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1 Attachment

Bruce 2012-Urinary nitrate, thiocyanate and perchlorate and serum thyrid endpoints based on NHANES 2001-2002.pdf

Tom,

Thank you for your guidance on this last week. As I mentioned, we think this article will be useful to the SAB. Please see the attached article and permission below.

Thank you,
Lisa

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**From:** Marjory Spraycar [redacted]
**Sent:** Monday, October 22, 2012 7:39 AM
**To:** Lisa Corey
**Cc:** Marianne Dreger; 'Brandt-Rauf, Paul W.'
**Subject:** RE: JOEM permission

Dear Lisa,
On behalf of ACOEM and the JOEM editorial office, I am writing to allow permission to you make this paper available to the EPA Scientific Advisory Board for scientific purposes. Please note that permission is being granted only for this particular use; all provisions of the JOEM copyright transfer agreement remain in effect.

Thank you very much for your work as a JOEM author. If I can provide further assistance, please call on me.

All the best,

Marjory Spraycar
Managing Editor
*Journal of Occupational and Environmental Medicine*
605 Worcester Road
Towson, MD 21286-7834
Voice: [redacted]
Fax: [redacted]

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**From:** Lisa Corey [redacted]
**Sent:** Tuesday, October 16, 2012 4:34 PM
**To:** [redacted]
**Subject:** JOEM permission

Hi Marjory,

Our paper, *Urinary Nitrate, Thiocyanate, and Perchlorate and Serum Thyroid Endpoints Based on NHANES 2001 to 2002*, was recently published Ahead of Print. Thank you again for all your help in answering questions as we went through the submission process.

The US EPA has convened a Science Advisory Board on perchlorate (Perchlorate - Approaches for Deriving Maximum Contaminant Level Goals for Drinking Water). We think this paper could be useful for their discussions and we would like to provide the SAB a copy. However, due to Federal Advisory Committee regulations, EPA cannot post the article without express copyright privileges from JOEM. Any guidance you can provide will be helpful.

Thank you,

**Lisa M. Corey, Ph.D.** Staff Toxicologist
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SCIENCE STRATEGY RESEARCH

# Urinary Nitrate, Thiocyanate, and Perchlorate and Serum Thyroid Endpoints Based on NHANES 2001 to 2002

*Gretchen M. Bruce, BS, Lisa M. Corey, PhD, Jeffrey H. Mandel, MD, MPH, and Richard C. Pleus, PhD*

**Objective:** To determine, on the basis of iodide uptake inhibition (IUI), whether associations between urinary concentrations of IUI agents (perchlorate, nitrate, and thiocyanate), as total perchlorate equivalent concentration (PEC), and serum thyroid parameters suggest functional thyroid abnormalities. Additional thyroid hormone measures were released to augment the National Health and Examination Survey (NHANES) 2001 to 2002 data set, which we used in this study. **Methods:** Enhanced thyroid hormone measures released to augment the National Health and Examination Survey (NHANES) 2001–2002 data set were used in this study. Multiple regression was used to assess the relationships among total thyroxine (TT4), free thyroxine, total triiodothyronine (TT3), free triiodothyronine, and thyroid- stimulating hormone (TSH) and PEC. **Results:** PEC was weakly and negatively associated with TT4, but not with TSH, TT3, or free hormone. This association with TT4 appears to be dominated by nitrate and thiocyanate. **Conclusion:** No evidence of functional thyroid abnormality (eg., low thyroid hormone coupled with high TSH) was seen with exposure to the combined IUI agents and enhanced estimates of thyroid function.

Assessing risk from multiple chemicals in risk assessment is not a new concept and is one that has been endorsed by the National Academy of Sciences,[1] US Environmental Protection Agency,[1] and World Health Organization.[2] Despite this, studies in the peer-reviewed literature often focus on the effects of a single chemical. For example, nitrate, thiocyanate, and perchlorate are among a class of chemicals that can cause iodide uptake inhibition (IUI) at sufficient dose. These three chemicals are environmentally ubiquitous and cause IUI through the exact same mechanism of action. Coexposures are common, yet previous studies have evaluated these chemicals independently, rather than additively.[3,4]

At sufficiently large doses for weeks or months, these agents can cause a decrease in thyroid hormones. The mechanism of action is the inhibition of iodide uptake from the blood as it competitively binds to the sodium iodide symporter (NIS), a membrane-bound protein on the basal side of the thyrocyte.[5] In the thyroid, iodide is organified to produce thyroid hormones (thyroxine [tetraiodothyronine; T4] and triiodothyronine [T3]), and if thyroidal iodide stores are sufficiently reduced, this could result in decreased production of thyroid hormones.[6] In general, decreased thyroid hormone production causes increased release of thyroid stimulating hormone (thyrotropin [TSH]) from the anterior pituitary gland, which increases the thyroid's ability to take up iodide and produce active thyroid hormone. The developing fetus, in particular, requires adequate thyroid hormone levels, but because the fetus is supported by maternal thyroid hormone, iodide stores in the adult thyroid prevent against short-term iodide uptake fluctuations. Iodide uptake inhibition must be reduced by at least 70% and sustained for months or longer to deplete existing stores.[6] Furthermore, when iodide uptake is decreased, the body adapts by increasing the number and efficiency of NISs to pump more iodide into the thyroid.[6] As an adaptive effect, IUI precedes clinical hypothyroidism by several biochemical steps.[7]

Nitrate and thiocyanate are less potent inhibitors of NIS activity than perchlorate;[8] nevertheless, typical exposure levels are much higher.[9] Nitrate is formed endogenously in humans and is found in drinking water and foods such as spinach and carrots. Thiocyanates are formed endogenously from cyanates in certain foods and cyanide in smoking tobacco, and are commonly found in cruciferous vegetables. Perchlorate is environmentally ubiquitous and is naturally formed or man-made. When considering exposures to all three agents it is estimated that perchlorate is responsible for less than 1% of thyroidal IUI.[10,11] The effect of perchlorate on IUI cannot be distinguished from the effects of other NIS inhibitors.[10,12] It is useful to normalize the potencies of nitrate and thiocyanate to that of perchlorate to facilitate the dose addition. Relative potency factors (RPFs) for IUI at the NIS have been reported by others.[8,9] These RPFs can be used to calculate a total perchlorate equivalent concentration (PEC) to provide a cumulative measure of the total IUI load contributed by nitrate, thiocyanate, and perchlorate.

The reversible inhibition of the NIS by these three agents is well documented both in vitro[7,8] and in vivo.[13,14] Inhibition of the NIS occurs with a sigmoidal dose–response curve—as dose increases, so does the response—and there is a threshold below which no measurable effect occurs.[6] If inhibition is functionally important, reduced thyroid hormone (e.g., total T4 [TT4], free T4 [fT4], total T3 [TT3], or free T3 [fT3]) would be accompanied by elevations in TSH.

Two studies reported associations between urinary perchlorate and limited serum measures of thyroid function (TSH and TT4) at perchlorate exposure levels below the no observable effect level (NOEL) for IUI identified in human volunteers in Greer et al.[7] and used as the basis for the US EPA reference dose, using the National Health and Nutrition Examination Survey (NHANES) 2001 to 2002 data set.[3,4] The Centers for Disease Control and Prevention (CDC) has been collecting information through NHANES since the early 1960s to assess the health and nutritional status of the US population, based on age, sex, race/ethnicity, and income. NHANES 2001 to 2002 provides data for 11,039 individuals of all ages, collected between January 2001 and December 2002. NHANES 2001 to 2002 oversampled low-income persons, persons aged 12 to 19 and more than 60 years, African Americans, and Mexican Americans.[15] Collected information included demographic, examination, laboratory, questionnaire, and dietary interview variables.

The NHANES 2001 to 2002 data set released in 2004 measured only TT4 and TSH. Clinically, these measures provide an

From Intertox, Inc. (Ms Bruce and Drs Corey and Pleus), Seattle, Wash; and Division of Environmental Health Sciences, School of Public Health, University of Minnesota (Dr Mandel), Minneapolis, Minn.

Funding for this study was provided by the Perchlorate Study Group (PSG), a consortium of manufacturers and users of perchlorate. The PSG member companies include Aerojet General Corporation, American Pacific Corporation, Alliance Techsystems, Inc., and Lockheed Martin Corporation. Neither the PSG nor any of its member companies had any role in the conduct of the study or in the writing or editing of the article.

Intertox is a health science research firm and has conducted work for the PSG and other users and/or manufacturers of perchlorate. Dr. Pleus has served as an independent expert and has provided expert testimony on the toxicology of perchlorate. Dr. Mandel has done general consulting for Lockheed Martin, unrelated to this topic.

The authors declare no conflict of interest.

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initial screen for thyroid function; nevertheless, follow-up testing is required to determine whether these changes reflect short-term fluctuations and are adaptive or whether thyroid function is impaired. Per the American Thyroid Association,[16] "The free T4 fraction is the most important to determine how the thyroid is functioning . . . " and "Combining the TSH test with the fT4 of fTI [FT4 index] accurately determines how the thyroid gland is functioning." CDC added testing for more specific thyroid measures, including fT4, to NHANES in later years.

In 2010, CDC released a new data set providing analyses of thyroid measures from surplus serum samples saved from the NHANES 2001 to 2002 subjects.[17] The samples were reanalyzed for TT4 and TSH, as well as fT4, TT3, fT3, thyroglobulin (Tg), Tg antibody, and thyroperoxidase antibody. The inclusion of these measures enables a more complete assessment of thyroid function.

The purpose of this evaluation was to assess the additive relationship among urinary nitrate, thiocyanate, and perchlorate, and serum thyroid parameters in the 2001 to 2002 data set. On the basis of the known mechanism of action, we hypothesized that serum thyroid parameters would be associated with total PEC. The assessment was made using reanalyzed measures of thyroid hormone and TSH with the addition of the free hormone measures, which best capture the active component of the hormone, and a correction for urinary creatinine to account for variation in urine dilution. The ubiquitous nature of these environmental agents and the potential impact on the newborn and/or developing fetus adds to the importance of this understanding.

## METHODS

### Study Design

In NHANES 2001 to 2002, perchlorate, nitrate, thiocyanate, and iodide were measured in stored urine from one third of persons aged 6 years and older in the sampled population. Samples were analyzed for perchlorate, nitrate, and thiocyanate using ion chromatography tandem mass spectrometry and for iodide using inductively coupled plasma–mass spectrometry.[18] Subsequently, a bias (−4.7%) in the reported sample volumes due to the aliquoting technique used in NHANES 2001 to 2004 was discovered by CDC, and urinary analyses for perchlorate, nitrate, and thiocyanate were adjusted by a factor of 1.049 and rereleased.[18] Stored NHANES 2001 to 2002 serum samples were also reanalyzed for CDC by the University of Washington, Seattle, for total and free T4, total and free T3, TSH, Tg antibodies, and thyroid peroxidase antibodies, in samples from participants older than 12 years, using immunoenzymatic assay techniques.[17] An explanation for the reanalysis was not provided.

Prior to statistical analysis, urinary results were normalized for creatinine as follows:

Urinary anion concentration ($\mu$g/L)/[(Urinary creatinine (mg/dL))/100] = $\mu$g anion/g Cr

### Statistical Analysis

Data were analyzed using Stata 11 (College Station, TX) statistical software with survey commands to account for survey design and weighting, using 2-year sample weights corresponding to the one-third subsample for urinary perchlorate, nitrate, and thiocyanate. Multivariate regression was used to identify variables associated with thyroid measures. The significance threshold was set at 0.05 (two-tailed).

Regression models were constructed separately for each thyroid measure (TSH, TT3, TT4, fT3, and fT4). The total PEC was calculated by multiplying the RPF for IUI at the NIS for each anion by the amount of the anion in the urine, and summing the PECs for each anion. The relative molar potency of perchlorate to inhibit $^{125}$I uptake at the NIS was 240 and 15 times that of free nitrate and thiocyanate, respectively, or 150 and 8.8 on a weight basis (to extrapolate from ingested doses rather than internal serum concentrations).[8] The RPF applied to ingested thiocyanate was adjusted to account for thiocyanate bound to albumin in human blood serum (50%), resulting in an RPF of 17.6.[11] Thus, the equation for PEC is as follows:

$$\text{PEC} = [\text{perchlorate}]_{\text{urine}} + [\text{thiocyanate}]_{\text{urine}}/17.6 + [\text{nitrate}]_{\text{urine}}/150$$

Perchlorate equivalent concentrations were calculated using the creatinine-corrected urinary concentration of each anion.

Independent variables were selected on the basis of a hypothesis-driven approach, including demographic variables and other independent variables collected in the NHANES database that have been shown to potentially influence thyroid status. Variables considered were age (in months), sex, race/ethnicity (non-Hispanic white [referent], non-Hispanic black, Mexican American, other Hispanic, other race [including multiracial]), fasting time (in hours), body mass index (BMI), kcal (divided by 1000), serum albumin concentration (indicative of serum protein levels and involved in the transport of thyroid hormones),[19] serum cotinine concentration (a biomarker of tobacco smoke exposure), serum C-reactive protein concentration (a marker of low-grade inflammation associated with overt and subclinical hypothyroidism),[20,21] urinary iodide concentration, and use of medications reported to potentially impact thyroid hormone or TSH concentrations (specifically, $\beta$-blockers, furosemide, and glucocorticoid steroids and androgens). Because of their skewed distributions, urine concentrations of iodide, perchlorate, nitrate, and thiocyanate and serum concentrations of cotinine and C-reactive protein, as well as BMI and thyroid function measures, were log transformed. Correlations among variables were computed to examine possible associations among the various factors.

## RESULTS

### Reanalysis Using Enhanced Thyroid Hormone Data From NHANES 2001 to 2002

A total of 1960 participants aged 12 years or older had urinary perchlorate as well as TSH and TT4 measures in the "new" data set. Individuals with a reported history of thyroid disease or current use of thyroid medications (including levothyroxine and "unspecified" thyroid drugs) as well as methimazole and propylthiouracil, were excluded from analysis (77 females and 31 males). Because estrogen increases the ability of thyroglobulin to bind circulating T4 and T3,[22] pregnant women and women currently taking estrogen-containing medications were also excluded (an additional 205 females). Six subjects with extreme TT4 or TSH levels were also removed because of the potential for intrinsic thyroid disease. These included four females (with TSH = 0.004 mIU/L and TT4 = 14.6 $\mu$g/dL; TSH = 39.776 mIU/L and TT4 = 4.4 ug/dL; TSH = 0.023 mIU/L and TT4 = 17 $\mu$g/dL; and TSH = 0.002 mIU/L and TT4 = 24.7 $\mu$g/dL) and two males (with TSH = 350.517 mIU/L and TT4 = 0.5 $\mu$g/dL; and TSH = 290.60 mIU/L and TT4 = 1.8 $\mu$g/dL). The resulting data set consisted of 758 females and 883 males. Only those who had information for all covariates were included in the regression analysis (711 females and 833 males). The number of individuals, records missing, and arithmetic means for normally distributed covariates, geometric means for log normally distributed covariates, or percent in category for binary covariates, are presented in Table 1.

Urinary perchlorate was detected above the detection limit (0.05 $\mu$g/L) in all samples in the data set. Three nitrate measurements and four thiocyanate measurements were reported at concentrations slightly below their detection limits of 700 $\mu$g/L and 20 $\mu$g/L, respectively. Median urinary iodide concentrations were 133.9 $\mu$g/g creatinine in females and 135.6 $\mu$g/g creatinine in males. For males, significant correlations ($P < 0.001$), with

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**TABLE 1.** Means and Percent in Category for Covariates in Regression Model

| Variable | *n* | No. Missing | Arithmetic Mean (95% CI) | Geometric Mean (95% CI) | Percent in Category (95% CI) |
|---|---|---|---|---|---|
| Age, mo | 1,641 | 0 | 435.2 (422.7–447.8) | | |
| Fasting, hr | 1,641 | 0 | 11.0 (10.7–11.2) | | |
| Serum albumin, g/dL | 1,640 | 1 | 4.33 (4.31–4.34) | | |
| Serum TSH, mIU/L | 1,641 | 0 | | 1.40 (1.36–1.44) | |
| Serum T3 (ng/dL) | 1,641 | 0 | | 116.4 (115.4–117.5) | |
| Serum T3 free (pg/mL) | 1,641 | 0 | | 3.32 (3.30–3.34) | |
| Serum T4, $\mu$g/dL | 1,641 | 0 | | 8.05 (7.99–8.12) | |
| Serum T4–free, ng/dL | 1,641 | 0 | | 0.78 (0.77–0.79) | |
| Serum thyroglobulin, ug/L | 1,641 | 0 | | 7.93 (7.55–8.33) | |
| Serum thyroid peroxidase antibodies, IU/mL | 1,632 | 9 | | 0.87 (0.80–0.93) | |
| Serum thyroglobulin antibodies, IU/mL | 1,641 | 0 | | 0.78 (0.75–0.82) | |
| Total calories, Mcal | 1,591 | 50 | 2.24 (2.19–2.29) | | |
| BMI | 1,590 | 51 | | 25.4 (25.2–25.7) | |
| Serum cotinine, $\mu$g/L | 1,632 | 9 | | 0.43 (0.36–0.51) | |
| Serum C-reactive protein, mg/dL | 1,641 | 0 | | 0.11 (0.10–0.12) | |
| Urine creatinine, mg/dL | 1,641 | 0 | | 115.1 (111.1–119.2) | |
| Urine iodine, $\mu$g/L | 1,640 | 1 | | 163.8 (156.4–171.4) | |
| Urine nitrate, mg/L | 1,640 | 1 | | 50.5 (48.7–52.4) | |
| Urine perchlorate, $\mu$g/L | 1,641 | 0 | | 3.87 (3.71–4.03) | |
| Urine thiocyanate, mg/L | 1,639 | 2 | | 1.52 (1.44–1.60) | |
| Race/ethnicity | | | | | |
| Non-Hispanic white | 1,641 | 0 | | | 42.7 (40.3–45.1) |
| Non-Hispanic black | 1,641 | 0 | | | 22.3 (20.3–24.3) |
| Mexican American | 1,641 | 0 | | | 26.7 (24.5–28.8) |
| Other race | 1,641 | 0 | | | 3.8 (2.9–4.7) |
| Medication usage | | | | | |
| Furosemide | 1,641 | 0 | | | 2.3 (1.6–3.0) |
| Glucocorticoids and androgens | 1,641 | 0 | | | 1.5 (0.93–2.1) |
| $\beta$-Blocker | 1,641 | 0 | | | 4.0 (3.1–5.0) |
| Other drug | 1,641 | 0 | | | 37.9 (35.6–40.3) |

All subjects aged ≥12 years, NHANES 2001–2002. Excludes women who are pregnant or taking estrogen, and subjects with missing TSH, T4, or perchlorate values, outlier values of T4 or TSH, or a history of thyroid disease, or currently taking thyroid (eg, levothyroxine) or anti-thyroid drugs (eg, methimazole or propylthiouracil). BMI, body mass index; CI, confidence interval.

coefficients greater than ±0.30, were apparent when comparing age with log BMI, serum albumin, and log C reactive protein (correlation coefficients, 0.33, −0.40, and 0.40, respectively), and when comparing log C reactive protein with log BMI and serum albumin (correlation coefficients, 0.48 and −0.38, respectively). For females, significant correlations ($P < 0.001$), with coefficients greater than ±0.30, were apparent when comparing age with log BMI and log C reactive protein (correlation coefficients, 0.31 and 0.45, respectively) and when comparing serum albumin with log BMI and log C reactive protein (correlation coefficients, −0.35 and −0.40, respectively).

Log serum cotinine concentration (a biomarker of tobacco smoke exposure) was considered for inclusion in the models but was excluded because it was significantly ($P < 0.001$) correlated with both log creatinine-corrected urinary thiocyanate (correlation coefficients, 0.45 and 0.51 for males and females, respectively) and log creatinine-corrected total PEC (correlation coefficients, 0.29 and 0.31 for males and females, respectively). Thiocyanate is expected to be greater in smokers independent of dietary exposures. Significant positive ($P < 0.001$), simple correlations were observed between the logs of creatinine-corrected urinary perchlorate and iodide (correlation coefficients, 0.38 in males and 0.38 in females), urinary perchlorate and nitrate (correlation coefficients, 0.33 in males and 0.35 in females), and urinary nitrate and thiocyanate (correlation coefficients, 0.33 in males and 0.30 in females). Correlation coefficients between the logs of creatinine-corrected IUI agents and thyroid parameters in males and females ranged from −0.15 to 0.19 (data not shown). Because of the correlation of iodide with urinary perchlorate, the impact of iodine status was evaluated by dividing the population into lower and higher iodide groups, using the median urinary iodide level for the population as the division. Potential correlations among urinary perchlorate, nitrate, and thiocyanate were addressed by evaluating exposure as total PEC.

Total PEC was not associated with TSH or fT4 (Table 2) or with TT3 or fT3 (Table 3) in males and females analyzed together or separately by sex. Total PEC was negatively associated with TT4 (coefficient, −0.0251; $P = 0.020$; Table 2) in the total population, but not in either males or females analyzed separately. To determine whether this association was driven by any one IUI agent, we analyzed the agents separately. In the combined population, there was a significant negative relationship between TT4 and both nitrate (coefficient, −0.018; $P = 0.045$) and thiocyanate (coefficient, −0.016; $P = 0.043$), but not between TT4 and perchlorate (coefficient, −0.005; $P = 0.516$).



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**TABLE 2.** Regression of Log TSH, Log TT4, and Log fT4 Measurements on Covariates in Males + Females, Males Alone, and Females Alone in NHANES 2001–2002

| | Males + Females ($n$ = 1544) | | | Males ($n$ = 833) | | | Females ($n$ = 711) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Thyroid Measure** | **Coefficient** | **SE** | ***P*** | **Coefficient** | **SE** | ***P*** | **Coefficient** | **SE** | ***P*** |
| Log TSH | $r^2 = 0.067$ | | | $r^2 = 0.040$ | | | $r^2 = 0.144$ | | |
| Intercept | −0.2825 | 0.2723 | 0.316 | −0.0112 | 0.2978 | 0.971 | −0.236 | 0.3116 | 0.460 |
| Log total PEC* | −0.0182 | 0.0411 | 0.665 | −0.0668 | 0.0525 | 0.223 | 0.015 | 0.0560 | 0.797 |
| Age in months | 0.0001 | 0.0000 | 0.082 | 0.0000 | 0.0000 | 0.978 | **0.000** | **0.0001** | **0.030** |
| Fasting time, hours | −0.0030 | 0.0016 | 0.080 | −0.0005 | 0.0031 | 0.876 | **−0.005** | **0.0014** | **0.001** |
| Log BMI | 0.1595 | 0.1232 | 0.215 | 0.1102 | 0.1212 | 0.378 | 0.112 | 0.2031 | 0.588 |
| Calories/1000 | −0.0075 | 0.0110 | 0.509 | −0.0057 | 0.0112 | 0.619 | −0.018 | 0.0147 | 0.245 |
| Non-Hispanic white | **0.0743** | **0.0153** | **<0.001** | **0.0723** | **0.0227** | **0.006** | **0.087** | **0.0225** | **0.001** |
| Serum albumin | 0.0620 | 0.0306 | 0.061 | 0.0378 | 0.0487 | 0.450 | **0.058** | **0.0262** | **0.044** |
| Log CRP | **0.0442** | **0.0107** | **0.001** | −0.0017 | 0.0194 | 0.929 | **0.088** | **0.0217** | **0.001** |
| $\beta$-Blocker use | 0.0477 | 0.0301 | 0.133 | 0.0673 | 0.0409 | 0.121 | 0.013 | 0.0557 | 0.823 |
| Furosemide use | −0.0100 | 0.0576 | 0.865 | −0.0032 | 0.0965 | 0.974 | 0.021 | 0.0650 | 0.752 |
| GC/androgen use | **0.1926** | **0.0428** | **<0.001** | 0.1654 | 0.0874 | 0.078 | **0.224** | **0.0445** | **<0.001** |
| Log TT4 | $r^2 = 0.089$ | | | $r^2 = 0.069$ | | | $r^2 = 0.133$ | | |
| Intercept | 0.9861 | 0.0843 | <0.001 | 1.0425 | 0.1272 | <0.001 | 0.890 | 0.1493 | <0.001 |
| Log total PEC* | **−0.0251** | **0.0097** | **0.020** | −0.0274 | 0.0159 | 0.105 | −0.022 | 0.0169 | 0.221 |
| Age in months | 0.0000 | 0.0000 | 0.335 | 0.0000 | 0.0000 | 0.789 | 0.0000 | 0.0000 | 0.308 |
| Fasting time, hours | 0.0012 | 0.0006 | 0.071 | 0.0015 | 0.0008 | 0.068 | 0.001 | 0.0006 | 0.129 |
| Log BMI | −0.0018 | 0.0244 | 0.942 | −0.0044 | 0.0401 | 0.914 | 0.002 | 0.0346 | 0.951 |
| Calories/1000 | **−0.0080** | **0.0027** | **0.010** | −0.0060 | 0.0030 | 0.069 | −0.011 | 0.0069 | 0.126 |
| Non-Hispanic white | **−0.0185** | **0.0059** | **0.007** | **−0.0144** | **0.0061** | **0.033** | **−0.024** | **0.0077** | **0.007** |
| Serum albumin | 0.0042 | 0.0156 | 0.789 | −0.0089 | 0.0170 | 0.608 | 0.027 | 0.0230 | 0.260 |
| Log CRP | **0.0241** | **0.0064** | **0.002** | **0.0225** | **0.0069** | **0.005** | **0.027** | **0.0072** | **0.002** |
| $\beta$-Blocker use | **0.0322** | **0.0140** | **0.037** | 0.0281 | 0.0211 | 0.204 | **0.047** | **0.0159** | **0.009** |
| Furosemide use | −0.0034 | 0.0262 | 0.897 | −0.0114 | 0.0350 | 0.750 | 0.005 | 0.0297 | 0.867 |
| GC/androgen use | −0.0286 | 0.0182 | 0.137 | −0.0142 | 0.0325 | 0.668 | **−0.049** | **0.0209** | **0.033** |
| Log fT4 | $r^2 = 0.031$ | | | $r^2 = 0.042$ | | | $r^2 = 0.052$ | | |
| Intercept | −0.0662 | 0.0794 | 0.418 | 0.1108 | 0.1056 | 0.310 | −0.239 | 0.1574 | 0.150 |
| Log total PEC* | −0.0066 | 0.0109 | 0.554 | −0.0019 | 0.0151 | 0.903 | −0.012 | 0.0108 | 0.271 |
| Age in months | 0.0000 | 0.0000 | 0.295 | 0.0000 | 0.0000 | 0.906 | 0.000 | 0.0000 | 0.206 |
| Fasting time, hours | 0.0009 | 0.0006 | 0.129 | 0.0010 | 0.0007 | 0.176 | 0.001 | 0.0007 | 0.216 |
| Log BMI | −0.0602 | 0.0326 | 0.084 | **−0.1063** | **0.0317** | **0.004** | −0.004 | 0.0499 | 0.940 |
| Calories/1000 | **−0.0051** | **0.0021** | **0.029** | **−0.0067** | **0.0022** | **0.009** | −0.005 | 0.0067 | 0.469 |
| Non-Hispanic white | −0.0131 | 0.0048 | 0.015 | −0.0080 | 0.0049 | 0.121 | **−0.018** | **0.0075** | **0.027** |
| Serum albumin | 0.0157 | 0.0134 | 0.260 | −0.0099 | 0.0142 | 0.495 | 0.039 | 0.0235 | 0.121 |
| Log CRP | **0.0095** | **0.0039** | **0.027** | **0.0122** | **0.0040** | **0.008** | 0.003 | 0.0066 | 0.646 |
| $\beta$-Blocker use | 0.0119 | 0.0080 | 0.156 | 0.0004 | 0.0117 | 0.972 | 0.037 | 0.0182 | 0.064 |
| Furosemide use | 0.0186 | 0.0182 | 0.324 | 0.0146 | 0.0176 | 0.419 | 0.020 | 0.0356 | 0.581 |
| GC/androgen use | 0.0076 | 0.0182 | 0.682 | 0.0022 | 0.0243 | 0.930 | 0.008 | 0.0191 | 0.696 |

$P < 0.05$ for data given in bold. BMI, body mass index; CRP, C reactive protein; fT4, free T4; GC, glucocorticoid; PEC, perchlorate equivalent concentration; SE, standard error; TSH, thyroid stimulating hormone; TT4, total thyroxin.

*Log total PEC = $[\text{perchlorate}]_{\text{urine}} + [\text{thiocyanate}]_{\text{urine}}/17.6 + [\text{nitrate}]_{\text{urine}}/150$, $\mu$g/g creatinine.

In the combined population with urinary iodide less than the median and in females analyzed separately regardless of iodine status, there were no significant relationships between total PEC and any thyroid variable, in the direction expected for an IUI agent. In females with urinary iodide less than the median, there was a significant positive relationship between total PEC and fT3 (coefficient, 0.020; $P = 0.033$); nevertheless, a positive relationship is in the opposite direction expected for an IUI agent.

In males analyzed separately with urinary iodine less than the median, there was a significant negative relationship between total PEC and fT3 (coefficient, −0.020; $P = 0.028$). Examination of the components of total PEC for this population revealed significant associations of fT3 with thiocyanate (coefficient, −0.014; $P = 0.005$), but not nitrate (coefficient, −0.007; $P = 0.479$) or perchlorate (coefficient, −0.0004; $P = 0.954$).

In the combined population and in males with urinary iodide greater than the median, total PEC was significantly negatively associated with TT4 (for combined population: coefficient, −0.057; $P < 0.001$; for males: coefficient, −0.049; $P = 0.026$). In both of these populations, TT4 was significantly associated with thiocyanate (for

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**TABLE 3.** Regression of Log TT3 and Log fT3 Measurements on Covariates in Males + Females, Males Alone, and Females Alone in NHANES 2001—2002

| | Males + Females ($n$ = 1544) | | | Males ($n$ = 833) | | | Females ($n$ = 711) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Thyroid Measure** | **Coefficient** | **SE** | ***P*** | **Coefficient** | **SE** | ***P*** | **Coefficient** | **SE** | ***P*** |
| Log TT3 | $r^2 = 0.144$ | | | $r^2 = 0.180$ | | | $r^2 = 0.124$ | | |
| Intercept | 1.9589 | 0.0825 | <0.001 | 1.9874 | 0.0979 | <0.001 | 2.1300 | 0.1283 | <0.001 |
| Log total PEC* | − 0.0089 | 0.0081 | 0.289 | − 0.0063 | 0.0095 | 0.516 | − 0.0069 | 0.0154 | 0.659 |
| Age, mo | **− 0.0001** | **0.0000** | **<0.001** | **− 0.0001** | **0.0000** | **<0.001** | **− 0.0001** | **0.0000** | **<0.001** |
| Fasting time, hr | 0.0004 | 0.0004 | 0.358 | 0.0001 | 0.0004 | 0.795 | 0.0005 | 0.0007 | 0.505 |
| Log BMI | 0.0472 | 0.0354 | 0.202 | 0.0555 | 0.0403 | 0.188 | − 0.0084 | 0.0472 | 0.862 |
| Calories/1000 | 0.0009 | 0.0032 | 0.789 | 0.0004 | 0.0040 | 0.922 | − 0.0097 | 0.0047 | 0.055 |
| Non-Hispanic white | − 0.0111 | 0.0053 | 0.054 | − 0.0070 | 0.0063 | 0.286 | − 0.0166 | 0.0084 | 0.067 |
| Serum albumin | **0.0273** | **0.0114** | **0.031** | 0.0207 | 0.0133 | 0.141 | 0.0062 | 0.0164 | 0.712 |
| Log CRP | **0.0091** | **0.0039** | **0.035** | 0.0098 | 0.0071 | 0.186 | 0.0064 | 0.0071 | 0.378 |
| $\beta$-Blocker use | 0.0103 | 0.0149 | 0.499 | 0.0171 | 0.0183 | 0.366 | − 0.0104 | 0.0223 | 0.649 |
| Furosemide | **− 0.0483** | **0.0180** | **0.017** | **− 0.0722** | **0.0263** | **0.015** | − 0.0151 | 0.0242 | 0.542 |
| GC/androgen use | − 0.0001 | 0.0302 | 0.997 | − 0.0028 | 0.0434 | 0.949 | 0.0106 | 0.0286 | 0.715 |
| Log fT3 | $r^2 = 0.272$ | | | $r^2 = 0.324$ | | | $r^2 = 0.244$ | | |
| Intercept | 0.3448 | 0.0397 | <0.001 | 0.4443 | 0.0552 | <0.001 | 0.4459 | 0.0748 | <0.001 |
| Log Total PEC* | − 0.0009 | 0.0054 | 0.875 | − 0.0045 | 0.0090 | 0.625 | 0.0090 | 0.0087 | 0.317 |
| Age, mo | **− 0.0001** | **0.0000** | **<0.001** | **− 0.0001** | **0.0000** | **<0.001** | **− 0.0001** | **0.0000** | **<0.001** |
| Fasting time, hr | **0.0007** | **0.0002** | **0.013** | **0.0009** | **0.0003** | **0.013** | 0.0005 | 0.0004 | 0.216 |
| Log BMI | **0.0558** | **0.0186** | **0.009** | 0.0403 | 0.0278 | 0.167 | 0.0268 | 0.0344 | 0.448 |
| Calories/1000 | 0.0021 | 0.0010 | 0.052 | − 0.0004 | 0.0017 | 0.837 | **− 0.0051** | **0.0023** | **0.044** |
| Non-Hispanic white | **− 0.0087** | **0.0025** | **0.003** | **− 0.0088** | **0.0039** | **0.040** | **− 0.0085** | **0.0039** | **0.044** |
| Serum albumin | **0.0309** | **0.0057** | **<0.001** | **0.0197** | **0.0050** | **0.001** | 0.0110 | 0.0090 | 0.241 |
| Log CRP | 0.0025 | 0.0032 | 0.445 | 0.0036 | 0.0023 | 0.135 | − 0.0017 | 0.0057 | 0.770 |
| $\beta$-Blocker use | − 0.0018 | 0.0068 | 0.789 | − 0.0050 | 0.0086 | 0.569 | − 0.0054 | 0.0084 | 0.529 |
| Furosemide | − 0.0115 | 0.0081 | 0.174 | − 0.0124 | 0.0155 | 0.436 | − 0.0073 | 0.0089 | 0.427 |
| GC/androgen use | − 0.0063 | 0.0128 | 0.631 | − 0.0189 | 0.0190 | 0.337 | 0.0076 | 0.0108 | 0.494 |

$P < 0.05$ for data given in bold. BMI, body mass index; CRP, C reactive protein; fT3, free T3; GC, glucocorticoid; PEC, perchlorate equivalent concentration; SE, standard error; TT3, total triiodothyronine.

*Log total PEC = $[\text{perchlorate}]_{\text{urine}} + [\text{thiocyanate}]_{\text{urine}}/17.6 + [\text{nitrate}]_{\text{urine}}/150$, $\mu$g/g creatinine.

combined population: coefficient, −0.037; $P < 0.001$; for males: coefficient, −0.039; $P < 0.001$), and in the combined population but not in males only, TT4 was also associated with nitrate (coefficient, −0.036; $P < 0.001$). TT4 was not associated with perchlorate in either population. In males with urinary iodide greater than the median, there was also a significant positive relationship between total PEC and TT3 (coefficient, 0.034; $P = 0.010$), in the opposite direction expected for an IUI agent.

Regression analyses were also run using the logarithm of PEC calculated with NIS inhibitor levels unadjusted for creatinine level and the log of creatinine level as a separate independent variable in the model. No substantial differences in model results were obtained using this approach.

## DISCUSSION

Using the newly released NHANES 2001 to 2002 data set for thyroid variables and urinary measures of chemical agents that cause IUI, we observed no consistent or functionally relevant association between PEC and thyroid measures, including TSH. Total PEC was associated with TT4 in the combined population, but the relationships did not remain in either males or females alone. When the impact of individual IUI agents on these thyroid variables was analyzed separately, TT4 was significantly negatively associated with both nitrate and thiocyanate but not perchlorate.

FT4 is the most important measure along with TSH to determine thyroid function.[16] Because there are many influences on measured thyroid hormone, the combined effect of both of these variables is critical in the determination of functional abnormalities involving thyroid hormone status and the pituitary gland. In these analyses, total PEC was not associated with fT4 or TSH in any group.

Even with statistical significance, the correlation coefficients between variables are low. The highest $r^2$ is 0.324, which means that only a relatively small amount of variance of the dependent variables (thyroid measures) is accounted for by the explanatory variables. Similar $r^2$ values have been reported for similar analyses.[3]

Iodide uptake inhibition resulting from perchlorate exposure is the same as IUI caused by the other agents. In the individual study subjects, perchlorate contributed an average of only 1.2% of the total PEC measured in urine (range, <1% to 18.0%), whereas nitrate contributed an average of 74.5% (range, 2.9% to 99.0%) and thiocyanate an average of 24.0% (range, 0.6% to 96.0%). This is similar to what others have reported.[10]

The findings based on the new CDC data set are not consistent with the results of other reports using the initially released NHANES 2001 to 2002 thyroid measures data set.[3,4] Using the NHANES 2001 to 2002 data, Blount et al[3] reported that perchlorate measured in spot urine samples was positively associated with serum TSH and negatively associated with serum TT4 in females aged 12 years and older



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with urinary iodide levels less than 100 $\mu$g/L. This relationship was more pronounced in current smokers.[4] Urinary nitrate was negatively associated with TT4 only in females with urinary iodide greater than 100 $\mu$g/L,[3] but was later reported to have no strong associations.[4] Urinary thiocyanate was negatively associated with TSH in females with urinary iodide greater than 100 $\mu$g/L.[3] In small subgroups of females with urinary iodide less than 100 $\mu$g/L, urinary thiocyanate was negatively associated with TT4 in a subgroup ($n = 78$) with urinary thiocyanate greater than 1800 $\mu$g/L and positively associated with TSH in a subgroup ($n = 107$) with midlevel urinary thiocyanate (751 to 1800 $\mu$g/L).[4]

We did not see a significant relationship between TSH and total PEC in the entire population, or in males or females analyzed separately or in these populations segregated by low and high urinary iodide. We did see a significant negative relationship between TT4 and total PEC in the entire population, but the association of the PEC variable with TT4 appears to be largely due to the contributions of nitrate and thiocyanate because TT4 was significantly negatively associated with nitrate and thiocyanate but not perchlorate. Furthermore, TT4 was only negatively associated with nitrate and thiocyanate in the subset with high urinary iodide, not the subset with low urinary iodide.

In addition to inclusion of nitrate and thiocyanate, our analyses differed from previous analyses of IUI agents and thyroid variables in NHANES 2001 to 2002 in several key respects. First, the data sets differed: the initially released NHANES 2001 to 2002 data set included TSH and TT4 data for 534 subjects not included in the new data release. These included 148 of the 1111 females older than 12 years evaluated in Blount et al.[3] Second, the new analyses were conducted at a different laboratory. Comparing the results of the original and new analyses for the samples in our data set, the geometric means for TT4 are 7.73 $\mu$g/dL and 8.05 $\mu$g/dL, respectively, and for TSH are 1.34 mIU/L and 1.39 mIU/L, respectively. The old and new means for both measures differ significantly ($P < 0.001$). Third, the current analysis normalizes for creatinine. For chemicals with a short biological half-life (eg, perchlorate, nitrate, iodide), concentrations in spot urine samples are known to be highly variable between samples, due to within- and between-day variations in urine volume and intake of exogenous compounds.[23] Factors shown to influence concentrations include fasting time, time of day, nature of the last meal, sample dilution, collection method, preservation method, sample interferences, and analytical method.[24] Urinary iodide concentrations in 24-hour samples vary up to threefold from one day to another. This suggests that reliance on a single sample without attempting to correct, for example, using creatinine, is insufficient to determine iodine status.[24] Without creatinine adjustment, population iodide excretion estimates require 100 to 500 spot urine samples for each group or subgroup, and fewer than 10 urine samples in an individual may be misleading.[25] This use of spot urine is an inherent limitation in using the NHANES data set when evaluating chemicals with short biological half-lives and although creatinine correction does not eliminate this issue, it is an enhancement compared with previous studies.

Finally, the current analysis segregates the populations into lower and higher iodide groups on the basis of the median creatinine-corrected urinary iodide level in each population, rather than a cut point of 100 $\mu$g/L. In the total study population of 1641 individuals, 442 (26.9%) had spot urinary iodide concentrations $<100$ $\mu$g/L. Of the 758 females, 273 (36.0%) had urinary iodide less than 100 $\mu$g/L, and of the 883 males, 169 (19.1%) had urinary iodide less than 100 $\mu$g/L. Dividing the groups on the basis of median provides larger sample populations for statistical analysis. The World Health Organization has stated that a median urinary iodide concentration within a population, based on spot samples, of less than 100 $\mu$g/L is indicative of iodine deficiency within the population.[26] The median urinary iodide concentration for this population was much higher (172.0 $\mu$g/L; 194.5 $\mu$g/L for males and 141.0 $\mu$g/L for females), indicating this population was not iodine-deficient.

The lack of consistent associations of IUI agents with thyroid measures in the NHANES data set is not surprising considering that measured urinary levels correspond to doses well below the established NOEL for perchlorate. The estimated 95th percentile intake of perchlorate, based on urinary perchlorate measures in the original NHANES 2001 to 2002 data set, was 0.234 $\mu$g/kg per day.[27] By comparison, the NOEL for perchlorate in adult humans has been reported to be 7 $\mu$g/kg per day, a level at which no significant increase was seen in IUI.[7] If IUI does not occur, no subsequent effects in the continuum of effects will occur, including changes in TT4, TSH, or any of the thyroid parameters measured in NHANES. In addition, the findings of associations between low urinary perchlorate levels and thyroid function[3,4] were not seen in other studies. For example, in studies of first trimester pregnancies using five independent data sets of women in Cardiff, Turin, Los Angeles, Cordoba, and Athens, no associations were reported between urinary perchlorate and serum TSH, fT4, and thyroperoxidase antibody.[28–30] Urinary perchlorate was also not associated with either fT4 or TSH in pregnant women in Chile.[31]

## CONCLUSIONS

In 2011, CDC released reanalyzed samples from their NHANES 2001 to 2002 data set. This new set provides updated results for the two thyroid measures (TSH and TT4) included in the initial release as well as additional thyroid measures, including fT3, fT4, TT3, thought to best reflect thyroid function.

The new data do not support a consistent relationship between exposure to the combination of IUI agents and thyroid measures. TT4 was associated with total PEC, but these relationships appeared to be determined by nitrate and thiocyanate. Perchlorate alone was not associated and would not be expected because the potential contribution of perchlorate to IUI is relatively low compared with nitrate and thiocyanate. No evidence of functional abnormalities in thyroid function (eg, low thyroid hormone coupled with high TSH) is seen in the enhanced data set despite the availability of more thorough measures of exposure to IUI agents and enhanced estimates of thyroid function.

As the mechanism of action of perchlorate on the thyroid is well understood and exposures to NHANES 2001 to 2002 participants were below the reported NOEL for perchlorate, no effects on thyroid parameters would be expected to occur. The findings of this investigation support this understanding.

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