INTRODUCTION

The fact that thyroid hormone is essential for cellular mechanisms associated with energy metabolism, growth and development (Kim, 2008), as well as normal physiological functioning of most mammalian tissues (Melzer et al., 2010) has created increasing clinical concern about potential thyroid hormone disruption from exposure to environmental toxicants (Diamanti-Kandarakis et al., 2009). Perfluorinated compounds have been of particular concern because of their persistence and widespread proliferation in water, air, soil, plants and animals (Lau et al., 2007); and because they have been shown to have endocrine disrupting influences on animals (Jensen and Leffers, 2008; Wade et al., 2002; Shi et al., 2007, 2009a and 2009b; Wei, 2007; Zhao, 2010) and humans (Knox et al., 2011). Perfluorooctanate (PFOA) and perfluorooctane sulfonate (PFOS) are two man-made surfactants that have been used in a number of household products such as food containers; stain- and water-resistant protection for clothing, furniture and carpets; paints; fire-fighting foam; photographic emulsifiers; and many others (Lau et al., 2007).

Animal studies in multiple species have shown that there are gender differences in the biological half-life of PFOA, with male-half-lives being up to 70 times longer than those of females (Kudo et al., 2002; Lee and Schultz, 2010). Although the half-life in humans is known to be long, the few studies published to date (Olsen et al., 2007a; Hölzer et al., 2009; Bartell et al., 2010) have not focused on gender differences. However, the C8 Health Study has reported moderate gender differences in median PFOA levels (Steenland et al., 2009) showing longer half-lives in men. An inverse association between serum PFOS and serum estradiol that is significant in women in the age ranges > 42-65 (Knox et al., 2011) has also been reported. Sex steroids modulate thyroid hormone levels and influence thyroid structure and function. Because of the importance of thyroid hormone, the present study assessed thyroid function in a cross-sectional analysis of 52,296 adults with a year or more of exposure to perfluorooctanoate (PFOA) from drinking water. Outcomes were: thyroxine, T3 uptake, and thyroid stimulating hormone (TSH). Analyses were stratified by gender and age group (< 20 - < 50 years and > 50). Both PFOA and perfluorooctane sulfonate (PFOS) were associated with significant elevations in serum thyroxine and a significant reduction in T3 uptake in all participants. There were also significant gender/PFOS interactions for T3 uptake and thyroxine, as well as gender/PFOA interactions for T3 uptake. Results provide evidence for disruption of thyroid function related to these common chemicals and possible mechanisms are discussed.

Key words: Perfluorocarbons, Thyroid function, Gender

Correspondence: Sarah S. Knox (E-mail: sknox@hsc.wvu.edu)

Original Article

Perfluorocarbon exposure, gender and thyroid function in the C8 Health Project

Sarah S. Knox1, Timothy Jackson2, Stephanie J. Frisbee1,3, Beth Javins1 and Alan M. Ducatman1

1West Virginia University School of Medicine, Department of Community Medicine, 1 Medical Center Drive, Morgantown, WV 26506, USA
2West Virginia University School of Medicine Department of Medicine, 1 Medical Center Drive, Morgantown, WV 26506, USA
3West Virginia University School of Medicine Center for Cardiovascular and Respiratory Sciences, 1 Medical Center Drive, Morgantown, WV 26506, USA

(Received March 4, 2011; Accepted May 14, 2011)

ABSTRACT — Perfluorocarbons from common household products such as food containers, stain-resistant protection for clothing, furniture and carpets, paints, and fire-fighting foams are found in soil, water, plants, animal and human serum worldwide. Previous research has shown a significant association between these chemicals and thyroid disease in women. The present data from the C8 Health Project assessed thyroid function in a cross-sectional analysis of 52,296 adults with a year or more of exposure to perfluorooctanoate (PFOA) from drinking water. Outcomes were: thyroxine, T3 uptake, and thyroid stimulating hormone (TSH). Analyses were stratified by gender and age group (< 20 - < 50 years and > 50). Both PFOA and perfluorooctane sulfonate (PFOS) were associated with significant elevations in serum thyroxine and a significant reduction in T3 uptake in all participants. There were also significant gender/PFOS interactions for T3 uptake and thyroxine, as well as gender/PFOA interactions for T3 uptake. Results provide evidence for disruption of thyroid function related to these common chemicals and possible mechanisms are discussed.

Key words: Perfluorocarbons, Thyroid function, Gender
by altering the clearance of thyroxine-binding globulin (TBG) (Tahboub and Arafah, 2009) produced in the liver, suggesting that gender differences in response to perfluorocarbons are an important issue to be addressed in human studies of thyroid function in response to perfluorocarbon (PFC) exposure.

In vitro modeling of human thyroid function shows that perfluorinated compounds can compete with thyroxine (T₄) for binding to the human thyroid hormone transport protein transthyretin (TTR), which has the potential to decrease thyroid hormone levels (Weiss et al., 2009). Despite this fact, studies of perfluorocarbons and thyroid function in human populations have been limited. They include exposure in occupational settings and from the surrounding environment, but the sample size of some of these studies has been small. A study of 31 licensed anglers in New York State showed no significant association between perfluorinated compounds and thyroid-stimulating hormone (TSH) or free thyroxine, however the authors indicated that the sample size was too small to detect differences (Bloom et al., 2010). A study of 371 individuals randomly selected from one of the six communities in the C8 Health Project with long-standing exposure (Emmett et al., 2006) also found no association between PFOA exposure and levels of TSH. A two-facility 3M Corporation study of fluorochemical workers during 1995 and 1997 enrolled 206 volunteers from one plant (~61% of eligible workers) and 263 from the other (~52% of eligible workers). Between 73-75% of them worked in production. Analyses were not stratified by gender (Olsen et al., 2003). The workers in the first plant had lower PFOA and PFOS values than those in the second but the study found no effect on thyroid function except for a small positive association with Triiodothyronine (T₃). Analysis of these same plants (N = 506, 93% male) in the year, 2000, also found no associations between thyroid function and PFOA (Olsen and Zobel, 2007b). Another small study (Pirali et al., 2009) of thyroid tissue in 28 patients with thyroid disease found no significant association of intrathyroidal concentrations of PFOA or PFOS and underlying thyroid disease when compared with thyroid tissue from autopsy controls. However, the National Health and Nutrition Examination Survey (NHANES) study using a larger probability-based sample reported significantly higher odds of self-reported thyroid disease in women in the upper quartile of serum PFOA levels compared with those in lower quartiles and a similar near significant trend in men (Melzer et al., 2010).

Animal studies have demonstrated significant decreases in serum T₃ at all dosages, no effect on serum TSH and increases of T₄ at low dosage (Yu et al., 2009; Chang et al., 2008). Because the function of thyroid hormones is complex, ranging from molecular to systemic interactions associated with multiple organ systems (Jugan et al., 2010), identifying the mechanisms by which perfluorinated compounds might disrupt function is a complex task. There are multiple levels which can be targeted by these chemicals (Jugan et al., 2010) and although animal studies have been very helpful in facilitating identification of mechanisms, associations may vary by species and are not always indicative of what happens in humans.

The objective of the present analyses was to investigate the extent to which PFOA and PFOS were associated with altered thyroid function in thyroid hormones in a large population based study. Data were obtained from the C8 Health Project, a study arising from a lawsuit after PFOA contamination of water supplies along the middle Ohio River Valley. The contamination was attributable to the DuPont Washington Works plant near the Ohio River, and the members of the Class action suit against DuPont won a settlement, $20 million of which was designated to be used for health and education projects. The data presented in this paper stem from the C8 Health Project.

**MATERIALS AND METHODS**

**Sample selection**

The C8 Health Project collected data on 69,030 subjects from six public water districts contaminated by PFOA from the DuPont Washington Works Plant near Parkersburg, WV between August 2005 and August 2006. Analysis revealed serum concentrations of PFOA that were 500% higher than national averages previously reported in NHANES data (Frisbee et al., 2009), although the PFOS concentrations at these same sites were similar to previously reported national data (Calafat et al., 2006). The current analyses were calculated on 25,026 men and 25,018 women age 20 and over who did not have thyroid disease.

The sample for this study resulted from a class action suit begun in 2001. The “Class” was defined as individuals in West Virginia or Ohio, whose drinking water had been contaminated by quantifiable levels of (PFOA or C8) from the DuPont Washington Works plant since 1951. An independent company, Brookmar, Inc., was created solely to carry out the Project under the supervision of a Court-appointed administrator.

The overall participation rate among current adult residents in 2005-2006, age 20 and older, was estimated using census data to be about 81% (Frisbee et al., 2009).
Gender, endocrine disruption & PFOA/PFOS

Eligibility
Class eligibility was defined by exposure to contaminated water, a combination of geographic and concentration criteria, and the duration of exposure. Two key criteria included:

1. Exposure to contaminated water from at least one of the six identified, public water districts (Lubeck Public Service District, WV; Mason County Public Service District, WV; City of Belpre, OH; Little Hocking Water District, OH; Tippers Plains-Chester Water District, OH; City of Pomeroy, OH) or exposure from any private water source within the geographical boundaries of the public water sources that was certified through testing to contain at least 0.05 ppb PFOA.

2. The ability to document a minimum of one year (12 months) of exposure to contaminated water between January 1951 and December 4, 2004 at either primary residence, place of employment, or school.

A more complete description of the study, its antecedents and methods has been published previously (Calafat et al., 2006).

Data collection and blood-processing procedures
Surveys were filled out online or at temporary modular office units established within each of the affected water districts for the purposes of validating eligibility and collecting serum. These units were staffed with nurses, phlebotomists, and other project staff, and equipped for venipuncture, blood processing, and short-term record and blood sample storage. A more detailed description of the survey instrument, clinical measures and data collection is available online at http://www.hsc.wvu.edu/som/cmed/c8/.

Thyroid hormones
Clinical laboratory tests were performed at a large, independent, accredited clinical diagnostic laboratory (LabCorp, Inc., Burlington, NC, USA). The measures used in the current analyses were thyroxine (total T4), T3 uptake, TSH and serum albumin, a protein that also binds thyroid hormone. Measures of thyroid-binding globulin were not included in the study protocol and were not available for analysis.

Because we believe that duration of exposure is important to interpretation of diagnosed thyroid disease, and because a model to calculate individual exposure duration is still under construction by investigators of the C8 Health Project, data on thyroid disease will not be reported here. However, participants with a diagnosis of thyroid disease were excluded from the analyses.

Perfluorocarbon measurement
The analytic procedure to quantify perfluorocarbons (PFOS and PFOA) in serum used a protein precipitation extraction combined with reversed-phase high-performance liquid chromatography/tandem mass spectrometry. Spectrometric detection employed a triple quadrupole mass spectrometer in a selected reaction monitoring mode, monitoring for the M/2 transitions of the specific perfluorocarbons and the 13C–PFOA surrogate. The primary laboratory performing PFC analysis (Exygen Research Inc., State College, PA, USA) was selected based on its ability to meet U.S. Food and Drug Administration (FDA) guidelines for bioanalytical method validation, a lower limit of quantification of 0.5 ng/ml, and 96-well-plate-based technology allowing for high throughput capability. The detailed methodology used by this laboratory, including validations has been previously detailed (Frisbee et al., 2009; Flaherty et al., 2005).

Cumulative exposure
Given the comparability of PFOS levels in the C8 participants with those from other national studies, we will focus the discussion of cumulative exposure on PFOA, the primary contaminant from the DuPont Works plant. Due to the cross-sectional nature of these measurements, cumulative exposures are difficult to assess because they are dependent upon multiple factors: duration of exposure, number of sources of exposure and levels of exposure at various time points. Because individual cumulative exposure cannot be directly calculated from the database, background information on exposure levels in this cohort have been extrapolated using multiple assessments to provide a background for the analyses (Seals, 2010). The results of this modeling showed that residential district explained 68% of the variance in PFOA levels, based primarily on the distances from the contamination site. The average increase in PFOA concentrations for each year of residence, after adjusting for sex, age, exercise, race/ethnicity, body mass index, smoking, and use of bottled water as a primary source of drinking water, was 1.2%. Emissions from the DuPont plant were not constant but increased over time, peaking in the 1990’s. Participants who had lived in multiple districts, had private wells or who also worked in a DuPont plant were excluded from the analyses, indicating that these estimates are probably conservative. In the case of former residents, the decay of serum PFOA levels was nonlinear.

Statistical analyses
Because sex differences in elimination of perfluorocarbons have been noted in multiple species (Lau et al.,
2007), T₄, TSH, and T₃ uptake were calculated separately for men and women in two age groups: 20 ≤ 50 and > 50. The age groups were chosen to approximate endocrine changes associated with menopause. TSH, T₃ uptake, T₄, and albumin were regressed on quintiles of log serum concentrations of PFOA and PFOS, adjusting for age, serum estradiol, and alcohol; excluding participants with diagnosed thyroid disease. Estradiol was included as a covariate because it has been shown to cause an increase in thyroid binding globulin and thus also total serum T₃ and T₄. The General Linear Models (SAS STAT Users Guide) procedure was used to calculate regression analyses and Analyses of Variance related to gender differences in thyroid hormones.

Because of reports that the effects of PFOA may vary in obese subjects (Lin et al., 2010), we also calculated the above regressions stratified by body mass index (BMI) using two groups: greater than or equal to a BMI of 30 and below 30. No consistent differences were found and they will not be reported here.

**RESULTS**

**Descriptive data**

A table showing the distribution of age, gender, alcohol consumption and smoking can be seen in Table 1. Consistent with animal data, serum concentrations of PFOA and PFOS were lower in women than in men and lowest in women 20 ≤ 50 yrs.

The breakdown of perfluorocarbon exposure level by water district can be seen in Table 2. The water districts farthest from the source of contamination have the lowest levels. This table illustrates that there was enough variation in exposure between districts to allow for meaningful statistical analyses.

**PFOA and thyroid hormones**

Although there was no significant association between

| Table 1. Means and standard deviations for PFOA, PFOS, age, BMI, smoking and alcohol consumption by age/gender group* |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Women 20-50     | Men 20-50       | Women > 50      | Men > 50        |
|                  | (N = 16,193)    | (N = 14,944)    | (N = 8,854)     | (N = 10,122)    |
| Mean PFOA (ng/ml) | 52.6 (192.8)    | 91.0 (261.5)    | 98.6 (230.2)    | 124.3 (380.8)   |
| Mean PFOS (ng/ml) | 17.3 (10.8)     | 24.8 (14.3)     | 25.7 (17.5)     | 29.1 (20.6)     |
| Mean age          | 35.3 (8.7)      | 35.7 (8.8)      | 62.1 (9.2)      | 62.1 (8.6)      |
| Mean BMI          | 28.2 (8.6)      | 28.6 (6.3)      | 29.1 (8.7)      | 29.0 (6.4)      |
| % Smoke           | 32.7            | 32.1            | 17.7            | 18.8            |
| % Drink alcohol   | 53.7            | 64.1            | 28.4            | 43.3            |

*After eliminating people diagnosed with thyroid disease.

| Table 2. Age and gender adjusted serum PFOS and PFOA concentrations by water districts* |
|-----------------|-----------------|-----------------|
| Water district   | PFOA            | PFOS            |
| City of Belpre (OH) | Mean (ng/ml) 42.96 | 23.18 |
|                  | Std. Error 2.48 | 0.16 |
| Little Hocking Water Association (OH) | Mean (ng/ml) 227.59 | 23.47 |
|                  | Std. Error 2.03 | 0.14 |
| Lubeck Public Service District (WV) | Mean (ng/ml) 92.36 | 24.96 |
|                  | Std. Error 1.78 | 0.12 |
| Mason County (WV) | Mean (ng/ml) 16.00 | 23.01 |
|                  | Std. Error 2.06 | 0.14 |
| Tuppers Plains (OH) | Mean (ng/ml) 42.07 | 22.29 |
|                  | Std. Error 1.96 | 0.13 |
| Village of Pomeroy (OH) | Mean (ng/ml) 15.96 | 20.97 |
|                  | Std. Error 3.83 | 0.25 |
| Private Well (WV or OH) | Mean (ng/ml) 132.56 | 26.15 |
|                  | Std. Error 18.41 | 1.22 |

*Adapted from Frisbee et al. (2009).
PFPA and TSH, there was a significant positive association between serum PFNA and thyroxine in women in both age groups (β = 0.05, p = < 0.0001; and β = 0.08, p < 0.0001 for women ≤ 50 and > 50, respectively) and in men > 50 (β = 0.06, p = 0.001), after adjustment for covariates. In both age groups of women there was a small but significant inverse association between PFNA and T3 uptake (β = -0.08, p = 0.0001, and β = -0.07, p < 0.005, respectively) and a similar inverse association in men > 50 (β = -0.04, p = 0.037). T3 uptake in both younger and older women was below 32%, the lower threshold of the range considered to be normal. Thyroxine-binding globulin, which binds about 75% of thyroxine (Diamanti-Kandarakis, 2009) was not available in the data base, so albumin (a non-specific binding protein that binds 5-10% of thyroxine) was measured. Albumin was positively associated with PFNA in all participants (p < 0.0001 in all groups) but had small β coefficients (rounded off to 0.02 in all groups). Graphs for thyroxine and T3 uptake can be seen below (Figs. 1 and 2).

The interaction between PFNA and gender was significant for: T3 uptake (F = 3.65, p < 0.006), which was lower in women than men, for TSH (F = 2.57, p = 0.036) which was lower in men than in women, and for albumin (F = 6.07, p < 0.0001), which was somewhat higher in men (Fig. 3). Although thyroxine levels were higher in women than in men, the difference reached only borderline significance (F = 2.20, p = 0.067).

PFOS and Thyroid Hormones

The associations between PFOS and thyroid hormones resembled their association with PFNA (see Figs. 4 and 5), i.e., PFOS is positively associated with thyroxine in both men and women of all ages (β = 0.14, p < 0.0001 and β = 0.08, p < 0.0001 for women ≤ 50 and > 50 respectively; β = 0.05, p = 0.0001 for men in both age groups). The association with TSH was not significant in men or in women in either group. PFOS was inversely associated with T3 uptake in women in both age groups (β = -0.21, p < 0.0001 and β = -0.17, p = 0.0001 in women ≤ 50 and > 50 respectively) and in men (β = -0.05, p = 0.009; and β = -0.09, p < 0.0001 respectively). Albumin was again significantly positively associated with PFOS in men and women of both age groups (p < 0.0001) (Fig. 6). Gender / PFOS interactions were significant for thyroxine (F = 4.17, p = 0.002), being higher in women than in men, for T3 uptake (F = 3.35, p < 0.010), being lower in women than in men, and for albumin (F = 3.52, p = 0.007), being lower in women than in men of comparable ages.
DISCUSSION

The C8 Health Study is the largest study to date of human environmental exposure to perfluorinated compounds in the natural environment. Consistent with the animal data showing faster clearance in females, gender comparisons of mean PFOA and PFOS concentrations indicate lower levels in women than men, highlighting the importance of stratifying data analyses by gender. One explanation for the gender differences is menstrual bleeding. Perfluorocarbons are eliminated with menstrual blood and the volume of blood is restored before the levels of toxicants. Since men don’t menstruate, their levels could be expected to be higher. Indeed, earlier work on this cohort (Knox, 2011) demonstrated that perfluorocarbon concentrations in women in perimenopausal and menopausal age groups were higher than those in younger women.

Our primary finding was that of significant gender differences in thyroxine and T3 uptake associated with exposure to PFOS and PFOA which have not previously been reported in the literature. The increase of total thyroxine and reduction of T3 uptake associated with perfluorocarbons in both men and women is most consistent with an increase in the production of TBG. Unfortunately, this could not be verified because TBG was not measured. The only thyroid hormone binding protein actually available for analysis was albumin, which only binds a small amount of thyroxine. Nevertheless, there were positive associations between albumin and both PFOA and PFOS in both genders and age groups. This is only suggestive because albumin is a non-specific protein binding globulin. Mechanistically, the associations of perfluorocarbons with total thyroxine and T3 uptake are consistent with a hepatic increase in production of TBG without significant influence from the hypothalamic pituitary thyroid axis. This is supported by the fact that there is no increase in thyroid stimulating hormone TSH associated with increases in PFOA or PFOS. The pattern found in these data is consistent with what occurs with the use of exogenous estrogens in patients, namely an increase in TBG but not TSH (Tahboub and Arafah, 2009; Sänger et al., 2008), an increase in total thyroxine and a decrease in T3 uptake.

Despite the limitation of not having measured thyroxine-binding globulin, making interpretation of mechanism inconclusive, the pattern of findings reported here is clinically disturbing. Given the important role of thyroid function to metabolic processes, these results raise serious concerns about what physiological mechanisms are being disturbed by perfluorocarbon exposure. A need for further research to clarify these questions is warranted.
REFERENCES


Wade, M.G., Parent, S., Finnson, K.W., Foster, W., Younglai, E., McMahon, A., Cyr, D.G. and Hughes, C. (2002): Thyroid toxici-