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Mendez Jr, William to
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Biomarkers of perchlorate exposure are correlated with circulating thyroid hormone levels in the 2007–2008 NHANES

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ABSTRACT

Previous epidemiological studies provide conflicting evidence as to whether environmental perchlorate exposure can affect levels of circulating thyroid hormones in the general population. We investigated the statistical relationships between biomarkers of perchlorate exposure and serum thyroid hormone levels in 2007–2008 National Health and Nutrition Evaluation Survey (NHANES) subjects. Generalized additive mixed models (GAMMs) were developed to estimate the relationships between T3 and T4 levels and creatinine-adjusted urinary perchlorate excretion. The models included covariates related to gender, age, ethnicity, income, smoking status, prescription medications, and biomarkers of exposures to other goitrogenic ions and phthalate ester metabolites. Where necessary, relationships between hormone levels and covariates were represented as nonlinear smoothed terms. The effect of the hypothalamic–pituitary–thyroid (HPT) axis on serum hormone levels was taken into account by including a term for thyroid stimulating hormone (TSH) in the models. Regression coefficients for perchlorate were significant and negative in GAMMs predicting total T4 and free T3 levels in males, females, and for the entire cohort when phthalate ester biomarkers and other covariates were included. Coefficients for perchlorate were also significant and negative in regressions predicting free T4 levels in males and in the entire study population. The consistency of these results suggests that HPT axis controls do not completely compensate for small changes in thyroid hormone levels associated with perchlorate and phthalate ester exposures.

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1. Introduction

Perchlorate is a ubiquitous environmental contaminant that occurs naturally in soils and groundwater as the result of geochemical reactions, particularly in arid regions (Dasgupta et al., 2005). Perchlorate also has been released into the environment as a consequence of nitrate fertilizer usage (Dasgupta et al., 2006), and as a result of pyrotechnic and propellant manufacturing (Soldin et al., 2001). Low levels of perchlorate

have been detected in 2.4 percent of all public drinking water sources and in drinking water systems in 25 states (U.S. EPA, 2008). Perchlorate also occurs widely in foodstuffs, including leafy green vegetables, grains (Sanchez et al., 2005a, 2005b), and dairy products (Kirk et al., 2005).

Because perchlorate is not appreciable bioconcentrated or produced naturally as a metabolite, urinary excretion can serve as a biomarker of recent exposures. From 2001 to 2004, urinary perchlorate excretion was measured in one-third of subjects age six years or older in the U.S. National Health and Nutrition Evaluation Survey (NHANES) conducted by the National Center for Health Statistics (NCHS, 2010). Beginning in 2005, urinary perchlorate was measured in all subjects age six or older, and drinking water perchlorate concentrations have also been measured in 50 percent of the NHANES subjects (NCHS, 2010, 2011). Blount et al. (2006) reported that the geometric mean and 95 percent confidence interval for urinary perchlorate were 2.84 (2.54–3.18) $\mu\text{g}/\text{l}$ in 1111 subjects from the 2001–2002 NHANES.

Using data from the U.S. Environmental Protection Agency's (EPA) National Contaminant Occurrence Database (NCOD) (US EPA, 2008) and dietary survey data, Mendez et al. (2010) estimated that the median perchlorate intake by reproductive-age women in the United States was 68 ng/kg day. They estimated the upper

Abbreviations: BMI, body mass index; BPA, bisphenol A; EPA, U.S. Environmental Protection Agency; GAMMS, generalized additive mixed models; HPT, hypothalamic–pituitary–thyroid; MCP, mono-(3-carboxypropyl) phthalate; MECCP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MIBP, mono-isobutyl phthalate; NCHS, National Center for Health Statistics; NCOS, National Contaminants Occurrence Database; NHANES, National Health and Nutrition Evaluation Survey; NIS, Sodium-iodide symporter; NSAID, non-steroidal anti-inflammatory drug; PIR, poverty–income ratio; RAIU, radioactive iodide thyroid uptake; SAS[®], Statistical Analysis System[®]; SSRIs, selective serotonin reuptake inhibitor; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone

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95th-percentile total intake to be 160 ng/kg day, below EPA's reference dose of 700 ng/kg day. The bulk of perchlorate came from food, primarily leafy vegetables, with 3–8 percent of the aggregate intake (3–13 ng/kg day) coming from drinking water. The median intake estimated from dietary and water sources closely matched estimates derived using urinary perchlorate excretion data from the 2001–2002 and 2003–2004 NHANES (56 and 45 ng/kg day, respectively). Blount et al. (2010) estimated that the U.S. population median perchlorate intake from drinking water was 9.11 ng/kg day, based on perchlorate water concentration and drinking water intake data from the 2005–2006 NHANES. They also concluded that drinking water contributed a relatively small proportion of the overall population perchlorate intake.

Perchlorate is of concern from a public health standpoint owing to its potential impacts on thyroid function. Although high doses of perchlorate were historically used to inhibit iodine uptake in thyroid function tests (Wolff, 1998), and the ability of perchlorate to inhibit thyroid iodide uptake by the sodium-iodide symporter (NIS) has been demonstrated in vitro (Tonacchera et al., 2004), the impacts of long-term low-dose exposures are less clear. Greer et al. (2002) reported a dose-dependent inhibition of radioactive iodide thyroid uptake (RAIU) in 37 healthy male and female adults receiving 0.007–0.5 mg/kg day perchlorate in drinking water for 14 day, which was fully reversible after cessation of exposure. The average perchlorate dose estimated to produce a 20-percent inhibition of RAIU ranged from 20.3 to 23.1 µg/kg day, depending on the timing of measurement relative to perchlorate administration. Greer et al. (2002) found no statistically significant association between serum thyroid hormone levels TSH, total tri-iodothyronine [T3], and free and total thyroxine [T4] and perchlorate dose among the study subjects. Similarly, Braverman et al. (2006) observed no effects on thyroid function (radioactive thyroid uptake, serum T3, T4 index, TSH, or thyroglobulin levels) among 14 healthy adult volunteers exposed to 0.5 or 3.0 mg potassium perchlorate daily for six months.

In addition, two occupational studies found no effect (Gibbs et al., 1998, Lamm et al., 1999) on thyroid hormone levels in workers exposed to 0.01–432 mg/kg day estimated perchlorate intake, while Braverman et al. (2005) identified small, reversible post-shift changes in RAIU, T3, and T4 levels in workers receiving an average of 0.3 mg/kg day perchlorate, compared to unexposed referents. Results of in vitro studies (Tonacchera et al., 2004) suggest that typical environmental exposures are unlikely to appreciably inhibit the sodium-iodide symporter, the putative key event in perchlorate thyroid effects.

Epidemiological studies of general populations exposed to low-to-moderate levels of perchlorate in drinking water generally have found no significant effects on thyroid function, although small sample size, large uncertainties in exposure characterization, and other design issues limit the sensitivity of most published studies (see NRC, 2005 for a review). Of particular concern, however, are potential impacts on reproductive-age women and on fetal development, as even relatively mild thyroid dysfunction (hypothyroxinemia) during pregnancy has been associated with increased risk of neurodevelopmental defects in offspring (Kooistra et al., 2006; Pop et al., 1999, 2003; Henrichs et al., 2010).

Enhancing this concern was the finding by Blount et al. (2006) of statistically significant relationships between urinary perchlorate excretion and total T4 and TSH levels in 1111 adult females included in the 2001–2002 NHANES, in regressions that controlled for age, ethnicity, body mass index (BMI), prescription medication use, biomarkers of exposure to other goitrogenic ions, and other covariates. The negative relationship with T4 was statistically significant only for women with low urinary iodine levels (less than 100 µg/L). The positive relationship with TSH was

significant both for women with low (< 100 µg/L) and sufficient (> 100 µg/L) urinary iodine levels. Steinmaus et al. (2007) also analyzed the 2001–2002 NHANES data and found that serum cotinine levels and urinary thiocyanate excretion, both indicators of tobacco smoke exposure, modified the association of perchlorate with total T4 levels, but the relationship remained significant irrespective of smoking status.

Interpretation of the Blount et al. (2006) and Steinmaus et al. (2007) studies is difficult because of limitations inherent in the 2001–2002 NHANES data. During this period, the survey measured only two indicators of thyroid function: total serum T4 and TSH. Serum levels of the active thyroid hormone, T3, were not measured, and “free” T3 and T4 (levels of hormones not bound to serum proteins) were not estimated, precluding a broader analysis of perchlorate impacts on circulating hormones and thyroid function. Perhaps most importantly, thyroid antibody levels, which can be important indicators of autoimmune thyroid disease and other thyroid disorders, were not assayed. These limitations, and negative findings in several recent studies of perchlorate impacts on thyroid hormone levels (Téllez Téllez et al., 2005; Gibbs and van Landingham, 2008; Pearce et al., 2010), have called into question the generality of the relationship between environmental perchlorate exposure and thyroid hormone levels.

The 2007–2008 NHANES provides much more comprehensive data for investigating relationships between thyroid hormone levels and urinary perchlorate excretion because this recent data set includes information from a complete thyroid function battery of tests and provides demographic, ethnicity, and socioeconomic data for each subject. Urinary excretion of perchlorate and other goitrogens (thiocyanate and nitrate) also were measured, along with urinary iodine and exposure biomarkers for pesticides, phthalate esters, and other chemicals suspected of affecting thyroid function. This paper presents results of an analysis of the relationship between covariate-adjusted perchlorate excretion and serum thyroid hormone levels in the 2007–2008 NHANES data and discusses the public health implications of these findings.

2. Methods

2.1. Data source

All data used in this study came from the 2007–2008 National Health and Nutrition Evaluation Survey (NHANES.) The NHANES program is an ongoing series of cross-sectional investigations conducted by NCHS of the U.S. Centers for Disease Control and Prevention (CDC) to assess the health and nutritional status of civilian, non-institutionalized adults and children in the United States. Each survey is based on a complex, multistage probability design that is used to select a nationally representative sample. The NHANES program combines interviews and physical examinations at a mobile examination center, during which blood and urine samples are obtained for analysis (NCHS, 2010).

2.2. Selection of study population

The relationships between perchlorate excretion and thyroid hormone levels were analyzed for a subset of the 2007–2008 NHANES subjects having (1) complete thyroid panel results, (2) urinary excretion data for perchlorate, thiocyanate, and nitrate, and (3) data on urinary excretion of phthalate ester metabolites and bisphenol A (BPA). Phthalate ester and BPA excretion data were included in the analysis because Meeker and Ferguson (2011) have reported that urinary excretion of these substances is correlated with thyroid hormone levels in an overlapping subset of the 2007–2008 NHANES subjects. Thus, controlling for phthalate ester excretion was necessary, to estimate the independent impact of perchlorate on hormone levels.

Thyroid panel data were available for 6265 of the 6917 NHANES subjects age 12 years and older from whom laboratory samples were obtained (NCHS, 2011). Urinary perchlorate excretion data were reported for a largely overlapping sample of 7629 of subjects age six years and older. Phthalate ester excretion data, however, were available only for a much smaller subsample (2531). Thus, the

final study population used in the analysis (the intersection of the above subsets) represented only a small proportion of the total 2007–2008 NHANES subjects.

Currently pregnant women were excluded from the analysis, as were individuals reporting current thyroid disease, and those reporting consumption of levothyroxine or other thyroid disease medication. Three women with apparent hyperthyroidism (total and free T4 levels above 99th percentiles in the study population, accompanied by low or undetectable TSH levels) were also excluded. The final data set included 1887 subjects; 970 males and 907 females. The imbalance between the numbers of men and women in the data set is due primarily to more women having reported current consumption of thyroid medications. Subjects included in the analysis were generally similar to those who were excluded with regard to other covariates (Supplementary Table 1).

2.3. Prescription medication use

Reported prescription medications were divided into categories known to affect thyroid function or thyroid hormone measurements, including oral estrogen preparations, nonsteroidal anti-inflammatory drugs, selective serotonin reuptake inhibitors (SSRIs), oral corticosteroids, and beta-blockers (Blount et al., 2006). Dummy variables were included in the analysis for each type of medication.

2.4. Frequency of analytical results below reporting limits

More than 99 percent of the laboratory results for the thyroid panel analytes and urinary excretion of perchlorate, thiocyanate, and nitrate were above analytical reporting limits (Supplementary Table 2). Nondetects were more frequent for antithyroid peroxidase antibody (5.6 percent of results) and antithyroglobulin antibody (88 percent), which is not unexpected, as undetectable levels of these antibodies represent “normal” levels. The frequency of nondetects was less than 7 percent for BPA and all phthalate ester metabolites, except mono-(2-ethylhexyl) phthalate, for which 34 percent of levels were below reporting limits. Analytical levels below reporting limits were retained in the data set at values equal to one-half the reporting limits.

2.5. Creatinine adjustment

Procedures typically used in analyzing NHANES urinary excretion data generally account for the use of “spot” urine samples by including creatinine excretion as an independent covariate in multiple regressions (Barr et al., 2005). Adjusting for creatinine concentration is intended to control for differences in urine concentration of samples due to gender, hydration status, activity levels, and muscle mass. Alternatives include direct creatinine standardization (dividing urinary metabolite concentrations by measured creatinine levels in the same sample) or estimating daily creatinine excretion based on gender, age, and body weight (Mage et al., 2008). Initial analyses of the 2007–2008 NHANES data set showed that the nonstandardized excretion measurements for perchlorate, thiocyanate, nitrate, BPA, and phthalate ester metabolites were highly correlated, due in large part because of their mutual correlations with creatinine excretion. Direct adjustment for creatinine excretion, however, reduced the magnitude of the correlations among the various metabolites, particularly those between perchlorate excretion and the phthalate esters (Supplementary Table 3). Fitting regressions using creatinine-adjusted metabolite excretion (micrograms metabolite per milligram creatinine) greatly increased the stability and statistical significance of the regression coefficients, and was in fact necessary to elucidate the separate relationships between perchlorate and phthalate ester excretion and thyroid hormone levels.

2.6. Statistical analysis

NHANES data were imported from the online NCHS database (NCHS, 2011) as SAS[®] (SAS Institute, Cary, NC) export files. Files were merged by subject identification (SEQN) numbers using the R computing environment version 2.1.12 (R Foundation, 2011) and Excel[®] spreadsheets. Plotting, normality testing, and correlation analyses were conducted using Statistica[®] version 9.1 (StatSoft, 2010). All other regression analyses were conducted using R.

All continuous variables were tested for normality using standard parametric procedures and graphical methods (probability plots). Right-skewed variables with long positive tails were log-transformed for statistical analysis, including all the urinary goitrogen, BPA, and phthalate ester data, iodine and creatinine excretion measurements, and serum TSH, thyroglobulin, and antithyroid antibody levels. The distributions of all serum thyroid hormone concentrations were also right skewed and were log-transformed, except total T3 levels in male subjects, for which the distribution was very close to a Gaussian.

To account for the complex, multistage sampling design, survey non-response, and post-stratification, the NHANES sample weights were used in all analyses, except as noted. Correlation analysis and generalized linear models with and without the NHANES sampling weights first were used to explore the relationships between metabolite excretion and thyroid hormone levels and to identify

important covariates. Covariates found to be consistently significant ($p < 0.05$) predictors of thyroid hormone levels were retained in the final models.

Relationships between predictors and thyroid hormone levels were estimated using generalized additive mixed models (GAMMs). GAMMs have two major advantages over standard linear regression and generalized linear models. First, they can estimate non-linear (smoothed) relationships between explanatory and dependent variables and, second, they can accommodate the clustered stratified sampling design used in the NHANES (Schwartz, 2001; Rehkopf et al., 2010). GAMMs were fit using the NHANES 2-year sampling weights, and the NHANES sampling locations were modeled as a random effect. Because of the variety of relationships among the many variables analyzed, a single model was developed that was fit to all circulating thyroid hormone levels. The standard regressions included variables related to age, ethnicity (with non-Hispanic White serving as the reference group), poverty-income ratio (PIR), BMI, serum cotinine, a dummy variable indicating whether the subject had ever been diagnosed with thyroid disease, variables describing prescription medication usage, log-transformed creatinine-adjusted goitrogen and phthalate ester metabolite excretion, and log-transformed serum thyroglobulin antibody and thyroid peroxidase antibody levels. A dummy variable representing oral estrogen consumption was added for models that included female subjects.

Because the objective of the analysis was to evaluate variations in thyroid hormones that have direct physiological impacts on peripheral tissues, the primary emphasis was on evaluating covariates and metabolite levels that affected free and total T3 and T4 levels. Levels of these hormones in serum and tissue are responsible for the regulatory effects of thyroid functions on growth and metabolism.

In recognition of its role in stimulating thyroid hormone release, TSH was evaluated primarily as an independent (predictive) variable in the regressions. In humans and other mammals, there is a negative feedback loop in the HPT axis whereby TSH and T3/T4 levels exert mutual and contrary effects on each other, but because the NHANES data are cross-sectional and rely on “spot” samples, it was not possible to explicitly model the temporal aspects of the HPT axis control in individual subjects. The regression coefficients derived for TSH represents the long-term average correlations between TSH and T3/T4 levels and do not reflect the short-term stimulation of T3 and T4 release. This issue is addressed on more detail in Section 4.

The R *mgcv* package version 1.7.3 (Wood, 2011) was used in fitting GAMMs in this analysis. The *gamm* function of *mgcv* uses thin-plate regression splines as the default method to fit nonlinear relationships. This program employs Bayesian methods to identify the degree of smoothing providing the best and most parsimonious fits. Variables were included as linear coefficients unless the GAMM results indicated that smoothing provided a better fit to the data. The magnitudes of the potential effects of perchlorate and phthalate ester exposures on thyroid hormone levels were estimated by calculating the model-predicted differences in serum total T4 concentrations associated with a 95th to 5th percentile change in biomarker levels in the male and female NHANES populations.

3. Results

3.1. Demographic characteristics and medication usage

The median ages of men and women in the study population were essentially equal (Table 1). There were slightly fewer Hispanic (“Mexican” plus “Other Hispanic”) men than women (34.28 percent) and more non-Hispanic white males than females (48 vs. 41 percent).¹ Median serum cotinine levels in men (0.57 ng/mL) were higher than in women (0.25 ng/mL), although the proportions of men and women living in households with one or more smokers were not significantly different. Prescription medication usage was similar for men and women, except for androgen/estrogen preparations, and more women than men reported taking SSRIs (8 vs. 4 percent). The data set includes 48 individuals (7 males, 41 females) that reported being “ever diagnosed with thyroid disease”, but who were not currently diagnosed or taking thyroid medications.

3.2. Thyroid function status of the study subjects

Analysis of T3, T4, and TSH levels confirm that the study population was predominantly euthyroid; most total T3 and T4, TSH, and thyroid antibody measurements for men and women fall

¹ The proportion of Hispanics in the study populations was higher than that in the general U.S. population because the 2007–2008 NHANES was structured so as to oversample Hispanics (NCHS, 2011).

Table 1
Characteristics of the study population.

Characteristic	Males ^a	Females ^a	p-value (male-female difference) ^b
Number of subjects	970	907	–
Age, years	43.5 (14, 79)	43 (14, 79)	N.S.
Hispanic (any)	274 (0.28)	305 (0.34)	0.012*
Non-Hispanic White	469 (0.48)	370 (0.41)	0.001**
Non-Hispanic Black	187 (0.19)	200 (0.22)	N.S.
Other ethnicity	40 (0.04)	32 (0.04)	N.S.
Poverty-income ratio (PIR)	1.94 (0.48, 5)	1.93 (0.46, 5)	N.S.
Body mass index, (kg/m ²)	27.3 (19, 39.4)	27.1 (18.9, 41.7)	N.S.
Serum cotinine (ng/mL)	0.57 (0.01, 367)	0.25 (0.01, 330)	< 0.001***
One or more smokers present in household	191 (0.20)	158 (0.17)	N.S.
Ever diagnosed with thyroid disease	7 (0.007)	41 (0.045)	< 0.001***
<i>Prescription Medications</i>			
Selective serotonin reuptake inhibitors (SSRI)	42 (0.04)	74 (0.08)	0.001**
Lithium	0 (0)	1 (0)	N.S.
Nonsteroidal anti-inflammatory drugs (NSAID)	58 (0.06)	47 (0.05)	N.S.
Amiodarone	3 (0)	0 (0)	N.S.
Androgen	4 (0)	0 (0)	–
Estrogen (oral)	0 (0)	66 (0.07)	–
Beta-blocker	88 (0.09)	76 (0.08)	N.S.
Corticosteroids (oral)	9 (0.01)	13 (0.01)	N.S.

NS=not significant, –=not calculated.

^a Values are median (5th, 95th percentiles) for continuous variables, and number (proportion) for dichotomous variables.

^b Student's *t*-test for independent samples (continuous variables), Mann-Whitney *U*-test (proportions).

* *p*-value: *p* < 0.05.

** *p*-value: *p* < 0.01.

*** *p*-value: *p* < 0.001.

Table 2
Proportions of study population with thyroid hormone and antibody measurements within reference ranges.

Thyroid indicator	Total tri-iodothyronine (T3) ^a	Total thyroxine (T4) ^a	Thyroid stimulating hormone ^a	Thyroglobulin antibodies ^b	Thyroid peroxidase antibodies ^b
Reference range	80–180 ng/dL	5.0–12.5 µg/dL	0.3–3.0 µIU/mL	< 4.0 IU/mL	< 9.0 IU/mL
<i>Proportion within reference range</i>					
Male (%)	95	98	86	96	94
Female (%)	93	97	84	93	87

^a Spencer (2010); reference ranges vary based on analytical methods used.

^b Reference ranges based on analytical methods used in 2007–2008 NHANES (NCHS, 2009a, 2009b).

within their respective reference ranges (Table 2). As shown in the table, the bulk of the thyroid antibody measurements (antithyroglobulin and antithyroid peroxidase) are also below upper bounds of reference ranges derived based on the specific analytical methods used in the 2007–2008 NHANES (NCHS, 2009a, 2009b). Slightly larger proportions of women have antibody levels above the reference range, but the proportions are typical of those observed in euthyroid populations.

3.3. Biomarkers of exposure and iodine sufficiency

Table 3 summarizes the urinary excretion data and thyroid hormone panel results for the study population. Geometric mean urinary perchlorate, thiocyanate, and nitrate excretion are slightly higher for men than for women. Average creatinine secretion is approximately 32 percent higher in men than in women, probably due to greater muscle mass; unadjusted perchlorate, thiocyanate, and nitrate excretion were also higher in men. Unadjusted urinary iodine secretion was significantly lower in women than in men (medians 166 and 155 µg/L, respectively). Approximately 23 percent of men and 31 percent of women in the study population excreted less than 100 µg/L iodine. Nevertheless, both men and women in the study population were “iodine sufficient” according to the World Health Organization definition, having median excretion levels greater than 100 µg/L (WHO, 2007). BPA and

phthalate ester excretion data are also summarized in Table 3. Generally, average excretion levels of the phthalate metabolites are quite similar for males and females.

3.4. Biomarker and covariate correlations with thyroid hormone levels

Creatinine-adjusted concentrations of all the goitrogenic ions, most phthalate ester metabolites, and serum thyroglobulin antibody and thyroid peroxidase antibody levels were found to be significantly correlated with at least one thyroid function indicator in both genders (Supplementary Table 4). For males, important covariates include age, Hispanic and non-Hispanic Black ethnicity, BMI, PIR, and non-steroidal anti-inflammatory drug and β-blocker consumption. For females, correlations of hormone levels with ethnicity are also significant, along with PIR and consumption of oral estrogen medication, SSRIs, and β-blockers. Interestingly, smoking indicators (serum cotinine, reported presence of smoker(s) in the household) were not significantly correlated with hormone levels in either gender.

3.5. Covariate-adjusted relationships between perchlorate excretion and thyroid hormone levels

Creatinine-adjusted urinary perchlorate was a consistently significant predictor of thyroid hormone levels in both men and

Table 3
Urinary excretion biomarkers and thyroid hormone levels for the study population^a.

Urinary biomarker excretion/thyroid panel results	Males (n=970)	Females (n=907)
Perchlorate (µg/L)	4.3 (1.0–18.4)	3.4 (0.7–15.6)
Nitrate (µg/L)	47,595 (13,227–12,4244)	42,500 (10,405–140,084)
Thiocyanate (µg/L)	1562 (257–10,301)	1135 (178–9136)
Creatinine (mg/dL)	119 (33–284)	90 (21–268)
Iodine (µg/L)	174 (43–742)	145 (31–567)
BPA (ng/mL) ^b	2.0 (0.3–11.7)	2.0 (0.3–12.2)
MNBP (ng/mL)	18.6 (3.2–91)	21.3 (2.7–136)
MCCP (ng/mL)	2.7 (0.4–19.5)	2.3 (0.3–15.5)
MEHHP (ng/mL)	22.2 (2.6–237)	20 (2.4–215)
MEHP (ng/mL)	2.7 (0.8–29.4)	2.5 (0.8–28.5)
MEOHP (ng/mL)	12 (1.2–122)	11.3 (1.2–120)
MECCP (ng/mL)	32.9 (4.7–287)	30.4 (4.2–257)
MIBP (ng/mL)	7.5 (1–38.5)	7.4 (0.7–45.6)
Free tri-iodothyronine (T3) (pg/mL)	3.3 (2.7–4.1)	3.1 (2.6–3.8)
Total tri-iodothyronine (T3) (ng/dL)	114 (82–158)	110 (79–162)
Free thyroxine (T4) (ng/dL)	0.8 (0.6–1.0)	0.8 (0.6–1.0)
Total thyroxine (T4) (µg/dL)	7.4 (5.4–9.9)	7.7 (5.5–10.6)
Thyroid stimulating hormone (µIU/mL)	1.6 (0.6–4)	1.5 (0.5–4.4)
Thyroglobulin (ng/mL)	8.9 (2.1–35.2)	10.5 (1.3–54.6)
Antithyroglobulin antibody (IU/mL)	0.7 (0.6–2.7)	0.9 (0.6–10.4)
Anti-thyroid peroxidase antibody (IU/mL)	0.8 (0.1–12.4)	1.1 (0.1–122.7)

^a Values=geometric mean (5th, 95th percentile).

^b BPA=bisphenol A, MNBP=mono-*n*-butyl phthalate, MCCP=mono-(3-carboxypropyl) phthalate, MEHHP=mono-(2-ethyl-5-hydroxyhexyl) phthalate, MEHP=mono-(2-ethylhexyl) phthalate, MEOHP=mono-(2-ethyl-5-oxohexyl) phthalate, MECCP=mono-(2-ethyl-5-carboxypentyl) phthalate, MIBP=mono-isobutyl phthalate.

Table 4
Generalized additive mixed model (GAMM) results for total thyroxine (T4).

Variable	Males, n=970			Females, n=907		
	Coefficient	Standard error	p-value	Coefficient	Standard error	p-value
Intercept	1.85	0.11	< 2 × 10 ⁻¹⁶ ***	1.84	0.10	< 2 × 10 ⁻¹⁶ ***
<i>Demographic, ethnic, economic covariates</i>						
Age	0.0008	0.0004	0.04*	– ^a	–	0.059
Hispanic	0.035	0.019	0.07	0.032	0.021	0.131
Non-Hispanic Black	–0.006	0.022	0.79	–0.019	0.022	0.396
Other ethnic	0.047	0.029	0.10	0.036	0.029	0.226
Poverty–income ratio	–0.002	0.004	0.61	–0.002	0.004	0.661
<i>Health/smoking covariates</i>						
Body mass index	0.0001	0.001	0.95	0.0053	0.001	0.00***
Serum cotinine	0.002	0.002	0.32	0.000	0.002	0.84
Ever thyroid disease	0.003	0.068	0.97	–0.013	0.027	0.63
<i>Antithyroid antibodies, thyroid stimulating hormone</i>						
Thyroglobulin antibody	0.0016	0.009	0.86	–0.0170	0.006	0.00***
Thyroid peroxidase antibody	0.001	0.005	0.88	–0.005	0.004	0.18
TSH	–0.042	0.010	0.00	–0.058	0.008	0.00
<i>Prescription medications</i>						
SSRI	–0.0568	0.029	0.05*	–0.0999	0.020	0.00***
Estrogen (oral)	–	–	–	0.1550	0.021	0.00***
β-Blocker	0.0216	0.023	0.35	–0.0082	0.025	0.74
<i>Urinary biomarkers (creatinine adjusted)^b</i>						
Iodine	–0.0146	0.008	0.08	0.0063	0.009	0.49
Perchlorate	–0.0195	0.008	0.01**	–0.0192	0.009	0.03*
Thiocyanate	–0.0149	0.007	0.03*	–0.0099	0.008	0.20
Nitrate	0.0196	0.014	0.15	0.0136	0.011	0.23
BPA ^b	0.0033	0.007	0.64	–0.0009	0.007	0.90
MCCP	–0.0135	0.007	0.05	0.0017	0.008	0.82
MEHHP	–0.1009	0.030	0.00	–0.1010	0.031	0.00***
MECCP	0.0186	0.018	0.30	–0.0203	0.017	0.25
MEOHP	0.0577	0.031	0.06	0.1021	0.030	0.00***
MIBP	0.0070	0.008	0.39	–0.0012	0.008	0.88
MEHP	0.0157	0.008	0.05*	0.0063	0.008	0.45

NS=not significant, –=not calculated.

^a Smoothed fit; effective d.f.=2.062, *p*=0.059.

^b Abbreviations as in Table 3.

* *p*-values: *p* < 0.05.

** *p*-values: *p* < 0.01.

*** *p*-values: *p* < 0.001.

women in the standard regressions. Table 4 summarizes the results of the GAMM models for total T4 in males and females. The coefficients for creatinine-adjusted perchlorate are significant and negative for both genders. In males, age, being “other ethnicity,” and taking SSRI medications are also significant predictors of log-transformed total T4. In addition, the coefficients for urinary biomarkers of both goitrogen (thiocyanate) and phthalate ester exposure (mono(2-ethylhexyl) phthalate [MEHP] and mono-(2-ethyl-5-hydroxyhexyl) phthalate [MEHHP]) are significant and negative. The fit to TSH is also highly significant; the relationship between TSH and total T4 is linear and strongly negative. The coefficient for thiocyanate was not significantly affected by inclusion or exclusion of serum cotinine from the model, despite the fact that both may be indicators of smoking status.

For females, in addition to perchlorate, other biomarkers that are significantly correlated with total T4 include two phthalate ester metabolites (MEHHP and mono-(2-ethyl-5-oxohexyl) phthalate [MEOHP]). Significant covariate predictors of total T4 include BMI, antithyroglobulin antibody levels, and consumption of oral estrogens or SSRIs. As for males, the correlation between total T4 and TSH is highly significant and negative. For females, the optimal fit to age is nonlinear with just over two effective degrees of freedom, as shown in Supplementary Fig. 1.

Table 5 presents the coefficients for creatinine-adjusted urinary perchlorate excretion when included as an explanatory variable in regressions for the various thyroid hormone measurements (free and total T3 and T4) in males, in females, and across the entire study population. The regression coefficients for perchlorate are statistically significant and negative for free and total T4 and for free T3 for the entire study population. In regressions for males and female subjects analyzed separately, the coefficients for total T4 and free T3 are also significant and negative. The perchlorate coefficient in the free T4 regression for males is negative and marginally significant ($p=0.073$), but perchlorate is not a significant predictor of free T4 in females. Perchlorate excretion is not a significant predictor of total T3 levels in any regressions for reasons that are unclear (see Section 4). The coefficient for TSH is significant and negative in all regressions, as expected based on its role in thyroid homeostasis.

In addition to perchlorate, other urinary biomarkers are also significant predictors of thyroid hormone levels. For men, coefficients for thiocyanate and for phthalate ester metabolites are significant negative predictors of one or more hormone metrics. In females, the pattern differs; goitrogens other than perchlorate are not significant predictors of free or total T3 or free T4, and phthalate ester metabolites are significantly correlated with free and total T4 but not with free or total T3 levels. Coefficients for urinary iodine are not significant in most of the regressions, but are significant and negative in the free T4 regression for males and in the free T3 regression for females (Table 5 and Supplementary materials) These results are discussed in more detail in the following section.

4. Discussion

Consistent, statistically significant relationships have been found between biomarkers of exposure to perchlorate, phthalate esters, and serum thyroid hormone levels (free T3, free and total T4) in male and non-pregnant female subjects aged 12 years and older in the 2007–2008 NHANES. These relationships were detected in regression (GAMM) models that also included age, ethnicity, income, and behavioral covariates (smoking, prescription medication consumption), and which also adjusted for the presence of biomarkers for phthalate ester exposures. Having a full range of thyroid status indicators (serum TSH, thyroglobulin, thyroid antibody levels) available for each subject enabled the potential effects of perchlorate to be distinguished from the known strong relationship among T3, T4, and TSH through the HPT axis, and also from factors related to age or disease, even in the predominantly euthyroid study population.

Perchlorate exposure was not a significant predictor of total T3 levels in either men or women, or of free T4 in women. Given the large number of covariates, the regressions might be overcontrolled for the simultaneous impacts of age, ethnicity, income, or even other chemical exposures, which could reduce the power to identify significant relationships in the moderately sized study cohorts. The finding of a significant coefficient for perchlorate in the regression for free T4 fit to data for the entire study cohort,

Table 5
Summary of GAMM regression coefficients for perchlorate in regressions for free and total T3 and T4.

Regression ^a	Perchlorate β estimate	<i>p</i> value	Significant smooth terms (nonlinear)	Other significant biomarkers (signs of coefficients) ^b
<i>All subjects (n=1877)</i>				
Total T4	-0.018	0.002**	-	Thiocyanate(-), nitrate(+), MEHHP(-), MEOHP(-)
Free T4	-0.011	0.021**	Age, TSH	Iodine(-), MEHHP(-), MEOHP(-)
Total T3	0	0.998	Age, TSH	Iodine(-), Nitrate(+), MEHHP(-)
Free T3	-0.013	0.000***	Age, TSH	Iodine(-), MCP(-), MECPP(-),
<i>Male subjects (n=970)</i>				
Total T4	-0.019	0.013*	-	Thiocyanate(-), MEHHP(-), MEHP, TSH(-)
Free T4	-0.011	0.073	Age	Iodine(-), TSH(-)
Total T3	0.00002	0.998	Age	MCP(-), MEHHP(-)
Free T3	-0.013	0.002*	Age	Thiocyanate(-) Nitrate(+), MECPP(+), TSH(-)
<i>Female Subjects (n=907)</i>				
Total T4	-0.019	0.033*	Age	MEHHP(-), MEOHP(-), TSH(-)
Free T4	-0.010	0.176	Age, TSH	MEHHP(-), MEOHP(+)
Total T3	-0.012	0.194	Age, TSH	-
Free T3	-0.014	0.005*	Age, TSH	Iodine(-)

NS=not significant, -=not calculated.

* *p*-values: $p < 0.05$.

** *p*-values: $p < 0.01$.

*** *p*-values: $p < 0.001$.

^a All regressions are fit to log-transformed hormone levels except for total T3 in males.

^b Abbreviations as in Table 3.

despite the marginal significance for males and the lack of significance for females, supports that possibility, at least for this indicator. The overall consistency of the relationships between creatinine-adjusted perchlorate excretion and circulating thyroid hormone levels reduces the likelihood that the observed correlations are spurious, and further supports the hypothesis that environmental exposures to perchlorate do affect thyroid hormone levels. The best-fitting models predict reductions in serum total T4 of 0.46 $\mu\text{g}/\text{dL}$ for males and 0.48 $\mu\text{g}/\text{dL}$ for female NHANES subjects associated with the 95th to 5th percentile change in creatinine-adjusted perchlorate excretion.

Excretion of phthalate ester metabolites (MEHHP, MEHP, MCPP, and MEOHP) was the other most consistently significant biomarker predictor of T3 and T4 levels. The findings of this analysis are similar to those of Meeker and Ferguson (2011) who estimated these relationships in an overlapping cohort of 1675 adults and adolescents using generalized linear models. Similarities with the previous analysis include the identities of the specific phthalate metabolites most commonly associated with hormone levels, the generally negative signs of their relationships to T3 and T4 levels, and the approximate magnitude of the observed relationships.

Meeker and Ferguson (2011) reported that a 75th- to 25th-percentile change in MEHHP excretion was associated with approximately 4.1 percent decrease in total T4. The regression for male subjects in the current analysis predicts an approximately 2.6 percent reduction (0.20 $\mu\text{g}/\text{dL}$) in total T4 for a similar change in MEHHP when perchlorate and other covariates are also included. For females, the predicted change is 1.9 percent (0.15). The smaller predicted effects of phthalate in this analysis may be due to differences in the data subsets used in the analyses, differences in the model specification (GAMM versus GLM), the specific covariates included, and the multicollinearity of the relationships between perchlorate, phthalate esters, and hormone levels.

The absolute and relative magnitudes of perchlorate and phthalate ester associations with thyroid hormones vary with gender and across the indicators (free and total T3 and T4) that were evaluated. Generally, the regression coefficients for perchlorate and the predicted impacts of both perchlorate and MEHHP are greater for total T4 than for the other biomarkers (Table 5). Also, the predicted effects of perchlorate vs. phthalate exposures are greater for total T4 than for other indicators, and greater for males than for females. (Complete GAMM results are presented in the [Supplementary materials](#)).

In contrast to previous analyses using earlier NHANES data (Blount et al., 2006; Steinmaus et al., 2007), where perchlorate impacts on T4 levels were limited to female subjects with low iodine secretion, significant relationships between perchlorate excretion and T3/T4 levels were detected in a population that was iodine-sufficient. Iodine secretion was found to be only weakly correlated with hormone levels. In the GAMM models, coefficients of urinary iodine excretion rarely achieved statistical significance when the perchlorate and phthalate biomarkers and other significant covariates were also included (see [Supplementary materials](#)) This is probably because, as discussed in Section 3.2, both the male and female NHANES subjects are predominantly iodine-sufficient, so that iodine intake is not a limiting factor in thyroid hormone production. As noted in Section 3.5, indicators of smoking status were also generally not significant predictors of thyroid hormone levels when other variables were included. The coefficient for serum cotinine was not significant in any regression for male or female subjects, while the coefficient for thiocyanate excretion was significant and negative (-0.015 , $p=0.026$) only in the GAMM model of total T4 levels in male subjects (Table 5 and [Supplementary materials](#)) The coefficient for the other goitrogenic biomarker, nitrate excretion, likewise was never significant in any model.

Limitations of this study include the relatively small subset of the total 2007–2008 NHANES subjects (fewer than 20 percent) for whom biomarker and covariate data were available. As noted in Section 3.2, however, the study population was generally similar, in terms of demographics and other covariates, to the excluded subjects. Children under age 12, women who were currently pregnant, and all subjects reporting current thyroid disease were excluded from the analysis. Thus, it was not possible to evaluate perchlorate impacts in these potentially sensitive populations. Also, the NHANES data are cross-sectional, precluding evaluation of the temporal relationships between perchlorate exposures, hormone levels and TSH, arising from HPT axis control mechanisms in individual subjects. The interpretation of our results depends on the extent to which “spot” thyroid panel results and urinary metabolite measurements provide reliable indices of thyroid status and recent exposures. As noted previously, the coefficients for TSH in the regression models represent the average long-term correlations between TSH and T3/T4 levels and do not reflect short-term impacts of TSH on T3/T4 excretion from the thyroid. Similarly, the regressions do not capture the feedback effects of euthyroid T3/T4 levels through the HPT axis to inhibit TSH release. Analyses using causal path modeling could provide a more systematic analysis of potential modes of action. Until such analyses are completed,² inferences related to causality must be drawn very cautiously. Results from the 2009–2010 NHANES, when they are released, should also help to further clarify the relationships among exposures to perchlorate, phthalate esters, and circulating thyroid hormone levels.

5. Conclusions

Perchlorate exposure is a statistically significant predictor of circulating thyroid hormone levels, although the magnitude of the effect appears to be modest. The consistent finding of statistically significant relationships between perchlorate and T3 and T4 levels in models that account for the effect of TSH suggests that homeostatic control of thyroid hormone levels might not compensate completely for the impacts of low-level goitrogen and phthalate exposures on thyroid hormone levels. The results of our analyses suggest that the combined impacts of perchlorate and phthalate ester exposures could be physiologically significant in the general population. While all the correlations were seen in a euthyroid population in which no subjects have clearly pathological thyroid hormone levels, the study cannot discern whether subjects with apparently normal thyroid status had in fact, been shifted far from their normal thyroid “set points” (Miller et al., 2009) by environmental exposures to perchlorate or phthalate esters. These findings raise the level of concern regarding sensitive individuals with mild or moderate hypothyroxinemia, overt thyroid disease, or who are simultaneously exposed to other HPT axis stressors, and with regard to reproductive age women, since even mild disturbances in thyroid function during pregnancy may be associated with neurodevelopmental effects on the fetus (Pop et al., 2003; Kooistra et al., 2006; Henrichs et al., 2010).

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² Path model studies are currently under way and will be submitted for publication shortly.

Human subjects disclaimer

All analyses are based on public release files from the U.S. NHANES; thus, informed consent requirements have been met.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.envres.2012.05.010>.

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