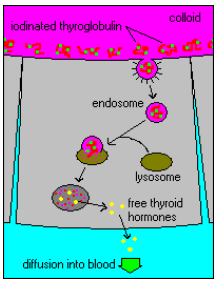
1.Iodination of tyrosines on thyroglobulin (also known as "organification of iodide").

2.Synthesis of thyroxine (or triiodothyronine) from two iodotyrosines.

Through the action of thyroid peroxidase, thyroid hormones accumulate in colloid, on the surface of thyroid epithelial cells. *Remember that hormone is still tied up in molecules of thyroglobulin - the task remaining is to liberate it from the scaffold and secrete free hormone into blood.*

Control of Thyroid Hormone Synthesis and Secretion

- **Thyroid hormone is released into the blood after being excised from the thyroglobulin scaffold**
- **Each of the processes appears to be stimulated by <u>Thyroid Stimulating Hormone</u> from the anterior pituitary gland. Binding of TSH to its receptors on thyroid epithelial cells stimulates synthesis of the iodine transporter, thyroid peroxidase and thyroglobulin.**



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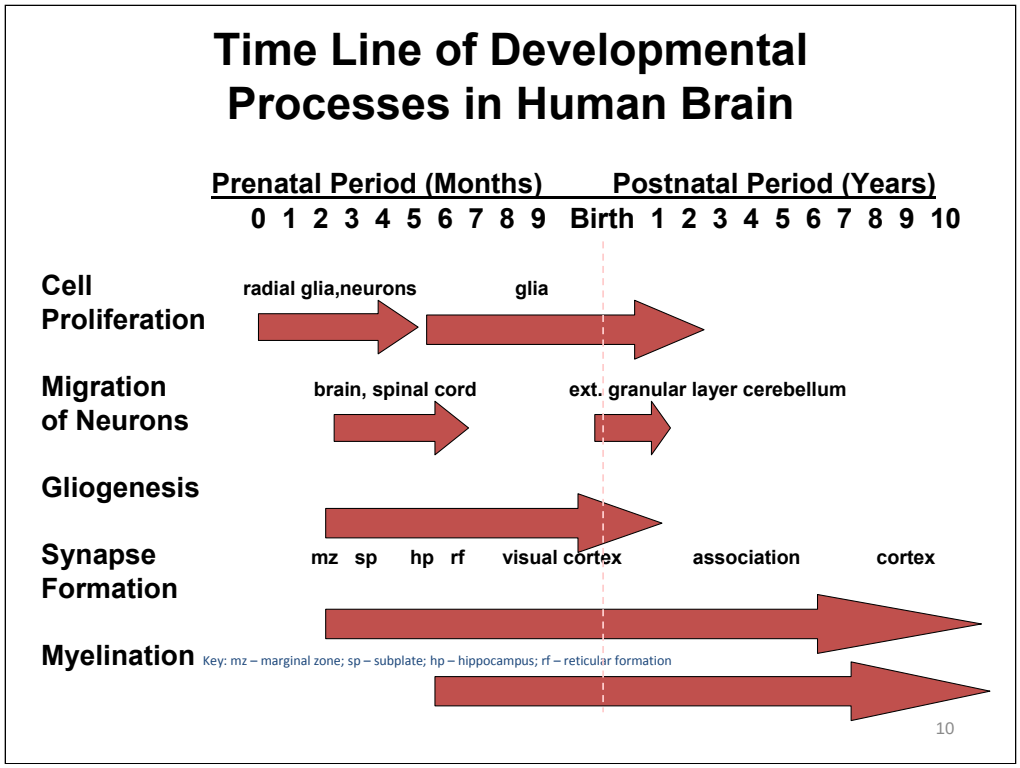


• Brain development involves the production of 1 billion nerve cells and 1 trillion glial, or support, cells. Once produced neurons undergo migration, synaptogenesis, cell loss, and myelination. Pruning of synapses occurs through adolescence. The peak number of synapses is reached in early childhood. Inhibition at one stage can cause alterations throughout the cascade. Various aspects of myelination occur from around 5 months gestation through 10 years of life.

• Compared to other organs, the brain remains vulnerable to developmental disruption over a prolonged period of time, extending from shortly after conception, until well after birth.

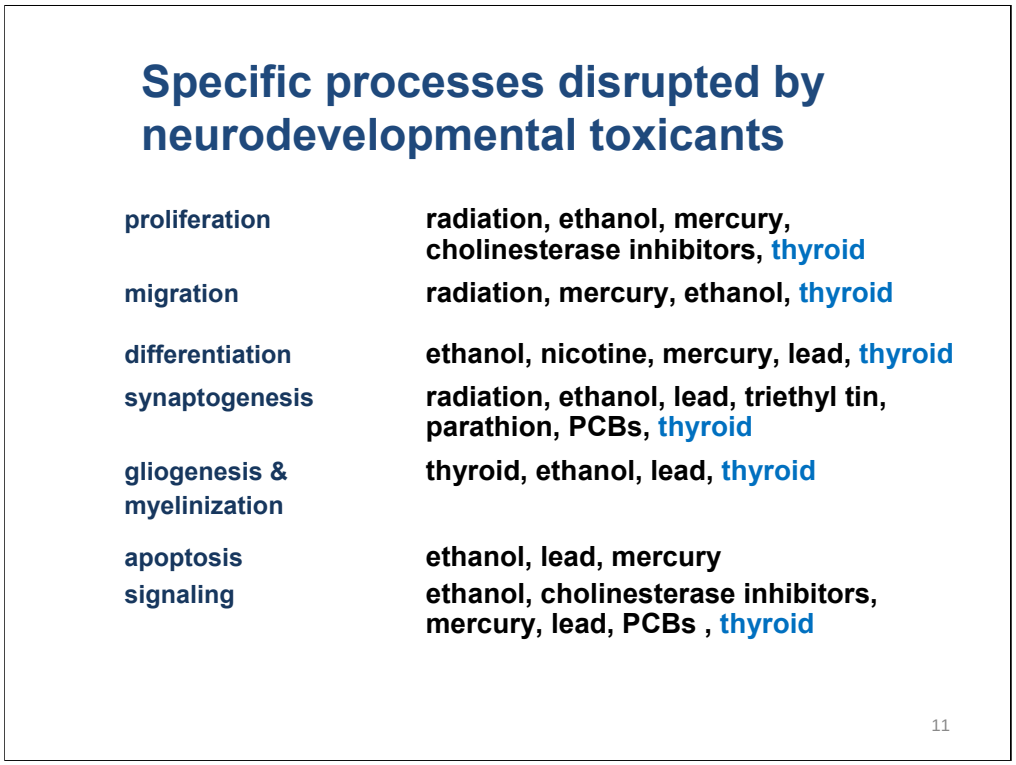
• This slide shows how the timing of processes involved in normal brain development vary during fetal life, infancy, childhood, and adolescence. Notice, for example, how cellular migration tends to be compressed into fairly short timeframes compared to cell proliferation, synapse formation or myelination.

• The clinical effect of disruption of the process by a developmental neurotoxic compound depends not only on the size of the dose but also on the timing and duration.

• EXAMPLE: Effects of the developmental neurotoxin, methylmercury, in large populations of people, include mixtures of developmental delays, learning disabilities, attention deficits, seizures, and mental retardation, depending on the size, timing, and duration of exposure.

•EXAMPLE: Thalidomide caused autism in some children only when the exposure occurred between gestational days 20-24. The window of vulnerability to limb development defects is much longer.

Source: Rice D, Barone S. Critical periods of vulnerability for the developing nervous system: evidence from human and animal models. Environ Health Perspect 108 (Suppl 3):511-533, 2000. *With author's permission.*



Toxic substances can interfere with one or several processes necessary for normal brain development. For example, lead can interfere with cell differentiation, synapse formation, myelinization, programmed cell death (apoptosis), and neurotransmitter levels. Similarly, mercury interferes with cell proliferation, migration, programmed cell death, and neurotransmitter levels.

Therefore the size, timing, and duration of exposures will determine which processes are disrupted and how brain function will be impaired.

Sources:
1. Rice D, Barone S. Critical periods of vulnerability for the developing nervous system: evidence from human and animal models. Environ Health Perspect 108 (Suppl 3):511-533, 2000.
2. Olney JW, Farber NB, Wozniak DF, Jevtovic-Todorovic V, Ikonomidou C.
Environmental agents that have the potential to trigger massive apoptotic neurodegeneration in the developing brain.
Environ Health Perspect. 2000 Jun;108 Suppl 3:383-8. Review.

MATERNAL THYROID DEFICIENCY DURING PREGNANCY AND SUBSEQUENT NEUROPSYCHOLOGICAL DEVELOPMENT OF THE CHILD
Haddow et al., 1999 NEJM

- Examined 2nd trimester blood for TSH in 25,216 women
  - 48 had untreated high TSH levels (above 98%)
  - compared to 124 matched controls
- Children at 7-9 years tested intelligence, attention, language, etc.
  - Full scale IQ decreased by 7 points (p 0.005)
  - 19% had scores of 85 or less (p 0.007)

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Even Sub-clinical Thyroid Hormone Levels Appear Harmful

- Being in the lowest 10% of maternal $\mathbf{T_4}$ (Nl. TSH) at 12 weeks gestation resulted in a decrease of 8 IQ points at 2 years
- Additional study confirms finding in infants (NBAS)
- Having antibodies to thyroid during pregnancy increased risk of low T_4 6 fold

Pop, *et al*., Clin Endocrin 2003
Pop, *et al*., Pediatrics, 2006

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Neonatal Effects of Maternal Hypothyroxinemia During Early Pregnancy

METHODS. Examined were 108 neonates who were born to mothers with low maternal free thyroid hormone (fT4 concentrations; 10th percentile) at 12 weeks' gestation (case patients) and 96 neonates who were born to women whose fT4 values were between the 50th and 90th percentiles, matched for parity and gravidity (control subjects). Newborn development was assessed at 3 weeks of age using the Neonatal Behavioral Assessment Scale. Maternal thyroid function (fT4 and thyrotropin hormone) was assessed at 12, 24, and 32 weeks' gestation.

RESULTS. Infants of women with hypothyroxinemia at 12 weeks' gestation had significantly lower scores on the Neonatal Behavioral Assessment Scale orientation index compared with subjects. Regression analysis showed that first-trimester maternal fT4 but not maternal TSH or fT4 later in gestation was a significant predictor of orientation scores.

CONCLUSIONS. This study confirms that maternal hypothyroxinemia constitutes a serious risk factor for neurodevelopmental difficulties that can be identified in neonates as young as 3 weeks of age.

Boston women have higher breast milk perchlorate and lower iodine than expected

- Mean BM Iodine =
  - 62mcg/L in smokers
  - 221 mcg/L non-smokers
  - Iodine ranged from 84-224mcg/L in formula
- 4 out of 49 had perchlorate in breast milk greater than 100 mcg/L
  - Infant formulas also contained perchlorate at up to 4.1 mcg/L

•Pearce et al., J Clin Endocrin Metab 2007

Breast milk in Boston

- IOM iodine adequate intake 110-130 mcg/d
- Estimated that as many as 47% of nursing women in the study may be providing insufficient iodine
- Median perchlorate 9.1mcg/L

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Pearce et al., J Clin Endocrin Metab 2007

Perchlorate content formula and estimated dose (perchlorate-free water)

Infant Formula	Iodide (µcg/L)	Perchlorate (µcg/L)	Perchlorate dose (µcg/kg-d)
Dairy based formula N=4	70-110	0.24 – 1.8	0.03 – 0.23
Dairy based lactose-free N=2	81/90	0.08/0.43	0.01/0.054
Soy N=4	19-96	0.11 – 0.47	0.015 – 0.06

RfD = 0.7 mcg/kg/d

- Blount and Valentin-Blasini, Thyroid 2007

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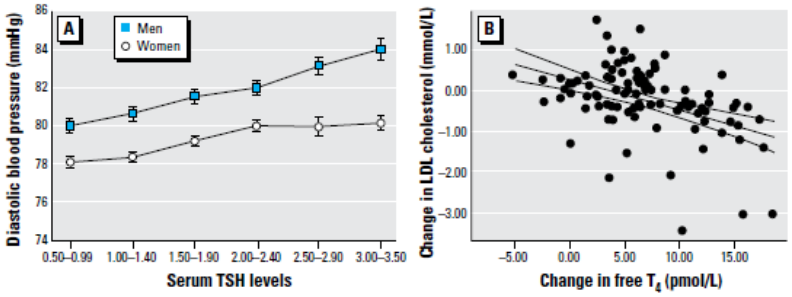
Blood Pressure and Lipid Values Change with Thyroid



Miller et. al. 2009 Env Health Persp redrawn with permission from Asvold (2007; *A) and from Razvi (2007; B) (Copyrights 2007, The Endocrine Society).*

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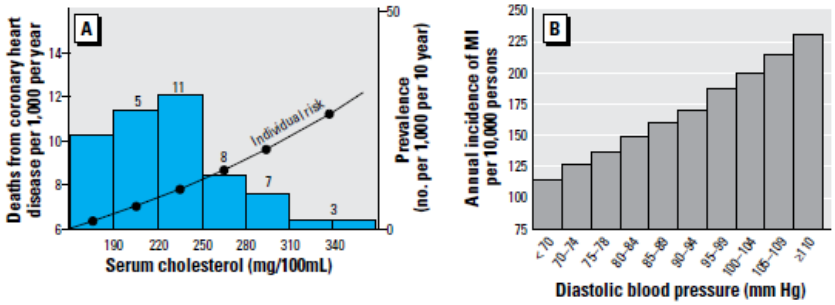
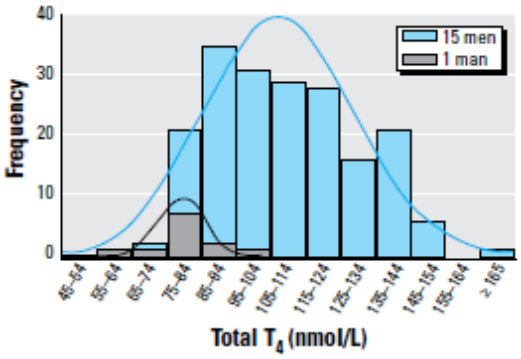
Population vs. Individual Impact



Miller et al., Env Health Persp. 2009 A (adapted from Rose 1981; with permission from the BMJ Publishing Group)
(*B) Death from MI associated with increased diastolic blood pressure in males 45–74 (age-adjusted rate) (adapted U.S. EPA 1985).*

Individual vs. Population Normal Range Thyroid



Miller et al., Env Health Persp. 2009 adapted from
Andersen et al. (2002); copyright 2002, The Endocrine Society

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Perchlorate in Foods: US FDA 2005-6 Survey

- Market basket survey each year involving grocery stores and fast food restaurants in four regions in the US, three cities in each region
- Detectable levels of perchlorate found in 213 of the 286 foods assessed

Food item	Ppb
Tomato, raw	78.0
Grapes (red/green), raw	72.7
Shrimp, boiled	55.0
Cantaloupe, raw/frozen	47.2
Watermelon, raw/frozen	42.6
Spinach, fresh/frozen, boiled	40.0
Tortilla, flour	31.8
Apple pie, fresh/frozen	27.5
English muffin, plain, toasted	26.8
Cauliflower, fresh/frozen, boiled	25.9
Cucumber, peeled, raw	25.3
Pork bacon, oven-cooked	25.2
Ham, cured (not canned), baked	17.1
Bagel, plain, toasted	16.8
Potato, french-fried, fast-food	14.5

Public Water Sources in California 2004-9 with Detectable Perchlorate Levels (≥ 4 ppb)

County	No. of Sources	No. of Systems	Peak Conc. (ppb)
Los Angeles	117	33	86
Riverside	68	9	73
San Bernardino	58	15	80
Orange	21	11	11
Tulare	9	6	24
Santa Clara	7	3	7
Kern	5	4	34
Sacramento	4	2	10
San Diego	5	2	8
Madera	2	1	7
San Joaquin	1	1	69
Tehama	1	1	82
San Luis Obispo	1	1	20
Monterey	1	1	7
Sutter	1	1	6
Ventura	1	1	5
TOTAL	297	92	-

CDPH (2009). http://www.cdph.ca.gov/certlic/drinkingwater/pages/Perchlorate.aspx

Medicinal doses: 800 mg/day (aplastic anemia?)

Selected High Dose Studies of Perchlorate and Thyroid Function

Study	Type	Dose	Outcome Assessed	Result
Greer et al, 2002	Clinical dosing	35 mg/d x 2 wk	T4, fT4, T3, TSH	No effect*
Lawrence et al, 2000	Clinical dosing	10 mg/d x 2 wk	T3, T4, TSH	No effect*
Lawrence et al, 2001	Clinical dosing	3 mg/d x 2 wk	T3, T4, TSH, FTI	No effect
Braverman et al, 2006	Clinical dosing	3 mg/d x 6 mo	T4, FTI, TSH	No effect
Gibbs et al, 1998	Occupational	2.5 mg/d[#]	T3, T4, FTI, TSH	No effect
Lamm et al, 1999	Occupational	1-34 mg/d[#]	T4, T3, TSH, FTI	No effect
Braverman et al, 2005	Occupational	12 mg/d[#]	T4, FTI, T3, TSH	No effect
Crump et al, 2000	Environmental	0.2 mg/d[#]	Child T4 and TSH	No effect
Tellez et al, 2000	Environmental	0.2 mg/d[#]	Mom/neonate T3, fT4, TSH	No effect
Amatai et al, 2007	Environmental	0.7 mg/d[#]	Neonatal T4	No effect

* Decreases in radioactive iodine uptake were seen, but no impact on thyroid hormone levels
[#] Estimated

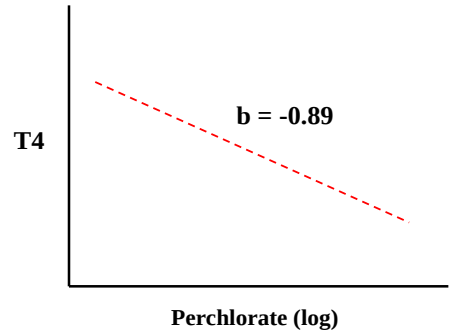
Blount et al. "Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States." Environ Health Perspect 114(12):1865-1871.

- Cross sectional study of urinary perchlorate and serum thyroid hormone levels (T4 and TSH)
- 2299 men and women in NHANES 2001-2
- Detectable perchlorate found in all urine samples tested.
- Mean urinary perchlorate = 2.84 ug/L (intake of about 5 ug/day)
- Excluded people with thyroid disease
- Adjusted for multiple potential confounding variables

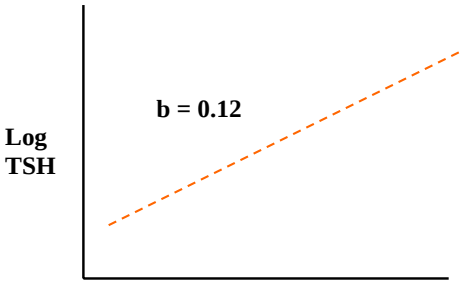
Regression coefficient (b) between perchlorate (log) and thyroid hormones (Blount et al. 2006)

	Total T4	TSH (log)
	b (p-value)	**b p-value**
Men	No effect	No effect
Women		
Iodine < 100*	-0.89 (p <0.0001)	0.12 (p = 0.001)
Iodine ≥ 100	No effect	0.11 (p = 0.02)

*35% of all women had urinary iodine levels < 100 ug/L



Low iodine women



All women

BIOLOGICAL PLAUSIBILITY OF THE FINDINGS:

- Perchlorate is known (at high doses) to decrease thyroid hormone production, which can increase TSH
- Perchlorate is known to inhibit iodine uptake
- Women have higher rates of thyroid disease than men
- Synergistic effect with thiocyante, another known NIS inhibitor

REMAINING QUESTIONS:

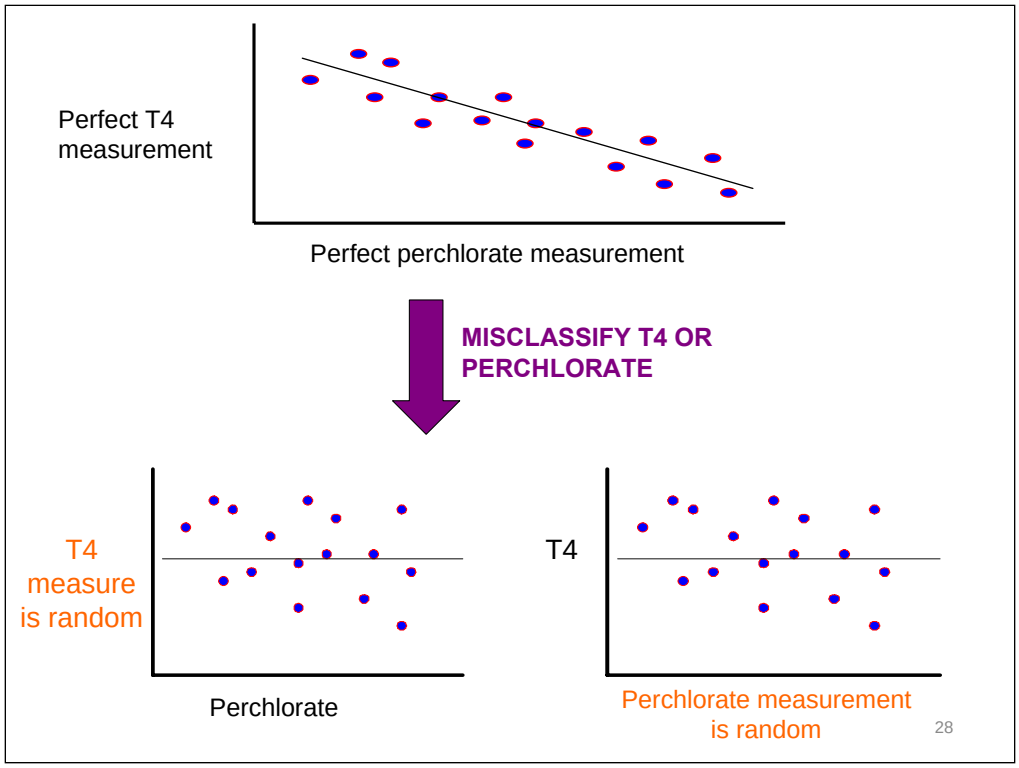
- Exposures in Blount et al. 2006 are very low
- No effect of nitrate, another NIS inhibitor
- Others

Some critiques of Blount et al. 2006

Misclassification of true thyroid status and true long-term perchlorate exposure

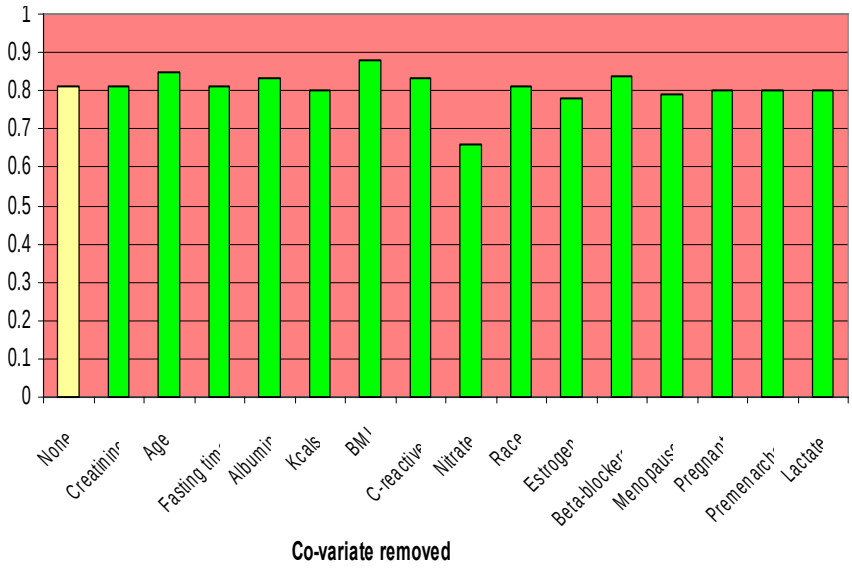
Effects may be due to confounding

Not consistent with other data

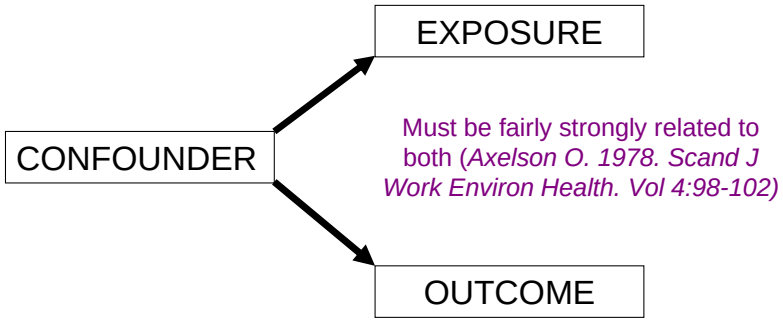

Perfect T4 measurement
Perfect perchlorate measurement
MISCLASSIFY T4 OR PERCHLORATE
T4 measure is random
Perchlorate
T4
Perchlorate measurement is random

Perchlorate-T4 Regression Coefficients (B) After Removing Each Co-variate From the Fully Adjusted Model.

[b]



Why aren't these things causing confounding if we know they are related to thyroid hormone levels?



Must be fairly strongly related to both (*Axelson O. 1978. Scand J Work Environ Health. Vol 4:98-102)*

What about other factors: PCBs?

CONSISTENCY: WHAT ABOUT STUDIES IN OTHER SUSCEPTIBLE GROUPS

Exposure:

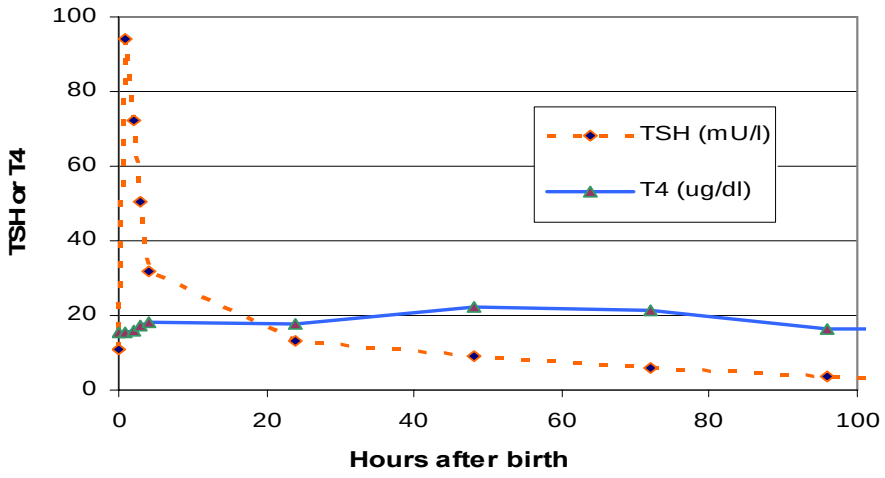
Perchlorate in Maternal Residential Drinking Water During Pregnancy

Outcome:

Newborn T4 and TSH levels

- Kelsh et al., Buffler et al., Schwartz, Brechner et al., Li et al., Tellez et al.
- Most are "negative"
- Some removed TSH or T4 measurements collected in the first 24 hours after birth**** *Does this reduce the ability to ID effects?*

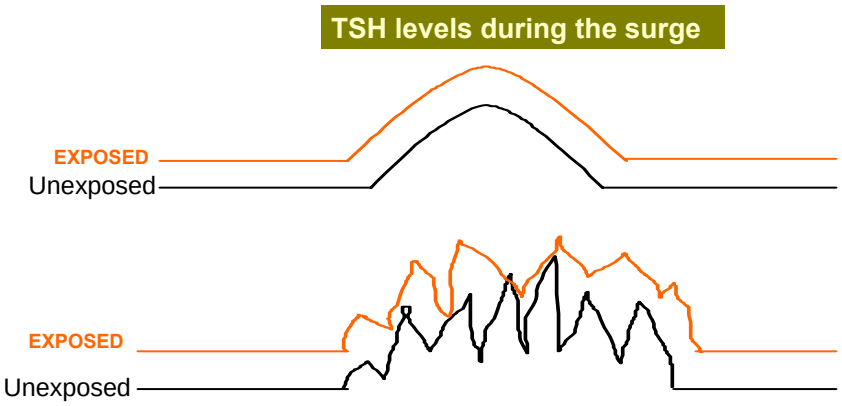
REMOVE MEASUREMENTS IN FIRST 24 HOURS AFTER BIRTH: SURGE IN THYROID HORMONES DURING THIS TIME



Abuid et al. 1973; Cavallo et al. 1980; Fisher and Odell 1969; Sack et al. 1976

1. Lots of "false positives" when determining which children need to be treated for congenital hypothyroidism with thyroid hormone.

2. Increased variability during the surge may make it more difficult to find a true effect



Perchlorate-TSH neonatal study results excluding the first 18-24 hours:

California: Odds ratio for a high TSH = 0.73 (95% CI, 0.40-1.23)

Redlands: Odds ratio for a high TSH = 0.69 (95% CI, 0.27-1.45)

Las Vegas: Mean TSH: Exposed = 12.8 Unexposed = 12.8

Israel*: Mean T4: High exposed = 14.5 Low exposed = 13.98

*Not excluded, but <10% in first 30 hours after birth

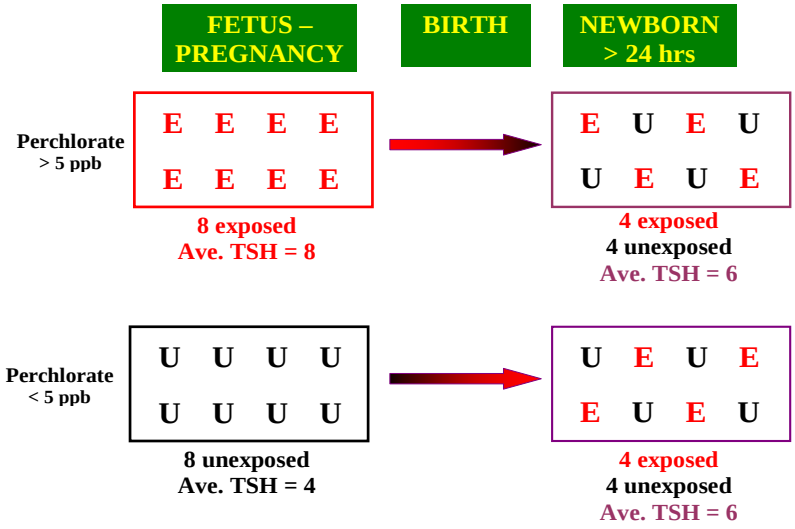
Refs: Amatai et al. 2007, Li et al. 2001, Kelsh et al. 2003, Buffler et al. 2006

First 24 hours may be the most relevant

- The half life of perchlorate is short (8 hours)[1]
- The half life of thyroid hormones is very short (a few hours)[2]
- Perchlorate exposure may change at birth: > 50% newborns receive some formula[3]

1. Greer et al. EHP 2002)
2. Fisher and Odell 1969, de Zegher et al 1994, van den Hove et al 1999
3. California 1998 unpublished

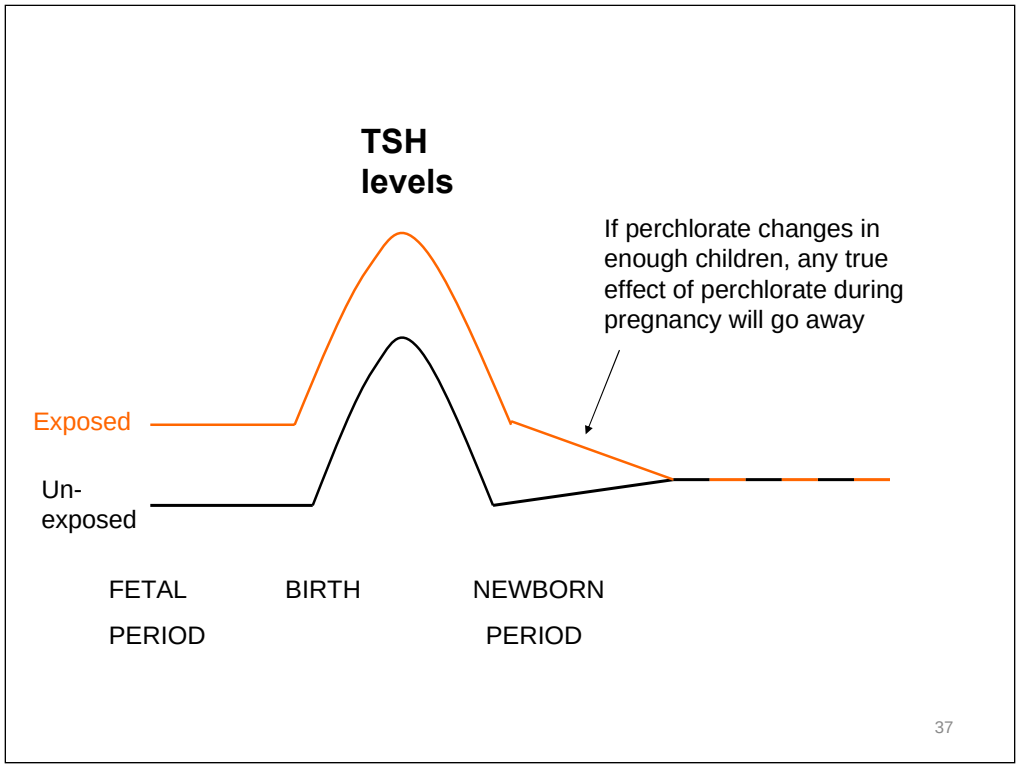
What can happen if exposure changes soon after birth



Exposure Sources:

Maternal only:
- Water
- Food

Multiple:
- Maternal (breast fed)
- Hospital and bottled
- Formula


TSH levels
If perchlorate changes in enough children, any true effect of perchlorate during pregnancy will go away
Exposed
Un-exposed
FETAL PERIOD
BIRTH
NEWBORN PERIOD

Selected Results in Using Data from First 24 Hour after Birth

Study	Who	Where	Outcome	Result: exposed vs. unexposed*	Levels
Unpublished	TSH measured ≤ 18 hours	Redlands 1983-97	High TSH	Odds ratio = 1.57	0-9 ppb
Unpublished	TSH measured < 24 hours	California 1998	High TSH	Odds ratio = 1.60	> 5 ppb
Schwartz 2001	All	California 1996	Ave. T4	Difference in T4 = 18.2 mg/dl	> 13 ppb
Brechner et al. 2000	All	Arizona 1994-7	Low T4 Ave TSH	Odds ratio = 1.18 19.9 vs. 13.4 mU/l	4-6 ppb

* $p < 0.05$ for all results

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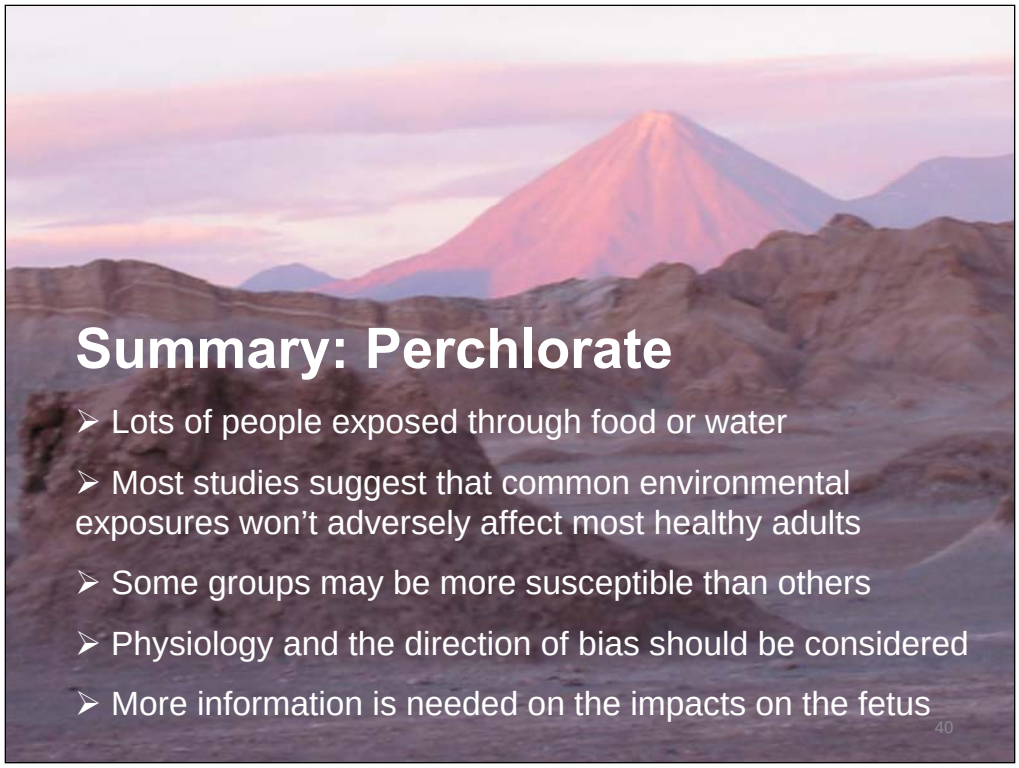
1. Lots of “false positives” when determining which children need to be treated for congenital hypothyroidism with thyroid hormone.

- Determining need for Rx. is not our only goal
- More subtle effects may also be important

2. Increased variability during the surge may make it more difficult to find a true effect

- This causes bias to the null, not towards a false effect


Summary: Perchlorate
➢ Lots of people exposed through food or water
➢ Most studies suggest that common environmental exposures won't adversely affect most healthy adults
➢ Some groups may be more susceptible than others
➢ Physiology and the direction of bias should be considered
➢ More information is needed on the impacts on the fetus
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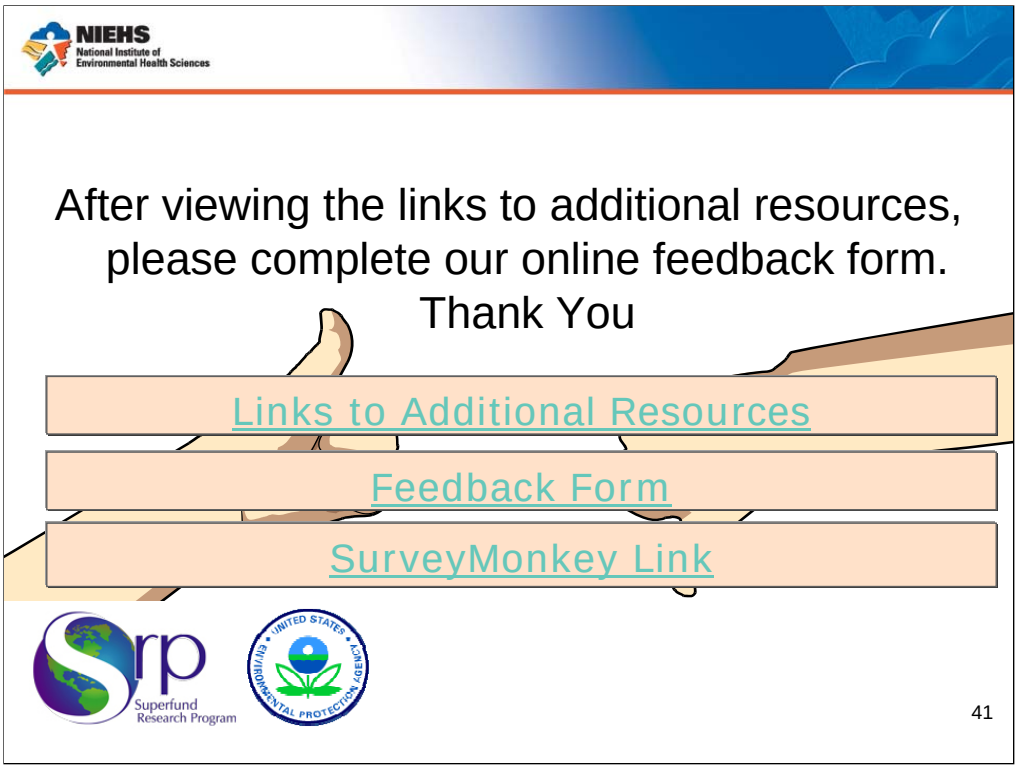
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