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Short running head: PFOA and thyroid disease

Key words: C8, human population, PFOA, PFOS, thyroid disease

Abbreviations

C8 alternative brief name for Perfluorooctanoic acid

PFOA Perfluorooctanoic acid

PFOS Perfluorooctane sulfonate

PFC Perfluorinated chemicals

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Abstract:

BACKGROUND:

Perfluorooctanoic acid (PFOA, 'C8') and perfluoroctane sulphonate (PFOS) are stable compounds with many industrial and consumer uses. Their persistence in the environment plus toxicity in animal models has raised concern over low-level chronic exposure effects to human health.

OBJECTIVES:

To estimate associations between serum PFOA and PFOS concentrations and thyroid disease prevalence in representative samples of the United States general population.

METHODS:

Analyses of PFOA/PFOS against disease status in the Health and Nutrition Examination Survey (NHANES) 1999-2000, 2003-2004 and 2005-2006. Included were 3974 adults with measured for PFC concentrations. Regression models were adjusted for age, sex, race/ethnicity, education, smoking status, body mass index, and alcohol intake.

RESULTS:

In women, the NHANES weighted prevalence of reporting any thyroid disease was 16.18% (n=292), for men 3.06% (n=69): prevalence of current thyroid disease taking related medication was 9.89% (n=163) for women and 1.88% (n=46) for men.

In fully adjusted logistic models, women with PFOA ≥5.7ng/ml (top population quartile '4') were more likely to report current treated thyroid disease: Odds Ratio = 2.24 (95%CI: 1.38 to 3.65, p=0.002) compared to PFOA ≤4.0ng/ml (quartiles 1&2). In men, there was a near significant similar trend OR=2.12 (CI 0.93 to 4.82, p=0.073).

For PFOS in men, a similar association was present comparing those with PFOS≥36.8 ng/ml (Q4) to those with PFOS concentrations ≤25.5 ng/ml (Q1&Q2): OR for treated disease 2.68 CI: 1.03 to 6.98, p=0.043. In women this association was not significant.

CONCLUSIONS:

Higher concentrations of serum PFOA and PFOS are associated with current thyroid disease in the US general adult population. More work is needed to establish the mechanisms involved and to exclude confounding and pharmacokinetic explanations.

Introduction

The perfluoroalkyl acids (PFAAs) are a family of synthetic, highly stable perfluorinated compounds with a wide range of uses in industrial and consumer products, from stain- and water-resistant coatings for carpets and fabrics to fast-food contact materials, fire-resistant foams, paints and hydraulic fluids (OECD 2005). The carbon-fluoride bonds that characterize PFAAs and make them useful as surfactants are highly stable and recent reports indicate the widespread persistence of certain PFAAs in the environment and in wildlife and human populations globally (Fromme 2009; Giesy and Kannan 2001; Lau 2007; Saito et al. 2004). Two of the PFAAs of most concern are the eight carbon chained perfluoroctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA, 'C8').

Most persistent organic pollutants are lipophilic and accumulate in fatty tissues, but PFOS and PFOA are both lipo- and hydrophobic, and following absorption will bind to proteins in serum rather than accumulating in lipid (Hundley 2006; Jones et al. 2003). The renal clearance of PFOA and PFOS is negligible in humans, leading to reported half lives in blood serum of 3.8 and 5.4 years for PFOA and PFOS respectively (Olsen 2007). Human biomonitoring of the general population in various countries has shown that in addition to the near ubiquitous presence of PFOS and PFOA in blood, they may also be present in breast milk, liver, seminal fluid and umbilical cord blood (Lau 2007).

Extensive laboratory studies of the toxicology of PFOA and PFOS have reported enlargement of the liver, modulation of sex hormone homeostasis, developmental and immune system toxicity, hypolipidemia and reduced body weight in rodent and non-

human primate models (reviewed in Lau 2004;Lau 2007). Research interest has focused on the ability of these compounds to bind to nuclear receptors including the peroxisome proliferator activating receptor (PPARα), and to disrupt serum protein ligand binding (Luebker 2002), highlighting PFOA and PFOS as potential endocrine disruptors (Jensen 2008).

Endocrine systems that may be targets of endocrine disrupting chemicals include the hypothalamus-pituitary-thyroid axis (HPT) (Boas 2006). Thyroid hormone is essential for the normal physiological function of nearly all mammalian tissues. Thyroid hormone status is controlled by a well-established feedback mechanism, in which thyroid-stimulating hormone (TSH) stimulates the thyroid to synthesize T_4, which is then converted to the biologically active T_3. The rate of release of TSH is regulated by the hypothalamus as well as by the circulating levels of T_3 and T_4. Therefore, multiple physiological steps including hormone biosynthesis, transport, metabolism or action on target cells are required for thyroid hormone homeostasis.

Numerous studies have now shown PFAAs to impair thyroid hormone homeostasis in animal studies. Depression of serum T_4 and T_3 has been reported by several authors in PFOS-exposed rats (Lau et al. 2003;Luebker 2005; Seacat 2003), without the concomitant increase in TSH that would be expected through feedback stimulation. Earlier mechanistic studies of the structurally related compound perfluorodecanoic acid (PFDA) showed that it could reduce serum thyroid hormone levels by apparently reducing the responsiveness of the HPT axis and by displacing circulating thyroid hormones from their plasma protein binding sites (Gutshall 1989). Whilst circulating hormone levels were depressed, the activities of thyroid hormone sensitive liver enzymes were elevated, suggesting that functional hypothyroidism was not occurring. A similar

mechanism for PFOS has been hypothesized (Chang 2007). A recent study of the mechanisms involved in PFOS-induced hypothyroxinemia in rats has indicated that increased conjugation of T_4 in the liver, catalysed by the hepatic enzyme uridine dihosphoglucuronosyl transferase (UGT1A1), and increased thyroidal conversion of T_4 to T_3 by type 1 deiodinase may be partly responsible for the effects seen (Yu 2009). Taken together, these findings suggest that the effects of PFAAs on thyroid hormone physiology are multiple and complex.

Extrapolations from animal laboratory studies such as these to an estimation of the risks posed by PFOA and PFOS to thyroid function in humans are complicated by the extreme variations reported in their toxicokinetic profile between species (Johnson 1979; Olsen 2007). The extremely long half lives of PFOA and PFOS in humans are in contrast with the relatively rapid elimination seen in animal models: the serum half life of PFOS in rats is around 100 days (Hundley et al. 2006), drawing attention to the potential risks to human health. Disruption to thyroid hormone balance was not found in previous studies of community exposure to PFOA (Emmett 2006; Olsen et al. 2003a) or PFOS (Inoue 2004) . Modest associations between PFOA and thyroid hormones (negative for free T_4 and positive for T_3) were reported in 506 PFOA production workers across three production facilities (Olsen and Zobel 2007). There were no associations between TSH or T_4 and PFOA and the free hormone levels were within the normal reference range.

Given the evidence from animal studies of thyroid hormone imbalance and the varied epidemiological results from community and occupational exposures, we aimed to explore the hypothesis that higher serum PFOA and PFOS concentrations would be

associated with thyroid disease in the general adult population. The U.S. Centers for Disease Control and Prevention (CDC) environmental chemical biomonitoring program, using samples from the US National Health and Nutrition Examination Survey (NHANES), provides large scale data on serum PFAA concentrations in population representative samples. Here, we use these data to estimate associations between PFOA/S concentrations and thyroid disease in representative samples of the general population of the USA.

Methods

Study population.

Data were from three independent cross-sectional waves of the US National Health and Nutrition Examination Survey (NHANES) 1999-2000, 2003-2004 and 2005-2006. NHANES surveys assess the health and diet of the non-institutionalized civilian population of the United States and are administered by the National Center for Health Statistics. The study protocol for NHANES was approved by the National Centers for Health Statistics Institutional Review Board.

Assessment of PFOA/S concentrations.

Solid phase extraction coupled to High Performance Liquid Chromatography-Turbo Ion Spray ionization-tandem Mass Spectrometry with isotope labeled internal standards was used for the detection of PFOA and PFOS, with a limit of detection of 0.2 ng/ml.

(Kuklenyik 2005). The laboratory methods and comprehensive quality control system were consistent in each NHANES wave, and documentation for each wave are available (1999-2000: http://www.cdc.gov/nchs/data/nhanes/frequency/sspfc_a.pdf ; 2003-2004: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/l24pfc_c.pdf ; and 2005-2006: http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/lab_methods_05_06.htm - accessed 2nd October 2009).

Serum polyfluorinated chemicals (PFCs) were measured in a one third representative random subset of persons 12 years and over in each NHANES wave. Data from individuals under 20 years old were excluded, as questions relating to disease prevalence were only asked of adults.

Disease outcomes.

In all NHANES waves, adult respondents were asked about physician diagnosed diseases. Associations were examined between PFOA and PFOS concentrations and thyroid disease outcomes. Individuals were asked whether they had ever been told by a doctor or health professional that they had a thyroid problem (in the 1999-2000 survey the questions related to goiter and other thyroid conditions), and whether they still had the condition. We further defined thyroid disease by considering those people who said they currently had thyroid disease and were taking any thyroid related medication, including levothyroxine; liothyronine; 'thyroid desiccated' and 'thyroid drugs unspecified' for hypothyroidism and propylthiouracil and methimazole for hyperthyroidism. No details were available on specific thyroid disease diagnosis and the PFC samples did not overlap with the thyroid hormone measurement sub-samples in NHANES.

To assess disease specificity, associations were examined between PFOA and the other NHANES disease categories elicited: Ischemic heart disease (combining any diagnoses of coronary heart disease, angina, and/or heart attack), diabetes, arthritis, current asthma, Chronic Obstructive Pulmonary Disease (COPD: bronchitis or emphysema) and current liver disease.

Statistical analysis.

NHANES uses a complex cluster sample design with some demographic groups (including less privileged socioeconomic groups and Mexican Americans) over-sampled to ensure adequate representation. Prevalence estimates and models were therefore survey weighted using the NHANES primary sampling unit, strata and population weights, unless otherwise stated.

Multivariate logistic regression modeling was used to estimate odds ratios of thyroid disease outcomes by quartile of PFOA and PFOS concentrations, and associations of other physician-diagnosed diseases. As thyroid disease prevalence is markedly higher in women, we used sex specific models. Because the distribution of PFC concentrations is skewed (with most people having relatively low-exposures and with considerably more variance at the higher exposure end), all available data were pooled and PFOA and PFOS concentrations were divided into population weighted quartiles. Using the Hsieh method (Hsieh 1998), our estimated power to detect an association of OR≥1.8 with current treated disease comparing the top PFOA quartile to bottom quartile is 67% in women. Combining the lowest two quartiles into a larger control group provides 80% power. The corresponding minimum detectable effect size in men is an OR>2.9. Assumptions for the

power calculations include a significance level of 5%, and a multiple correlation coefficient of 0.2 relating PFOA exposure to potential confounders.

Models were adjusted for the following potential confounding factors: year of NHANES study; age; gender; race/ethnicity, from self-description and categorized into: Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other race (including multi-racial); education, categorized into: less than high school, high school diploma (including GED), more than high school, and unknown education; smoking (from self-reported status asked in those aged 20 and over), categorized into: never smoked, former smoker, smoking some days, smoking every day, and unknown smoking status; Body Mass Index ('BMI', measured weight in kilograms divided by the square of measured height in meters), categorized into: underweight (BMI <18.5), recommended weight (BMI 18.5 to 24.9), overweight (BMI 25.0 to 29.9), obese I (BMI 30.0 to 34.9), obese II (BMI 35.0 or above), and unknown BMI; alcohol consumption (in adults aged 20 and over - based on responses to the question "In the past 12 months, on those days that you drank alcoholic beverages, on the average day, how many drinks did you have?"), categorized into: 0, 1, 2, 3, 4, 5 or more drinks per day, and unknown alcohol consumption. Regression analyses were conducted using STATA SE 10.1.

Results

Serum concentrations of PFOA were available for n=3974 individuals aged 20 years and older from NHANES waves 1999-2000 (n=1040), 2003-2004 (n=1454) and 2005-2006 (n=1480). In age, gender, NHANES wave and ethnicity adjusted analyses, mean levels of PFOA were higher in men than women (by 0.76ng/ml (95% CI: 0.73 to 0.80) p<0.0001),

and there were significant differences between ethnic groups (Table 1). Individuals with more education had higher PFOA levels (highest vs. lowest education: 1.1ng/ml (95% CI: 1.03 to 1.19, p=0.008). Increased alcohol consumption levels were also associated with higher PFOA concentrations (e.g. those drinking 5 or more drinks per day had mean PFOA levels 1.24ng/ml higher than non-drinkers (95% CI: 1.14 to 1.37, p<0.0001)).

In age, gender, NHANES wave and ethnicity adjusted analyses of the full sample (men and women), mean levels of PFOA were higher in men than women (p<0.0001), and there were significant differences between ethnic groups (Table 1). Individuals with more education had higher PFOA levels (p=0.008). Increased alcohol consumption levels were also associated with higher PFOA concentrations (p<0.0001)). Similar differences were found in PFOS concentrations. Mean levels of PFOS were higher in men (p<0.0001), with significant differences in levels between ethnic groups, and individuals with more education had higher PFOS levels (p=0.008).

Eight individuals did not answer questions about thyroid disease, so the included sample size was n=3966, with 1900 men and 2066 women (Table 2). In women, the overall (unweighted) numbers reporting any thyroid disease was n=292 and the NHANES weighted but unadjusted prevalence was 16.18%: for men. n=69, weighted prevalence 3.06%. The study weighted prevalence of current thyroid disease taking medication was necessarily lower (n=163, 9.89% for women; n=46, 1.18% for men).

Population weighted quartiles of PFOA and PFOS concentrations were computed for men and women separately (Table 2): the highest quartile (Q4) of PFOA for women

ranged from 5.7 to 123.0 ng/ml and for men 7.3 ng/ml to 45.9 ng/ml. Study weighted but unadjusted prevalences of current thyroid disease taking related medication in women varied across the quartiles, but with wide confidence intervals: Q1=8.14% (95%CI 5.75 to 10.53), Q4=16.19% (CI 11.74 to 20.62). In men, unadjusted prevalence rates were far lower throughout (prevalence = 2.27% in Q1 and Q4). For PFOS the prevalence of treated thyroid disease ranged from 8.14% (Q1) to 12.55% (Q4) in women and for men 1.85% to 3.89%.

In logistic regression models adjusting for age, ethnicity and study year (Table 3), there were associations between PFOA quartiles and both definitions of thyroid disease in women. For logistic models additionally adjusted for educational status, Body Mass Index, smoking status and alcohol consumption, these associations remained significant: e.g. comparing those with PFOA concentrations ≥5.7ng/ml (Q4) to PFOA ≤2.6ng/ml (Q1) the odds ratio for current thyroid disease on medication was OR=1.86 (95%CI: 1.12 to 3.09), p=0.018. Comparing Q4 to the larger control group of PFOA≤4.0ng/ml (Q1 & Q2) the estimated odds ratio for treated thyroid disease was OR=2.24 (CI 1.38 to 3.65), p=0.002. In men, there was a similar suggestive trend, but it narrowly missed significance: comparing PFOA ≥7.3ng/ml (Q4) to those with PFOA concentrations ≤5.2ng/ml (Q1&Q2) the odds ratio for treated disease was OR=2.12 (0.93 to 4.82), p=0.073.

For PFOS concentrations, in women odds ratios for disease trended in a similar direction, but were far from significance. However, in men as association was present comparing

those with PFOS≥36.8 ng/ml (Q4) to those with PFOS concentrations ≤25.5 ng/ml (Q1&Q2): odds ratio for treated disease was OR=2.68 (1.03 to 6.98), p=0.043.

Sensitivity analyses.

As a sensitivity analysis we computed a logistic regression model including both men and women, testing an interaction term between gender and PFOA levels for treated thyroid disease risk: the interaction term was not significant (p-value for interaction = 0.152.

As a post-hoc analysis we examined associations between chemical concentration quartile and any of the other major disease categories covered in NHANES: arthritis, asthma, Chronic Obstructive Pulmonary Disease (COPD), diabetes, Heart disease, or liver disease. Combining men and women (to reduce multiple testing and because these diseases are less gender related) we found no significant associations for PFOA (Table 4) except for comparisons of the intermediate quartiles to Q1 for arthritis: this did not reach significance in the top quartile.

For PFOS, there were no 'positive' associations between higher serum concentrations and higher prevalence of disease. There was one statistically significant 'negative' association suggesting that people reporting having Chronic Obstructive Pulmonary Disease (COPD) may be less likely to be in the highest PFOA concentration quartile (OR=0.58 CI 0.43 to 0.76, p=0.0003).

Discussion

This study aimed to determine whether increased serum PFOA or PFOS concentrations were associated with thyroid disease in a general adult US population sample. The prevalence of thyroid disease is markedly higher in women than in men, and therefore we have estimated sex specific associations. We have shown that across all the available data from NHANES, thyroid disease associations with serum PFOA concentrations are present in women and are strongest for those currently being treated for thyroid disease. For men, a near significant association between PFOA and treated thyroid disease was also present: an interaction term analysis suggests that the PFOA trends in men and women are not significantly different, despite the relative rarity of thyroid disease in men. In addition, a nominally significant association was present between PFOS concentrations and treated thyroid disease in men, but not in women.

The presence of associations with both PFOA and PFOS raises the issue of how best to perform risk assessments for combinations of perfluorochemicals. The somewhat divergent risk patterns for the two compounds supports their separate risk assessment (Scialli et al. 2007), given that current legislative advice (US EPA 2000; MDH 2008) is to consider the combined effects of chemicals only when two or more chemicals in a mixture affect the same tissue, organ or organ system.

Our results are important because PFAAs are detectable in virtually everyone in society (Kannan et al. 2004) with ubiquitous presence across global populations (Calafat et al. 2006). Occupational exposure to PFOA reported in 2003 showed mean serum values of

1,780 ng/ml (range 40-10,060 ng/ml (Olsen et al. 2003b) and 899 ng/ml (range 722-1,120 ng/ml, (Olsen et al. 2003c)) Production of PFOS was halted in 2002 in the USA by its principal producer, due largely to concerns over bioaccumulation and toxicity. Since then, voluntary industry reductions in production and usage of other perfluorinated compounds, such as the US EPA initiated PFOA Stewardship Programme (US EPA 2006) have contributed to a decreasing trend in human exposure for all perfluorinated compounds (with the notable exception of perfluorononanoic acid, PFNA) (Calafat et al. 2007; Olsen et al. 2007) . In May 2009, PFOS was listed under the Stockholm Convention on persistent organic pollutants (http://chm.pops.int/).

Our results can be compared with previous studies of human populations and also of non-human primates. A six month study of cynomolgus monkeys chronically exposed to PFOA showed no associations between PFOA and thyroid parameters, at mean serum PFOA concentrations higher than those reported in NHANES, although only male monkeys were involved (Butenhoff et al. 2002). The largest human study of PFOA is centered on an industrial facility in Washington, West Virginia, from which PFOA spread to the population through air, water, occupational and domestic exposure in a 'point source' contamination. The C8 Health Project (Steenland et al. 2009) has measured PFOA concentrations in over 69,000 residents. Markedly high concentrations were found, with an arithmetic mean of 83 ng/ml and a median concentration in serum of 28 ng/ml (http://c8sciencepanel.org/pdfs/Status_Report-factors_associated_with_C8_levels_Oct2008.pdf), far higher than the NHANES concentrations in the general population. Preliminary analyses report associations between PFOA and total cholesterol, LDL and triglyceride concentrations in multivariate

models adjusting for age, body mass index, sex, education, smoking, alcohol and regular exercise. Comprehensive cross-sectional and follow-on analyses of associations with thyroid disease have not yet been reported but are expected to be released in 2010-2011 (http://www.C8sciencepanel.org/studies.html#2).

Importantly, disruption to thyroid hormone balance was not found in other studies of populations exposed to PFOA, despite the considerably higher levels reported in some studies (Emmet et al. 2006; Olsen et al. 2003a). Emmet et al. (2006) studied 371 residents of a community with long standing environmental exposure to PFOA. They found a median serum PFOA concentration of 181-571 ng/ml, but there was no association between serum PFOA and a history of thyroid disease. In a study which included thyroid hormone levels, a positive association was reported between serum PFOA concentration and T_3 levels in occupationally exposed workers, although there were no changes in other thyroid hormones (Olsen et al. 2001). Modest associations between PFOA and thyroid hormones (negative for free T_4 and positive for T_3) were reported in 506 PFOA production workers across three production facilities (Olsen and Zobel 2007). There were no associations between TSH or T_4 and PFOA and the free hormone levels were within the normal reference range.

A linear extrapolation of the findings reported here would be expected to lead to associations being more evident at higher exposure levels, yet this is not supported by the literature. Non-linearity of response is not uncommon for receptor mediated systems such as endocrine-signalling pathways that act to amplify the original signal. Large changes in cell function can occur in response to extremely low concentrations, but which may

become saturated and hence unresponsive at higher concentrations (vom Saal and Hughes 2005; Welshons et al. 2003).

The mechanisms involved in thyroid homeostasis are numerous and complex and there are multiple potential targets for disruption of thyroid hormone homeostasis (Schmutzler et al. 2007). These include thyrotropin receptor (Santini et al. 2003), iodine uptake by the sodium iodide transporter (Schroder van der Elst et al. 2004), type 1 5'-deiodinase (Ferreira et al. 2002), transthyretin (Kohrle et al. 1988), thyroid hormone receptor (Moriyama et al. 2002) and the thyroid hormone dependent growth of pituitary cells (Ghisari and Bonefeld-Jorgensen 2005). Depression of serum T_4 and T_3 has been reported by several authors in PFOS-exposed rats (Lau et al. 2003; Luebker et al. 2005; Seacat et al. 2003). One mechanism by which PFAAs may deplete T_4 is through induction of the hepatic uridine diphosphoglucuronosyl transferase (UGT) system, which is involved in hepatic metabolism of thyroid hormone and biliary clearance of T_4 as T_4-glucuronide (Barter and Klaassen1994). Since PFOA is an agonist for PPARα, it is plausible that induction of hepatic UGT in PFAA-exposed rats (Yu et al. 2009) could represent a PPARα mediated response. The involvement of another PPARα agononist, WY 14643, in enhancing the hepatic degradation of thyroid hormone has recently been shown (Weineke et al. 2009).

A growing body of data describes the *in vitro* binding affinity of PFOA to human serum binding proteins (Chen and Guo 2009), PPAR α, β and γ, and other nuclear receptors (Vanden Huevel et al. 2006), but the contribution of these mechanisms to PFOA's

thyroid-mediating effects in humans remains to be established. Many cellular and metabolic processes including lipid metabolism, energy homeostasis and cell differentiation are controlled by PPARα. Early studies of the effects of PFAAs in rodents showed that a single dose lowered heart rate and body temperature and depressed T_4 and T_3. Replacement of T_4 did not reverse the clinical symptoms of hypothermia (Gutshall et al. 1988; Langley and Pilcher 1985). Although circulating thyroid hormone levels were low, liver enzymes responsive to thyroid hormone levels were elevated, suggesting that thyroidal homeostasis was not functionally compromised. Chang et al. (2007) found that exposure to PFOS for up to 3 weeks did not affect functional thyroid status, as free T_4, TSH and various thyroid-responsive liver enzymes were all unaffected. These findings, and later results have led to proposals that displacement of circulating thyroid hormones from plasma protein binding sites and a reduced responsiveness of the HPT axis contribute significantly towards PFOA's hypothyroid-inducing effects (Lau et al. 2007). Whatever the mechanisms involved, it is clear that more research is merited to clarify the pathways involved.

The feedback mechanism by which the rate of release of TSH and the circulating levels of T_3 and T_4 are regulated tends to show a low level of individual variation (Felt-Rasmussen et al. 1980). Therefore subtle disruption of the thyroid axis within normal reference ranges may have negative health consequences for the individual, whilst remaining within normal reference values, highlighting the importance of including both clinical and laboratory endpoints in such studies. The NHANES data do not allow specification of precise type of thyroid disease present, since it does not report on

individual hormone levels. PFOA concentration was positively associated with free T4 and negatively associated with T3 levels in a cohort of 506 exposed workers, with a near significant association with TSH levels (Olsen et al. 2007b), although all effects were regarded as modest.

The limitations of these analyses should be noted. The PFOA and PFOS measures were based on a single serum sample. Although PFOA has a half life of four years {Olsen 2007) and therefore a single sample is likely to represent medium term internal dose, samples taken at several time points might be more accurate in classifying exposure. Any misclassification from single measures would tend to decrease power and under-estimate the real strengths of association. Secondly, the PFOA concentrations were measured at the same time as disease status, making attribution of causal direction difficult. This raises the possibility of reverse causation. One might hypothesize that following onset of thyroid disease, changes in the nature of exposure or in the pharmacokinetics of PFOA might occur (including patterns of absorption, distribution (including protein binding) or excretion). As the associations we report were present in people who were on thyroid hormone replacements, which effectively mimic normal thyroid function, a mechanism for reverse causation through changes in pharmacokinetics is difficult to imagine. Confounding by unmeasured factors is also possible, but it is unlikely that confounding could explain similar findings reported from some of the diverse experimental and observational studies discussed above.

Post-hoc association testing with other common diseases (necessarily involving multiple statistical testing) did not identify other robust associations of higher PFC concentration

with increased disease prevalence, suggesting specificity of our findings for thyroid disease. An apparent association between higher PFOS concentrations and lower prevalence of COPD requires replication, to exclude a false positive result from multiple testing. In addition to the limitations of our analyses, the strengths should also be noted: this is the first large-scale nationally representative general adult population analysis of directly measured serum concentrations of PFOA and PFOS. In addition, the associations present are strongest for the most specific identification of thyroid disease, based on reported diagnosis with current use of thyroid specific medication. The NHANES study also supported adjustment of models for a range of potential confounding factors, which in fact made relatively minor differences to the key estimates, suggesting that the associations are robust.

Further work is clearly needed to characterize the PFOA and PFOS associations with specific thyroid diagnoses and thyroid hormone levels in the general population, and clarify whether the associations reflect pathology, changes in exposure or altered pharmacokinetics. Longitudinal analyses are also needed to establish whether high exposures predict future onsets of thyroid disease, although concurrent alteration of thyroid functioning would still be a cause for concern.

Conclusions

Higher PFOA and PFOS concentrations are associated with thyroid disease (and being on thyroid related medication) in the NHANES US general adult population representative

study samples. More work is needed to establish the mechanisms underlying this association and to exclude confounding and pharmacokinetic explanations.

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Table 1: Survey weighted characteristics of sample with survey weighted back-transformed geometric mean concentrations of PFOA and PFOS.

	MEN			WOMEN		
	n (% within group)	*Mean PFOA (95% CI)*	*Mean PFOS (95% CI)*	n (% within group)	Mean PFOA (95% CI)	Mean PFOS (95% CI)
	1900	4.91 (4.64 to 5.2)	25.08 (23.63 to 26.62)	2066	3.77 (3.52 to 4.04)	19.14 (17.8 to 20.58)
Age						
20 to 49	928 (62.6%)	5.30 (5.02 to 5.59)	24.2 (22.66 to 25.84)	1134 (59.8%)	3.38 (3.13 to 3.64)	16.72 (15.37 to 18.19)
50 to 69	553 (27.0%)	4.46 (4.12 to 4.82)	26.97 (24.87 to 29.25)	545 (26.7%)	4.62 (4.15 to 5.14)	22.92 (20.81 to 25.23)
70 +	419 (10.4%)	3.99 (3.53 to 4.52)	25.8 (23.22 to 28.67)	387 (13.4%)	4.13 (3.78 to 4.51)	24.39 (22.19 to 26.81)
Ethnicity n (survey weighted %)						
Mexican American	432 (8.3%)	3.73 (3.48 to 4)	18.44 (16.79 to 20.25)	481 (7.3%)	2.44 (2.21 to 2.7)	12.04 (10.91 to 13.29)
Other Hispanic	62 (4.2%)	5.63 (4.77 to 6.65)	27.73 (21.89 to 35.14)	92 (5.9%)	3.6 (3.17 to 4.09)	16.28 (14.29 to 18.56)
Non-Hispanic White	969 (73.5%)	5.13 (4.83 to 5.45)	25.7 (24.04 to 27.47)	1008 (71.8%)	4.13 (3.84 to 4.45)	20.08 (18.57 to 21.72)
Non-Hispanic Black	382 (9.9%)	4.43 (3.92 to 5)	27 (24.3 to 30)	415 (10.8%)	2.98 (2.66 to 3.34)	20.52 (17.97 to 23.42)
Other Race	55 (4.1%)	4.32 (3.47 to 5.38)	22.77 (17.3 to 29.97)	70 (4.3%)	3.3 (2.61 to 4.17)	19.64 (15.04 to 25.64)
Education n (survey weighted %)						
Less than high school	637 (20.9%)	4.14 (3.82 to 4.49)	22.08 (20.47 to 23.82)	628 (19.5%)	3.53 (3.11 to 4)	19.26 (17.1 to 21.69)
High school graduate	478 (29.3%)	4.94 (4.56 to 5.35)	25.22 (23.56 to 27)	498 (25.9%)	4 (3.69 to 4.34)	19.9 (18.15 to 21.83)
More than high school	782 (49.7%)	5.26 (4.93 to 5.62)	26.38 (24.54 to 28.36)	937 (54.6%)	3.76 (3.49 to 4.04)	18.74 (17.32 to 20.28)
Unknown	3 (0.0%)	2.22 (1.31 to 3.74)	17.59 (8.5 to 36.41)	3 (0.0%)	1.84 (1.32 to 2.56)	12.64 (7.25 to 22.02)
BMI Categories n (survey weighted %)						
Underweight (BMI 0-18.5)	23 (1.2%)	4.42 (2.78 to 7.01)	17.95 (10.79 to 29.87)	35 (2.4%)	3.78 (3.07 to 4.65)	18.39 (14.05 to 24.08)
Normal (BMI 18.5-25)	525 (28.8%)	4.89 (4.44 to 5.39)	24.36 (21.99 to 26.98)	594 (32.6%)	3.84 (3.52 to 4.19)	18.97 (17.53 to 20.52)
Overweight (BMI 25-30)	778 (39.8%)	5.01 (4.67 to 5.38)	25.28 (23.55 to 27.13)	615 (27.8%)	3.64 (3.3 to 4.01)	19.48 (17.86 to 21.24)
Obese (BMI 30+)	545 (29.0%)	4.9 (4.54 to 5.29)	26.28 (24.42 to 28.28)	784 (36.0%)	3.83 (3.49 to 4.2)	19 (17 to 21.22)
Unknown	29 (1.2%)	3.1 (2.23 to 4.3)	17.51 (10.34 to 29.66)	38 (1.2%)	3.36 (2.78 to 4.05)	21.81 (17.13 to 27.78)
Smoking status n (survey weighted %)						
Smoked <100 cigarettes in lifetime	761 (41.1%)	5.01 (4.68 to 5.37)	26.16 (24.19 to 28.29)	1300 (58.6%)	3.63 (3.36 to 3.93)	18.91 (17.43 to 20.52)
Former smoker	664 (30.0%)	4.67 (4.28 to 5.09)	25.66 (23.7 to 27.79)	417 (21.3%)	3.81 (3.46 to 4.19)	19.22 (17.33 to 21.32)

Table 1 continued

Some days	90 (4.8%)	5.12 (4.23 to 6.19)	21.33 (17.97 to 25.31)	46 (2.4%)	4.05 (3.36 to 4.89)	18.29 (15.06 to 22.22)
Every day	385 (24.1%)	5.01 (4.7 to 5.33)	23.43 (21.45 to 25.59)	302 (17.8%)	4.17 (3.73 to 4.67)	19.9 (17.76 to 22.29)
Unknown				1 (0.0%)		
Drinking (average # drinks per day in past 12 months) n (survey weighted %)						
Non-drinker	334 (15.8%)	4.44 (3.89 to 5.06)	25.38 (22.78 to 28.28)	857 (35.1%)	3.5 (3.22 to 3.8)	18.84 (16.81 to 21.11)
1 drink / day	298 (15.7%)	4.67 (4.22 to 5.17)	26.39 (23.19 to 30.02)	316 (17.9%)	3.87 (3.47 to 4.32)	20.37 (18.12 to 22.9)
2 drinks / day	282 (17.5%)	5.4 (4.86 to 6.01)	27.7 (24.76 to 30.99)	291 (18.0%)	4.15 (3.79 to 4.55)	19.82 (17.95 to 21.87)
3 drinks / day	181 (11.1%)	5.2 (4.79 to 5.65)	23.73 (21.2 to 26.56)	124 (6.6%)	3.82 (3.4 to 4.29)	18.07 (15.81 to 20.65)
4 drinks / day	97 (6.0%)	5.58 (4.94 to 6.31)	25.05 (21.27 to 29.5)	64 (3.3%)	3.96 (3.19 to 4.92)	15.71 (12.89 to 19.14)
5+ drinks / day	283 (16.4%)	5.25 (4.88 to 5.65)	23.09 (20.92 to 25.49)	80 (4.8%)	4.63 (3.62 to 5.91)	18.13 (14.76 to 22.27)
Unknown	425 (17.6%)	4.44 (4.07 to 4.84)	24.04 (21.44 to 26.96)	334 (14.4%)	3.58 (3.22 to 3.98)	19.26 (17.29 to 21.46)

Table 2: Gender specific summary statistics with thyroid disease prevalence by weighted quartile* of PFOA and PFOS.

	Summary statistics by quartile				Thyroid disease ever			Current thyroid disease with thyroid medication		
		Unweighted		Weighted	N	Unweighted	Weighted	N	Unweighted	Weighted
	N	Range (ng/ml)	Mean (sd) ng/ml	Mean (95% CI) ng/ml	(case/total)	Prevalence (%)	prevalence (%) (95% CI)	(case/total)	Prevalence (%)	prevalence (%) (95% CI)
WOMEN PFOA – All and population based quartiles										
All	2066	0.1 to 123.0	4.25 (4.92)	4.84 (4.35 to 5.33)	292/2066	14.13	16.18 (14.44 to 18.09)	163/2066	7.89	9.89 (8.32 to 11.72)
Q1	689	0.1 to 2.6	1.71 (0.66)	1.79 (1.72 to 1.85)	65/689	9.43	12.62 (9.66 to 15.57)	34/689	4.93	8.14 (5.75 to 10.53)
Q2	550	2.7 to 4.0	3.32 (0.40)	3.33 (3.28 to 3.38)	71/550	12.91	13.87 (9.66 to 18.08)	35/550	6.36	7.27 (4.37 to 10.16)
Q3	441	4.1 to 5.7	4.79 (0.48)	4.78 (4.72 to 4.85)	72/441	16.33	15.98 (11.81 to 20.15)	39/441	8.84	8.25 (4.97 to 11.52)
Q4	386	5.7 to 123.0	9.47 (9.38)	9.7 (8.43 to 10.98)	84/386	21.76	22.57 (17.44 to 27.71)	55/386	14.25	16.18 (11.74 to 20.62)
WOMEN PFOS – All and population based quartiles										
All	2066	0.14 to 406.0	23.24 (23.13)	24.78 (22.6 to 26.9)	292/2066	14.13	16.18 (14.44 to 18.09)	163/2066	7.89	9.89 (8.32 to 11.72)
Q1	616	0.14 to 12.4	8.13 (2.82)	8.49 (8.06 to 8.93)	68/616	11.04	15.14 (10.82 to 19.46)	32/616	5.19	8.7 (5.45 to 11.96)
Q2	523	12.5 to 19.4	15.75 (2.02)	15.92 (15.71 to 16.13)	71/523	13.58	16.23 (12.58 to 19.89)	40/523	7.65	9.85 (6.58 to 13.13)
Q3	466	19.5 to 29.8	24.21 (2.89)	24.49 (24.03 to 24.94)	62/466	13.30	12.69 (8.59 to 16.78)	39/466	8.37	8.47 (4.98 to 11.97)
Q4	461	29.9 to 406.0	50.96 (35.15)	50.48 (45.81 to 55.16)	91/461	19.74	20.66 (16.46 to 24.87)	52/461	11.28	12.55 (8.86 to 16.23)
MEN PFOA – All and population based quartiles										
All	1900	0.1 to 45.9	5.23 (3.41)	5.79 (5.41 to 6.18)	69/1900	3.63	3.06 (2.40 to 3.88)	46/1900	2.42	1.88 (1.30 to 2.69)
Q1	643	0.1 to 3.6	2.47 (0.85)	2.51 (2.43 to 2.58)	24/643	3.73	3.49 (2.01 to 4.97)	16/643	2.49	2.27 (1.25 to 3.30)
Q2	517	3.7 to 5.2	4.42 (0.45)	4.44 (4.39 to 4.50)	20/517	3.87	3.44 (1.48 to 5.41)	13/517	2.51	2.14 (0.79 to 3.49)
Q3	381	5.3 to 7.2	6.12 (0.55)	6.19 (6.12 to 6.26)	11/381	2.89	1.51 (0.38 to 2.63)	7/381	1.84	0.77 (0.17 to 1.37)
Q4	359	7.3 to 45.9	10.39 (4.20)	10.3 (9.72 to 10.89)	14/359	3.90	3.71 (1.67 to 5.75)	10/359	2.79	2.27 (0.22 to 4.33)
MEN PFOS – All and population based quartiles										
All	1900	0.3 to 435.0	29.57 (22.11)	30.36 (28.2 to 32.5)	69/1900	3.63	3.06 (2.40 to 3.88)	46/1900	2.42	1.88 (1.30 to 2.69)
Q1	529	0.3 to 18.0	12.29 (4.30)	12.35 (11.94 to 12.76)	18/529	3.40	3.22 (1.86 to 4.57)	10/529	1.89	1.85 (0.82 to 2.89)
Q2	480	18.2 to 25.5	21.82 (2.13)	21.83 (21.63 to 22.03)	13/480	2.71	1.64 (0.40 to 2.87)	8/480	1.67	0.80 (0.12 to 1.48)
Q3	454	25.6 to 36.7	30.81 (3.18)	30.93 (30.57 to 31.29)	15/454	3.30	2.68 (1.26 to 4.10)	11/454	2.42	1.62 (0.55 to 2.69)
Q4	437	36.8 to 435.0	57.73 (29.4)	56.45 (52.85 to 60.04)	23/437	5.26	4.69 (2.44 to 6.95)	17/437	3.89	3.24 (1.07 to 5.40)

Note: *quartiles defined to reflect the US population, accounting for population weighting in NHANES

Table 3: Gender specific, survey weighted associations between PFOA and PFOS concentrations and thyroid disease.

		PFOA		PFOS	
		Models adjusting for age, ethnicity & study year: OR (95% CI), p-value	Fully adjusted models *: OR (95% CI) p-value	Models adjusting for age, sex, ethnicity & study year: OR (95% CI), p-value	Fully adjusted models *: OR (95% CI) p-value
Women - Thyroid disease ever					
Quartiles of PFC	Q1	1	1	1	1
	Q2	0.98 (0.65 to 1.50), p=0.936	0.95 (0.62 to 1.47), p=0.825	1.04 (0.63 to 1.71), p=0.875	1.01 (0.63 to 1.6), p=0.972
	Q3	1.09 (0.66 to 1.81), p=0.729	1.11 (0.67 to 1.83), p=0.679	0.68 (0.4 to 1.17), p=0.155	0.64 (0.39 to 1.05), p=0.078
	Q4	1.63 (1.07 to 2.47), p=0.024 **	1.64 (1.09 to 2.46), p=0.019 **	1.11 (0.66 to 1.86), p=0.69	1.15 (0.7 to 1.91), p=0.568
Top quartile (Q4) vs Q1&2		1.64 (1.12 to 2.41), p=0.013 **	1.68 (1.14 to 2.49), p=0.011 **	1.08 (0.73 to 1.61), p=0.681	1.15 (0.78 to 1.7), p=0.48
Women - Thyroid disease current with medication					
Quartiles of PFC	Q1	1	1	1	1
	Q2	0.77 (0.45 to 1.32), p=0.334	0.7 (0.41 to 1.22), p=0.205	1.11 (0.58 to 2.14), p=0.747	1.05 (0.55 to 2), p=0.89
	Q3	0.86 (0.47 to 1.57), p=0.607	0.89 (0.49 to 1.59), p=0.676	0.85 (0.46 to 1.59), p=0.609	0.81 (0.44 to 1.51), p=0.496
	Q4	1.83 (1.13 to 2.95), p=0.015**	1.86 (1.12 to 3.09), p=0.018**	1.27 (0.69 to 2.32), p=0.435	1.31 (0.72 to 2.36), p=0.369
Top quartile (Q4) vs Q1&2		2.09 (1.34 to 3.26), p=0.002 **	2.24 (1.38 to 3.65), p=0.002 **	1.19 (0.77 to 1.85), p=0.417	1.27 (0.82 to 1.97), p=0.269
Men - Thyroid disease ever					
Quartiles of PFC	Q1	1	1	1	1
	Q2	1.17 (0.64 to 2.15), p=0.600	1.11 (0.62 to 1.99), p=0.729	0.50 (0.22 to 1.17), p=0.107	0.51 (0.23 to 1.14), p=0.097
	Q3	0.58 (0.21 to 1.59), p=0.283	0.57 (0.19 to 1.66), p=0.291	0.81 (0.40 to 1.61), p=0.536	0.88 (0.43 to 1.84), p=0.736
	Q4	1.58 (0.79 to 3.16), p=0.191	1.58 (0.74 to 3.39), p=0.233	1.51 (0.70 to 3.22), p=0.284	1.58 (0.72 to 3.47), p=0.251
Top quartile (Q4) vs Q1&2		1.45 (0.68 to 3.09), p=0.323	1.5 (0.66 to 3.39), p=0.324	1.6 (0.57 to 4.46), p=0.360	1.78 (0.58 to 5.52), p=0.309
Men - Thyroid disease current with medication					
Quartiles of PFC	Q1	1	1	1	1
	Q2	1.18 (0.55 to 2.54), p=0.668	1.12 (0.52 to 2.39), p=0.767	0.42 (0.16 to 1.10), p=0.077	0.43 (0.17 to 1.08), p=0.073
	Q3	0.51 (0.20 to 1.32), p=0.162	0.49 (0.18 to 1.38), p=0.171	0.82 (0.29 to 2.27), p=0.694	0.95 (0.34 to 2.70), p=0.926
	Q4	1.74 (0.63 to 4.78), p=0.275	1.89 (0.60 to 5.90), p=0.268	1.72 (0.73 to 4.05), p=0.211	1.89 (0.72 to 4.93), p=0.190
Top quartile (Q4) vs Q1&2		2.02 (0.89 to 4.58), p=0.092	2.12 (0.93 to 4.82), p=0.073	2.44 (1.04 to 5.74), p=0.041 **	2.68 (1.03 to 6.98), p=0.043 **

Notes: * Models adjusted for age, ethnicity, education, BMI, smoking status, alcohol consumption; ** Significant association with 95% confidence

Table 4: Associations between PFOA & PFOS concentrations (population based quartiles) and other diseases in fully adjusted logistic regression models (by self-reported disease status).

	PFOA			PFOS		
	n (survey weighted %)	OR (95% CI)	p-value	n (survey weighted %)	OR (95% CI)	p-value
Arthritis ever	1006/3960 (22.8%)			1006/3960 (22.8%)		
Q1	287/1310 (19.2%)	1	1	219/1132 (19.0%)	1	1
Q2	298/1036 (27.6%)	1.63 (1.24 to 2.14)	0.001	267/1009 (23.5%)	1.19 (0.91 to 1.54)	0.193
Q3	231/857 (22.7%)	1.31 (1.03 to 1.66)	0.029	260/916 (26.8%)	1.29 (1.00 to 1.66)	0.054
Q4	190/757 (21.8%)	1.28 (0.97 to 1.68)	0.082	260/903 (22.0%)	0.74 (0.53 to 1.04)	0.085
Asthma ever	471/3961 (13.2%)			471/3961 (13.2%)		
Q1	138/1313 (11.9%)	1	1	139/1133 (14.0%)	1	1
Q2	128/1036 (14.2%)	1.25 (0.92 to 1.70)	0.154	140/1013 (15.6%)	1.16 (0.80 to 1.68)	0.427
Q3	122/856 (15.8%)	1.44 (1.01 to 2.05)	0.045	111/914 (13.1%)	0.97 (0.65 to 1.43)	0.867
Q4	83/756 (11.2%)	0.93 (0.64 to 1.36)	0.716	81/901 (10.3%)	0.79 (0.50 to 1.26)	0.320
COPD ever	302/3953 (8.2%)			302/3953 (8.2%)		
Q1	81/1310 (7.7%)	1	1	83/1131 (8.8%)	1	1
Q2	93/1033 (8.8%)	0.91 (0.58 to 1.43)	0.677	85/1008 (8.5%)	0.84 (0.56 to 1.25)	0.384
Q3	66/853 (8.3%)	0.88 (0.54 to 1.43)	0.593	67/914 (7.7%)	0.67 (0.41 to 1.09)	0.103
Q4	62/757 (8.2%)	0.85 (0.54 to 1.34)	0.473	67/900 (7.9%)	0.58 (0.43 to 0.76)	0.0003
Diabetes ever	459/3964 (8.7%)			459/3964 (8.7%)		
Q1	186/1314 (10.9%)	1	1	122/1133 (8.6%)	1	1
Q2	127/1035 (9.2%)	0.80 (0.55 to 1.17)	0.242	119/1012 (9.3%)	1.02 (0.70 to 1.47)	0.928
Q3	83/857 (7.7%)	0.74 (0.48 to 1.15)	0.177	103/916 (7.7%)	0.76 (0.50 to 1.18)	0.218
Q4	63/758 (7.0%)	0.69 (0.41 to 1.16)	0.158	115/903 (9.4%)	0.87 (0.57 to 1.31)	0.491
Heart disease ever[a]	321/3966 (5.8%)			321/3966 (5.8%)		
Q1	93/1314 (5.7%)	1	1	69/1134 (4.8%)	1	1
Q2	93/1037 (6.1%)	0.95 (0.59 to 1.51)	0.816	85/1013 (5.1%)	0.77 (0.49 to 1.23)	0.270
Q3	78/857 (5.9%)	1.02 (0.65 to 1.61)	0.917	80/916 (5.7%)	0.83 (0.46 to 1.51)	0.540
Q4	57/758 (5.4%)	1.08 (0.70 to 1.69)	0.715	87/903 (7.4%)	0.91 (0.50 to 1.64)	0.745
Liver Disease current	57/3942 (1.4%)			57/3942 (1.4%)		
Q1	24/1307 (1.4%)	1	1	22/1127 (1.7%)	1	1
Q2	11/1028 (1.0%)	0.66 (0.25 to 1.74)	0.391	10/1007 (0.9%)	0.49 (0.18 to 1.32)	0.154
Q3	17/855 (2.5%)	1.93 (0.96 to 3.88)	0.065	13/910 (1.6%)	0.94 (0.41 to 2.16)	0.880
Q4	5/752 (0.8%)	0.61 (0.21 to 1.78)	0.355	12/898 (1.5%)	0.95 (0.39 to 2.29)	0.907

[a] any report of coronary heart disease, and/or angina, and/or heart attack