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January 9, 2013

TSCA Confidential Business Information Center (7407M)
EPA East - Room 6428, Attn: Section 8(e)/FYI
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

Re: TSCA Section 8(e) And FYI Submission Re: PFOA – Human Health Effects

To TSCA 8(e)/FYI Database:

We hereby submit the following information to USEPA, pursuant to its TSCA Section 8(e) and "FYI" submission procedures, providing information relating to human health effects linked to exposure to perfluorooctanoate acid ("PFOA," a/k/a "C-8"):

1. Javins, B., *et al.*, "Circulating Maternal Perfluoroalkyl Substances during Pregnancy in the C8 Health Study," *Environ. Sci. & Tech.* (doi: 10.1021/es3028082) (on-line Jan. 7, 2013);
2. Dong, G-H., *et al.*, "Serum Polyfluoroalkyl Concentrations, Asthma Outcomes, and Immunological Markers in a Case-Control Study of Taiwanese Children," *Environ. Health Perspec.* (dx.doi.org/10.1289/ehp.1205351) (on-line Jan. 8, 2013); and
3. Vieira, V.M., *et al.*, "Perfluorooctanoic Acid Exposure and Cancer Outcomes in a Contaminated Community: A Geographic Analysis," *Environ. Health Perspec.* (dx.doi.org/10.1289/ehp.1205829) (on-line Jan. 8, 2013).

Very truly yours,

Robert A. Bilott

RAB:mdm
Enclosures



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Circulating Maternal Perfluoroalkyl Substances during Pregnancy in the C8 Health Study

Beth Javins, Gerald Hobbs, Alan M. Ducatman, Courtney Pilkerton, Danyel Tacker, and Sarah S Knox

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Circulating Maternal Perfluoroalkyl Substances during Pregnancy in the C8 Health Study

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ABSTRACT

Perfluoroalkyl substances (PFAFs) are man-made chemicals used in many consumer products and have become ubiquitous in the environment. Animal studies and a limited number of human studies have demonstrated developmental effects in offspring exposed to PFAFs *in utero* but the implications of timing of *in utero* exposure have not been systematically investigated. The current study investigated variation in perfluorocarbon levels of 9,952 women of childbearing age who had been exposed to perfluorooctanoic acid (PFOA) in drinking water contaminated by industrial waste. An analysis of variance with contrast was performed to compare the levels of PFOA and perfluorooctanesulfonic acid (PFOS) in pregnant and non-pregnant women overall

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2
3 and during each trimester of pregnancy. We found that pregnant women had lower circulating PFOA and
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5 PFOS concentrations in peripheral blood than non-pregnant women and that PFOA levels were consistently
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7 lower throughout all trimesters for pregnancy, suggesting transfer to the fetus at an early stage of gestation.
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9 These results are discussed in the context of the endocrine disrupting properties of perfluoroalkyl substances
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11 that have been characterized in animal and human studies. Our conclusion is that further, systematic study of
12
13 the potential implications of intrauterine perfluorocarbon exposure during critical periods of fetal
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15 development is urgently needed.
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19 INTRODUCTION

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21 Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are man-made perfluoroalkyl
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23 substances (PFASs) historically used in many consumer and industrial products. PFOA has been used
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25 primarily in the linings of cookware and food containers, and PFOS in stain resistant clothing and
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27 textiles, cleaning agents (waxes and floor polishes), and paint and varnish [1]. Because of their widespread
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29 use, PFASs have become ubiquitous in the environment and can be found in soil, water, wildlife, and in
30
31 measurable quantities in most humans [2], even those living in remote areas [3]. Data from a
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33 representative random sample (NHANES) in the United States indicate that 98% of participants had
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35 measurable quantities of PFOA and PFOS [4].
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41 Several animal studies have addressed the issue of reproductive toxicity. Leubker [5] and Grasty [6]
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43 found that pup PFASs were proportional to serum concentrations in the mothers, indicating that females
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45 transferred PFASs to their offspring. It has also been shown that daily PFAS exposure in pregnant rats,
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47 mice, and rabbits resulted in complications varying from altered feed consumption, to hepatomegaly,
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49 decreased litter size, and increased litter reabsorption [6-
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53 10]. Additional studies have found complications in offspring exposed to PFASs *in utero* that included a
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55 decreased rate of survival [11], decreased fetal weight [10], and developmental and birth defects (e.g.,
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3 delayed ossification of bones, enlarged right atrium, cleft palate, and inhibition of lung maturation) [8-
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5 11].
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8 Results from these studies have raised concerns about the health consequences of PFAS
9 exposure in pregnant women. Although human research is limited, PFOA was documented in all
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11 100 umbilical cord blood samples of one study [12] and an association between maternal and fetal
12 exposure to PFASs has been documented through umbilical cord blood concentrations at birth that were
13 proportional to maternal serum concentrations [13-18]. The Danish Cohort Study
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15 reported that maternal serum levels of PFOA and PFOS decreased in the second trimester compared to the
16 first [16] and a small study of 105 babies detected both PFOS and PFOA in umbilical cord blood. That
17 same study showed that mid-pregnancy maternal serum PFOS levels were higher than the maternal values
18 at delivery [17]. Together these data lend strong support for transfer PFAFs to the fetus in the latter
19 stages of pregnancy. Further human research has found an inverse association between gestational age and
20 circulating PFASs in pregnant mothers [16, 17] and data from a small sample of women in the National
21 Health and Nutritional Examination Survey (NHANES) indicate that pregnant women may have lower
22 PFAS levels than non-pregnant women [19].
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39 Thus, accumulating human and animal research raises concerns about *in utero* exposure to PFASs.
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41 The purpose of the present study was to investigate variation in PFAS levels in pregnant women during
42 gestational trimester and compare them with those of non-pregnant women in a large geographic area where
43 exposure to PFOA in drinking water had resulted from toxic waste disposal. Based on earlier animal and
44 human data, we hypothesized that redistribution of PFASs to the fetus during gestation would result in
45 lower circulating serum PFAS levels in the peripheral blood of mothers during all trimesters of
46 pregnancy compared with levels found in the non-pregnant women.
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55 MATERIALS AND METHODS

Participants

Participants were 9,952 women between the ages of 18 and 42 years enrolled in the C8 Health Project, which resulted from a class action suit. The "Class" was defined as individuals in West Virginia or Ohio who had consumed contaminated drinking water at a residence, place of employment, or school within a specific area of West Virginia and Ohio for at least a year between 1950 and December 3, 2004. Details have been described elsewhere [20]. Because of the circumstances of the study, the average level of exposure in these participants was higher than in NHANES [19]. Eligibility requirements for inclusion of women in the study were: that their residential water level exposure was classified as consistent with respect to geographic area prior to measurement (as described below), that they were of childbearing age (defined as 18-42 years), and that they had responded to the question on pregnancy status. There were 498 pregnant women and 9,454 non-pregnant women who met the inclusion criteria. Eligible enrolled participants filled out surveys with demographic, medical and other information and submitted a voluntary blood sample between August 1, 2005 and August 31, 2006. A detailed description of the consent, surveys, blood processing, and blood sample storage is available online at <http://www.hsc.wvu.edu/som/cmed/c8/>. Month of pregnancy was measured by self-report. The study was cross-sectional, meaning that perfluorocarbon measurements were done once in each woman.

Residential History of Exposure

Because this was an exposure study, a complete residential history was obtained from each participant to provide an indication of consistency and amount of exposures. Participants were eligible for the study if they had regularly consumed drinking-water from one of the six water districts of PFOA exposure based on residence, or having regularly worked or gone to school there. Interpreting the exposure of participants who had never lived in the C8 Health study water districts but were included due to regular exposure in their

workplaces or schools would have been difficult. Also, including women who had moved between districts with high, medium or low levels of exposure would also have made interpretation difficult. Therefore, non-residents and inconsistent residents were excluded from the analyses. There were 1,604 women excluded

due to not having resided in any of the C8 Health Study water districts, and 1,750 excluded due to inconsistent residential history. This left 498 pregnant women and 9,454 non-pregnant women whose data were analyzed.

Blood Sample Processing and Laboratory Methods

Participants voluntarily submitted up to 26mL of blood for analysis in the C8 Health Study, and the resulting serum was banked during the collection period (2005-2006). PFAS concentrations were quantified at Exygen Research Inc., State College, PA, USA. The analytic protocol was a modification of a previously described procedure combining protein precipitation extraction and reverse-phase high-performance liquid chromatography-tandem mass spectrometry. Spectrometric detection was performed using a triple quadrupole mass spectrometer in selected reaction monitoring mode, monitoring for the individual m/z transitions for each of the 10 PFASs and the ^{13}C -PFOA internal standard. A description of the perfluoroalkyl acid analytic techniques and quality assurance protocols for the C8 Health Project has been published elsewhere [20].

Statistical Analyses

Statistical analyses were performed using JMP/PRO 10 Visualization Software (SAS Institute Inc., Cary NC). Because month of gestation was self-report and calculating exactly when the pregnancy started was not exact, months one and two were combined in the analyses. An analysis of variance and covariance with a contrast statement comparing pregnant and non- pregnant women was calculated to investigate the

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3 differences in PFOA and PFOS concentrations between pregnant and non-pregnant women. Separate
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5 analyses were also calculated to compare non-pregnant women with those in each trimester of pregnancy
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7 (defined as months 1-3, 4-6, and
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10 7-9). The natural logarithm for PFOA and PFOS was used in all analyses to mitigate the effects
11 of outliers. All analyses were controlled for level of education and income, (proxies of socioeconomic status
12 that could influence simultaneous exposure to other toxicants based on living area within the water
13 district); age (older women may have longer exposure times); parity (increased number of pregnancies may
14 decrease perfluorocarbon levels [16]), smoking and alcohol consumption (which can impair blood flow
15 to the placenta [21, 22]), as well as a surrogate for plasma volume, which changes during pregnancy
16 [23] and could potentially affect interpretation of the PFAS measurements. Direct measures of plasma
17 volume such as Evan's Blue Dye [24] were not available in the dataset, so we performed plasma volume
18 adjustments using two different surrogate measures: hematocrit and the hemoglobin/hematocrit ratio.
19 Hematocrit is a crude estimate of overall plasma volume, i.e., the percentage of blood volume consisting
20 of packed red blood cells, and the hemoglobin-hematocrit ratio adds additional information concerning
21 whether red blood morphology might be contributing to a hemoglobin value. If the hemoglobin-hematocrit
22 ratio decreases and there is no other reason for a reduced red blood cell mass, the reduction is attributed
23 to increased plasma volume. Equations were calculated with both versions with similar results. The proxy
24 for plasma volume described in the results section is hemoglobin-hematocrit ratio. Covariates were allowed
25 to enter the equation in order of importance.
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47 RESULTS

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49 Descriptive characteristics of pregnant and non-pregnant women are listed in Table 1. Their PFOS and
50 PFOA concentrations are listed in Table 2. Both PFOS and PFOA are lower in pregnant women than in
51 non-pregnant women and this is reflected also in the covariates in Table
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Table 1. Select characteristics of the study population. Unless indicated otherwise, results are presented as: median, mean $\pm$ standard deviation. Percentages are rounded to the nearest decimal.

	Non-Pregnant Women (n=9,454*)			Pregnant Women (n=498*)		
	Median	Mean	Std. Dev.	Median	Mean	Std. Dev.
Age (Years)	30.74	30.44	± 7.00	26.26	26.93	± 5.14
Hematocrit (%)	40.90	40.79	± 3.04	36.90	37.06	± 3.30
Hemoglobin (g/dl)	13.80	13.74	± 1.05	12.50	12.54	± 3.30
Hemoglobin/Hematocrit Ratio	0.34	0.34	± 0.01	0.34	0.34	± 0.01
Parity (Number of Times Pregnant)	1.00	1.57	± 1.48	2.00	1.75	± 1.44
	% Non-Pregnant Women	PFOA [†]	PFOS [†]	% Pregnant Women	PFOA [†]	PFOS [†]
Highest Level of Education Obtained						
Less than a high school diploma	8.4%	32.29	15.00	11.3%	22.01	11.08
High school diploma or GED	34.1%	44.56	16.88	29.8%	22.03	12.93
Some college	41.5%	44.18	17.60	41.0%	22.11	16.41
Bachelor's degree or higher	16.0%	40.21	17.60	17.9%	35.84	15.70
Health Behaviors						
Currently smokes	31.6%	42.73	15.11	22.4%	19.54	12.62
Currently drinks	53.2%	45.73	17.20	32.2%	25.62	14.93
Average Yearly Household Income						
<\$10,000	14.6%	34.21	15.58	17.8%	20.76	12.76
\$10,000-19,999	16.8%	39.28	16.10	20.0%	19.26	14.10
\$20,000-29,999	15.8%	40.28	16.80	15.2%	22.18	16.30

\$30,000-39,999	13.7%	41.67	17.36	9.7%	33.04	15.34
\$40,000-49,999	11.0%	43.86	17.74	13.3%	26.83	15.29
\$50,000-59,999	8.8%	55.67	17.85	8.3%	26.25	14.56
\$60,000-69,999	7.0%	48.28	18.03	5.7%	23.22	17.66
≥ \$70,000	12.4%	51.31	18.68	10.0%	40.06	13.95
Residential History of PFAS Exposure						
High	41.0%	77.14	17.83	37.0%	45.61	15.58
Medium	30.8%	27.56	16.52	34.5%	15.87	13.91
Low	24.2%	11.18	16.80	28.5%	8.54	14.57
*This number reflects the total number of participants for the respective group. However, it varies slightly across cells due to missing values.						
†Mean PFOA and PFOS levels are in units of ng/ml.						

Table 2. PFOA and PFOS levels in pregnant and non-pregnant women.

	PFOA (ng/ml)				PFOS (ng/ml)			
	Mean	Median	Min.	Max.	Mean	Median	Min.	Max.
All women (n=9,952)	42.26	17.50	0.25	8,162.80	17.01	15.20	0.25	106.60
Non-Pregnant Women (n=9,454)	43.19	17.80	0.25	8,162.80	17.13	15.30	0.25	106.60
Pregnant Women (n=498)	24.49	12.20	1.10	295.60	14.71	12.90	0.25	56.00

Women in First Trimester (n=128)	25.42	12.40	1.50	227.30	16.32	15.00	1.80	56.00
Women in Second Trimester (n=193)	24.92	12.50	1.10	295.60	14.46	12.40	0.25	46.50
Women in Third Trimester (n=166)	23.69	12.00	1.40	194.50	13.63	12.10	1.30	41.50

Comparison of PFOA and PFOS in Pregnant vs. Non-Pregnant Women

Assumptions for normality in this population were met. After controlling for all covariates (age, alcohol consumption, smoking, educational level, hemoglobin-hematocrit ratio, parity, residential exposure, and income), the results of the ANOVA using month as a predictor (months

1 and 2 were combined) showed significant differences between pregnant and non-pregnant women in PFOA ($F=17.33$; $p<0.001$) AND PFOS ($F=9.78$; $p=0.0018$). Pregnant women in the study population had lower circulating levels of PFOA [15] than non-pregnant women. Mean values for pregnant and non-pregnant women are shown in Figure 1. In the figures, natural log values have been converted to analog values to make interpretation easier.

Insert Figure 1 about here

Perfluorocarbon Concentrations by Trimester of Pregnancy vs. Non-Pregnancy

To examine PFAS concentrations during different stages of fetal development, we also

analyzed the data by trimester. After controlling for the same covariates, comparison of non-pregnant women to women in each trimester of pregnancy showed significantly lower levels of PFOA in pregnant women in the second and third trimesters of pregnancy (second trimester $T=2.81$, $p=0.005$ and third trimester $T=2.67$, $p=0.008$, than in non-pregnant women and a strong trend towards lower concentrations in the first trimester ($T=1.85$ $p=0.06$). However, PFOS concentrations in pregnant women were significantly lower than those in non-pregnant women only during the third trimester $T=3.05$, $p=0.002$, although the tendency in the second semester was strong ($T=1.79$, $p=0.07$). The PFAS concentrations by trimester of pregnancy compared with non-pregnant women are shown in Figure 2.

Insert Figure 2 about here

DISCUSSION

This data from a population of women exposed to PFOA in drinking water substantiate the earlier indications from NHANES that circulating levels do indeed drop in pregnant women [19]. Pregnant women in our study had consistently lower levels of circulating PFOA in all trimesters of pregnancy (strong tendency in the first trimester and significant in the second and third); as well as lower concentrations of PFOS that reached significance during the 3rd trimester of pregnancy. Since pregnant women do not menstruate, they might have been expected, based on earlier reported hysterectomy data [25], to exhibit higher levels of circulating PFASs than non-pregnant women. Not only were their PFOA levels not higher, they were lower. One plausible explanation for the lower peripheral blood levels in pregnant women is redistribution to the fetus. This explanation is consistent with existing animal data,

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2
3 which have demonstrated transfer of PFOS to the fetus [5, 6] and with human data showing
4 the presence of both PFOS and PFOA in the cord blood of neonates from exposed mothers
5 [14-17]. Furthermore, a recent cross-sectional study in humans reported steadily increasing
6 PFOS in amniotic fluid by gestational week [26]. The results of these studies further support
7 our hypothesis that maternal PFASs are offloaded into the fetus throughout the course of
8 pregnancy.
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17 There are a number of potential consequences of fetal exposure to PFASs that could have
18 major consequences for subsequent child development. Our data show that the first trimester is
19 the beginning of a decline in maternal circulating PFOA levels, indicating that redistribution of
20 this chemical occurs during a critical period of gestational development. This is a period during
21 which structures of the nervous system, cardiovascular system, digestive system, respiratory
22 system, endocrine system, and kidneys are being formed [27], and when the potential for
23 epigenetic influences is particularly critical. Extensive data indicate that epigenetic influences
24 during critical periods of fetal development can cause permanent changes in metabolism and
25 chronic disease susceptibility [28]; and an increasing body of research shows that a complex
26 combination of adult health-related disorders can originate from developmental events that occur
27 in utero even without direct effects on pregnancy or birth weight [29]. A great many epigenetic
28 changes that occur in utero do not manifest until later in child- or even adulthood and may
29 therefore not be immediately identifiable as birth outcomes. Because animal research has shown
30 that perfluorocarbon exposure to the fetus is, in fact, associated with epigenetic changes, this
31 issue is important. It has been demonstrated in rat L02 liver cells [30], that there is a dose-related
32 increase in methylation (a process that turns genes off) of the glutathione-S-transferase (GSTP)
33 promoter, a gene that encodes an enzyme involved with detoxification metabolism and
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3 susceptibility to cancer) [31]. DNA methylation in human cord serum has also been
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5 demonstrated to be inversely associated with serum PFOA [32]. The implications of these
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7 methylation changes have not been investigated but given the increasing evidence of maternal –
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9 fetal transfer of these chemicals, the need for such research is urgently needed. Animal research
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11 on other toxicants (e.g. Vinclozolin) has demonstrated that embryonic exposure was associated
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13 with tissue abnormalities including prostate, kidney, immune system, and testis, as well as tumor
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15 development in the adult F1 generation as well as in subsequent generations (F2-F4) [33].
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19 The relevance of Vinclozolin is that it is an endocrine disruptor and endocrine disruption has
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21 been an important focus of research related to perfluoroalkyl substances [34, 35]. Although
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23 endocrine disrupting consequences of fetal exposure to PFASs, have to our knowledge not yet
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25 been investigated, the fact that exposure to other endocrine disrupting chemicals in utero has
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27 been associated with the above mentioned abnormalities as well as with human urogenital
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29 malformations and cancer [36-38], impaired reproductive function and infertility [38], increased
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31 risk of breast cancer [39], and intellectual impairment and neurodevelopmental changes
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33 manifesting in children [40], raises cause for concern. Whether or not these effects also
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35 generalize to PFOA and PFOS remains to be seen, however, accumulating data suggest that this
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37 is an important area for future investigation.
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43 The C8 Health Study has reported that in adults, PFOS and PFOA are associated with
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45 endocrine disruption related to thyroid dysfunction [41]. In analyses stratified by age and
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47 gender, both PFOA and PFOS were shown to be associated with significant elevations in serum
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49 thyroxin and a significant reduction of T3 uptake in all participants. These effects were
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51 significantly stronger in women. The pattern found in those data were interpreted as being
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53 consistent with what occurs with the use of exogenous estrogens in patients, namely an increase
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3 in thyroid binding globulin (TBG) but not thyroid stimulating hormone (TSH), an increase in
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5 total thyroxine and a decrease in T3 uptake. The limitation of that study was that the only binding
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7 protein actually measured was albumin, which binds a much smaller amount of thyroxine than
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9 TBG. However, albumin showed the same positive association with serum PFOA and PFOS that
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11 would have been expected from TBG.
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15 The fact that PFOS and PFOA are associated with thyroid dysfunction in adults has
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17 implications for pregnant mothers. It has been demonstrated in clinical studies that thyroid
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19 hormone reaches the fetus and affects gene expression in the fetal brain [42]. In fact, thyroid
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21 hormones control neuronal and glial proliferation in certain brain regions, and contribute to the
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23 regulation of neuronal migration and differentiation [43]. Thus, factors that disrupt thyroid
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25 function in the mother have the potential to affect brain development in the fetus [42]. In fetal
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27 fluids, a major proportion of T4 is not protein-bound (i.e., it is 'free') and is correlated to that in
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29 maternal serum [44]. The primary research focus with respect to maternal thyroid function and
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31 subsequent fetal and child development has been on the damaging effects of hypothyroidism to
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33 the central nervous system and cognitive development [45-49]. Hypothyroidism has not been
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35 observed in the C8 dataset. Rather the effect of perfluoroalkyl substances on thyroid function
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37 was an increase in T4 and circulating T3 (based on a reduction in T3 uptake). However, also
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39 increases in maternal thyroid hormones are associated with biochemical disturbances in the fetus,
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41 including effects on the neurotransmitters acetylcholine, dopamine and serotonin [50]. Again,
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43 the accumulating data indicate the need for more systematic research on fetal exposure to PFOS
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45 and PFOA.
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53 Endocrine disruption is not the only threat from exposure to PFOS and PFOA. These
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55 chemicals have also been shown to be associated with increased total and low-density lipoprotein
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(LDL) cholesterol in children and adults [51, 52]. The significance of this is that maternal hypercholesterolemia is associated with the increased formation of fatty streaks in human fetal arteries [53] as well as accelerated atherosclerosis progression in childhood [54].

Overall, studies investigating the effects of intrauterine PFAS exposure on child development in humans are limited and most have not investigated outcomes associated with endocrine disruption. Studies of infant allergies and infectious diseases from mothers exposed to PFASs during pregnancy have found an inverse association between maternal PFOA levels during pregnancy and IgE levels in cord blood of infant girls [55]; as well as negative associations between PFOS and PFOA and antidiphtheria prebooster antibody concentrations [56]. Consistent with the Developmental Origins of Disease hypothesis, PFOA concentrations in pregnant mothers have also been found to be positively associated with BMI and waist circumference among their 20 year old daughters [57] and with the daughters' biomarkers of adiposity (e.g. insulin, leptin, and leptin-adiponectin ratio) while being inversely associated with their adiponectin [57].

The comparison of maternal PFOS and PFOA concentrations during pregnancy with those of non-pregnant women is complex because of the significant changes in plasma volume that occur. One weakness of our study is that we were forced to use a proxy for plasma volume in our analyses because the dataset did not contain a direct measure. Interestingly, previously published data from this same cohort reported that levels of PFASs were higher in women who had had hysterectomies (i.e., were not menstruating) [25], the implication is that our comparison may actually be conservative. If non-menstruating women accumulate more perfluorinated substances, then pregnant women who also do not menstruate may actually be transferring higher concentrations to the fetus than would be expected from comparing concentrations between

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3 pregnant and non-pregnant women as a whole.
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5 A second limitation to our study is that the month of pregnancy is measured by self-report.
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7 Because women use more than one way to measure pregnancy onset (e.g., date of last menstrual
8 period, date of conception), our designation of trimesters is not exact. Despite this fact, there is
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10 no reason to assume any systematic bias with respect to this variable. Another study limitation is
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12 that we were not able to adjust for thyroid binding globulin, which varies greatly during
13 pregnancy, because it was not part of the dataset. Problems of this type with covariates are often
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15 a trade-off in large population databases. These databases provide a lot of power for
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17 investigating population effects but do not always contain the covariates one would desire. We
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19 nevertheless, believe these analyses are informative. The fact that the results were stronger for
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21 PFOA than for PFOS may reflect the results of a Chinese study which reported a higher partition
22
23 ratio of PFOA through placental barrier and lactation than for PFOS. There is an additional
24
25 covariate we would have liked to analyze but couldn't, namely history of lactation. Breast
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27 feeding could potentially influence the level of maternal PFAFs by off-loading some of them into
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29 the baby. This is a limitation of the study. Fortunately there is no reason to believe that history of
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31 lactation would vary by water district and thus introduce systematic bias. The major strength of
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33 this study is that the population was large enough to compare perfluorocarbon levels at different
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35 trimesters of pregnancy with those of non-pregnant women.
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45 Although there has been some human research with regard to PFASs and developmental
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47 outcomes, it has not been systematic. Study designs have tended to measure exposure at more
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49 advanced stages of gestation, and reports of clinical outcomes have not, for the most part, been
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51 theory driven. Because of the tremendous strides that have been made in the field of epigenetics,
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53 a whole new avenue for investigating fetal origins of adult disease has emerged. Given the
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3 accumulating data on PFASs and endocrine disruption and the indications that PFOA is entering
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5 the fetus in the first trimester of development, more systematic research needs to be applied to
6
7 the epigenetic effects of these chemicals on the developing fetus.
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23 24 Author Contributions

25
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28 individual authors area as follows: Beth Javins contributed to the statistical analyses and wrote
29
30 the first draft of the manuscript, Gerald Hobbs was the statistician, Alan M. Ducatman
31
32 contributed to editing the document, interpretation of results and was the technical consultant on
33
34 PFOS and PFOA, and Courtney Pilkerton contributed to discussions concerning implications of
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ABBREVIATIONS

PFAS, Perfluorinated Chemicals; PFOS, Perfluorooctanesulfonic acid; PFOA, Perfluorooctanoic acid

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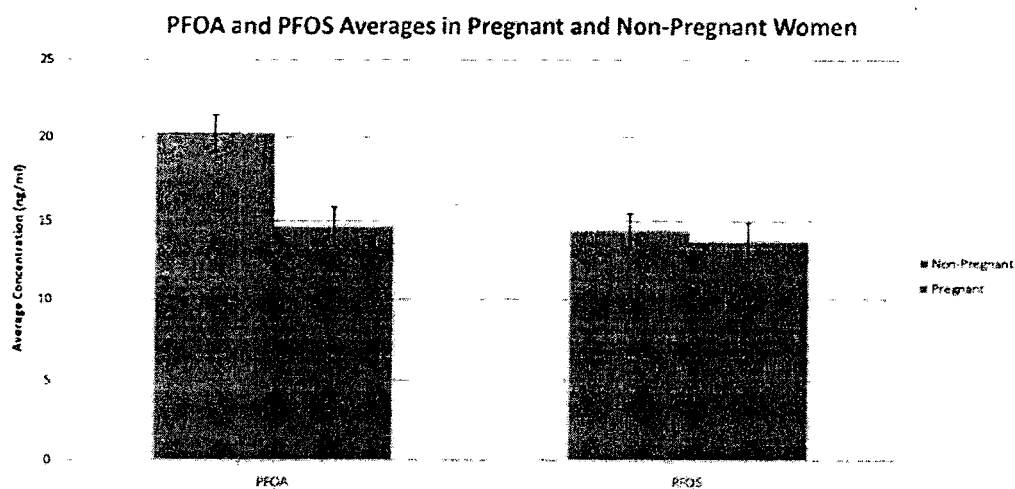
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Figure Legends

Figure 1. Average PFOS and PFOA Concentrations in Pregnant and Non-Pregnant Women

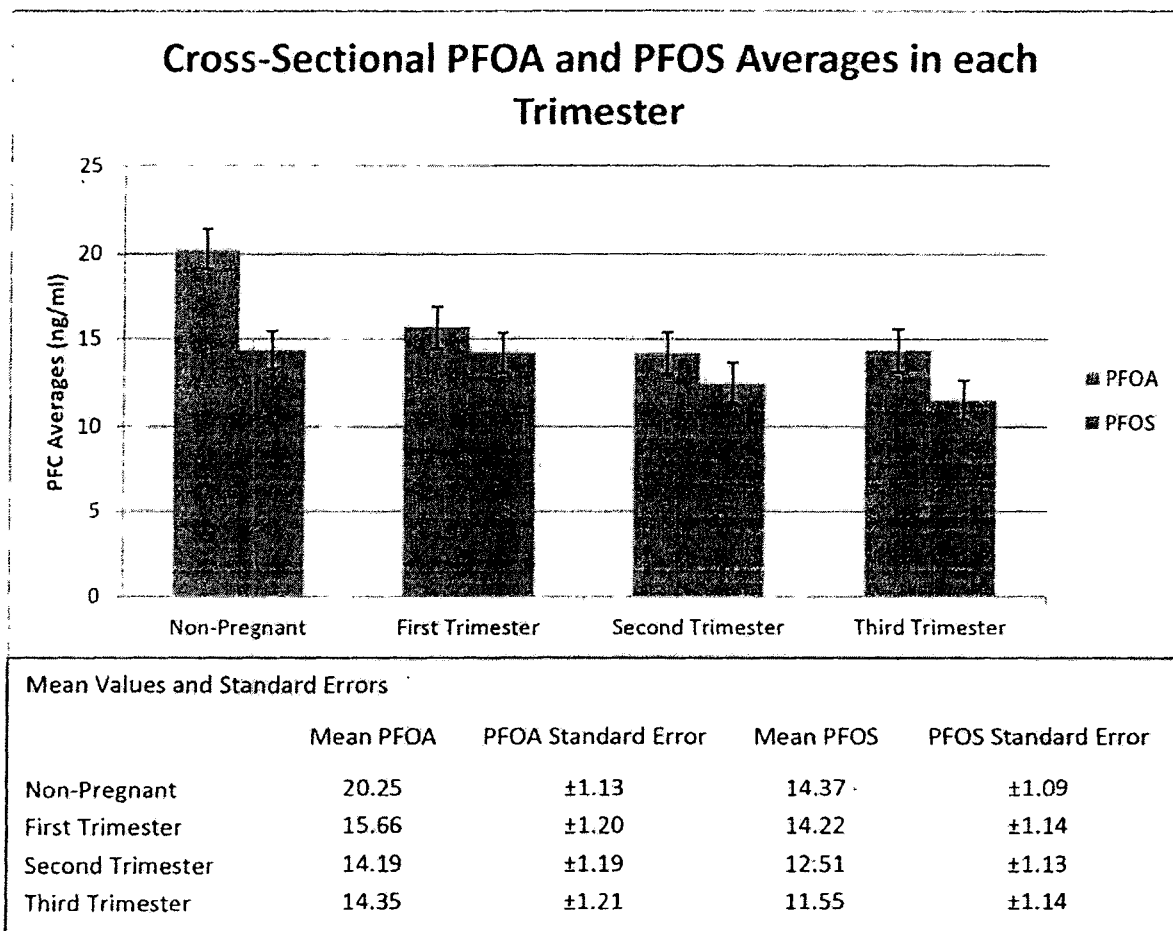
Figure 2. Cross-Sectional Maternal Perfluorocarbon Concentrations by Trimester

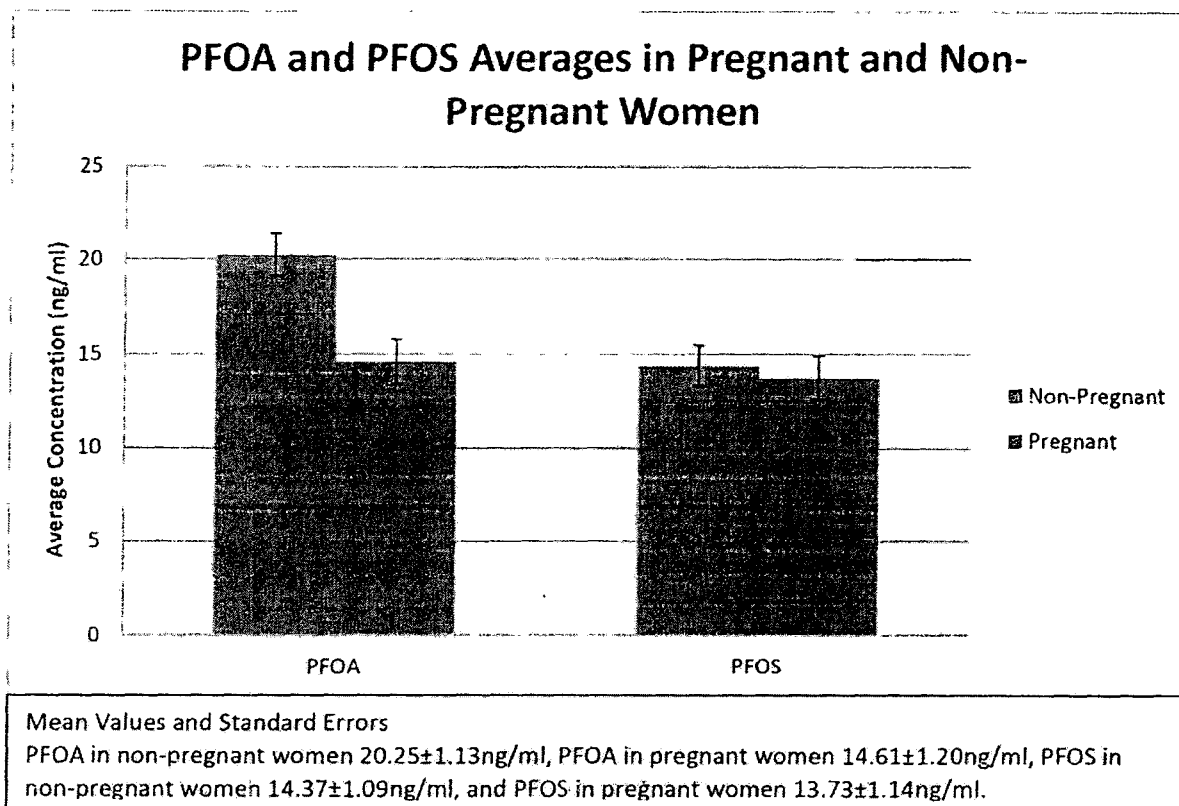
Figure 1. Perfluorocarbon Averages in Pregnant and Non-Pregnant Women

**Mean Values and Standard Errors**

PFOA in non-pregnant women 20.25 ± 1.13 ng/ml, PFOA in pregnant women 14.61 ± 1.20 ng/ml, PFOS in non-pregnant women 14.37 ± 1.09 ng/ml, and PFOS in pregnant women 13.73 ± 1.14 ng/ml.

Figure 2. Cross-Sectional Maternal Perfluorocarbon Concentrations by Trimester





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**Serum Polyfluoroalkyl Concentrations, Asthma
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**Serum Polyfluoroalkyl Concentrations, Asthma Outcomes, and Immunological Markers in
a Case-Control Study of Taiwanese Children**

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Running Head: PFCs and Asthma Outcomes in Children

Competing Interests: The authors declare that they have no competing interests.

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Key Words: Asthma, AEC, ECP, IgE, Perfluorinated compounds

Abbreviations:

ACT: Asthma Control Test

AEC: absolute eosinophil counts

ECP: eosinophilic cationic protein

GBCA: Genetic and Biomarkers study for Childhood Asthma

IQR: interquartile range

IgE: immunoglobulin E

ORs: odds ratio

95%CI: 95% confidence intervals

PFBS: perfluorobutane sulfonate

PFCs: perfluorinated compounds

PFDA: perfluorodecanoic acid

PFDoA: perfluorododecanoic acid

PFHpA: perfluoroheptanoic acid

PFHxA: perfluorohexane acid

PFHxS: perfluorohexane sulfonate

PFNA: perfluorononanoic acid (PFNA)

PFOA: perfluorooctanoic acid

PFOS: perfluorooctane sulfonate

PFTA: perfluorotetradecanoic acid

ABSTRACT

Background: Perfluorinated compounds (PFCs) are ubiquitous pollutants. Experimental data suggest that they may be associated with adverse health outcomes, including asthma. However, there is little supporting epidemiological evidence.

Methods: A total of 231 asthmatic children and 225 non-asthmatic controls, all from Northern Taiwan, were recruited in the Genetic and Biomarker study for Childhood Asthma. Structure questionnaires were administered by face-to-face interview. Serum concentrations of 11 PFCs and levels of immunological markers were also measured. Associations of PFC quartiles with concentrations of immunological markers and asthma outcomes were estimated using multivariable regression models.

Results: Nine PFCs were detectable in most children ($\geq 84.4\%$), of which perfluorooctane sulfonate (PFOS) was the most abundant (median serum concentration of 33.9 ng/mL in asthmatics and 28.9 ng/mL in controls). Adjusted odds ratios for asthma among those with the highest versus lowest quartile of PFC exposure ranged from 1.81 (95%CI: 1.02, 3.23) for the perfluorododecanoic acid (PFDoA) to 4.05 (95%CI: 2.21, 7.42) for perfluorooctanic acid (PFOA). PFOS, PFOA, and subsets of the other PFCs were positively associated with serum IgE concentrations, absolute eosinophil counts (AEC), eosinophilic cationic protein (ECP) concentrations, and asthma severity scores among asthmatics.

Conclusions: This study suggests an association between PFC exposure and juvenile asthma. Due to widespread exposure to these chemicals, these findings may be of potential public health concern.

INTRODUCTION

Perfluorinated compounds (PFCs) include a class of man-made organic chemicals comprised of a fluorinated carbon backbone of varying length, terminated by a carboxylate or sulfonate functional group. Such PFCs are extremely stable, thermally, biologically, and chemically, and additionally possess hydrophobic and lipophobic characteristics that enable products coated in them to repel both oil and water, and resist staining (Conder et al. 2008; Hoffman et al. 2010). Accordingly, PFCs are widely used, for example in surfactants, emulsifiers, food packaging, nonstick pan coatings, fire-fighting foams, paper and textile coatings, and personal care products (Lau et al. 2007; Lindstorm et al. 2011; Renner 2001).

This combination of extreme resistance to degradation and environmental ubiquity has raised concerns in recent years (Giesy and Kannan 2001; Lau et al. 2007). Furthermore, studies have shown that PFCs accumulate among the higher trophic level of the food chain, such as predators and human beings (Conder et al. 2008; Houde et al. 2006; Noorlander et al. 2011). Although data from the National Health and Nutrition Examination Survey have indicated a decrease in serum PFC concentrations in the general U.S. population since the production of some PFCs has been phased out (for example, PFOS decreased from 30.4 ng/mL in 1999 to 13.2 ng/mL in 2008) (Kato et al. 2011), PFCs are still manufactured abroad (Paul et al. 2009). PFCs bioaccumulate by binding to proteins in the liver and serum, in contrast with many other persistent organic pollutants that persist primarily in adipose tissue (Conder et al. 2008), and they are slowly eliminated without biotransformation (Lau et al. 2007). Estimated serum half-life estimates in an occupationally exposed cohort ranged from 5.4 years for perfluorooctane sulfonate (PFOS) to 8.5 years for perfluorohexane sulfonic acid (PFHxS) (Olsen et al. 2007).

Several attempts have been made to understand the toxicological hazards that may be

associated with exposure. Early animal studies focused almost exclusively on exposure to PFOS and perfluorooctanoic acid (PFOA), two of the most common PFCs. Evidence of several potential effects has been reported based on experimental studies, including hepatotoxicity, immunotoxicity, developmental toxicity, reproductive toxicity, neurotoxicity, endocrine toxicity, and tumors of the liver, thyroid, and mammary glands (Corsini et al. 2011; Lau et al. 2004, 2007; Olsen et al. 2009).

Preliminary data suggest that PFCs have the potential to exacerbate atopic diseases such as asthma. In a murine model of asthma, Fairley et al. (2007) found that PFOA increased serum levels of immunoglobulin E (IgE) and enhanced the hypersensitivity response to ovalbumin, suggesting that PFOA exposure may augment the IgE response to environmental allergens. Another recent study reported that PFOS exposure decreased baseline airway resistance but significantly increased airway responsiveness in an allergic murine model (Basu et al. 2011). Furthermore, in our experimental studies, *in vivo* exposure to PFOS was associated with decreased secretion of T_H1 -type cytokines (IL-2 and IFN- γ) and increased secretion of T_H2 -type cytokines (IL-4 and IL-10) and IgE, which suggested that PFOS exposure might shift the host's immune state toward a more T_H2 -like state (Dong et al. 2011). T_H1/T_H2 polarization (towards T_H2 response) is a hallmark of atopy diseases (Colavita et al. 2000), and IgE is known to play a role in mediated type I hypersensitivity reactions, including asthma (Platts-Mills 2001). Accordingly, we hypothesized that exposure to PFCs may have a role asthma development in humans.

Asthma is the most common respiratory disease in young children, and although recent studies indicated that asthma prevalence has plateaued or may be declining (Montefort et al. 2011; Pearce et al. 2007), it is still a major public health problem among young people. The

case-control design of the Genetic and Biomarkers study for Childhood Asthma (GBCA) provided an opportunity to explore the association between PFCs exposure and asthma in children. In addition, two validated questionnaires [asthma severity score questionnaire and Asthma Control Test (ACT)] were used to examine the association between PFCs and asthma severity and control in asthmatic children.

METHODS

Study population

The Genetic and Biomarkers study for Childhood Asthma (GBCA) was conducted between 2009 and 2010. A total of 231 ten to fifteen-year-old children with physician-diagnosed asthma in the previous year were recruited from two hospitals in Northern Taiwan. Controls were selected from our previous cohort study population in seven public schools of Northern Taiwan (Tsai et al. 2010). These schools had diverse geographical and socioeconomic settings, being located in city, rural, and high altitude communities, respectively. In each targeted school, children of the same age range and without a personal or family history of asthma were invited to participate, and 225 non-asthmatic controls enrolled in the study (response rate 72% among those contacted by phone). Information pertaining to demographic variables, environmental exposures and asthma outcomes were collected from questionnaires. We also collected urine and serum samples for each child after 8 hours of fasting. A trained field worker measured each child's height, weight, waist circumference and blood pressure. All participants and their parents signed written informed consents. The study protocol was approved by the Institutional Review Board (National Taiwan University Hospital Research Ethics Committee), and complied with the principles outlined in the Helsinki Declaration (Declaration of Helsinki., 1990).

Serum IgE, AEC, ECP level detection

Venous blood was placed into EDTA tubes, a peroxidase coloration was performed, and the absolute eosinophil counts (AEC) were calculated using automatic analyzer (>10000 cells/mL; Sysmex, XE2100, Japan). Serum samples were stored at -80°C until total IgE and eosinophilic cationic protein (ECP) levels were analyzed. Serum total IgE levels were determined using a Pharmacia UniCap assay test system (Pharmacia Diagnostics, Uppsala, Sweden). Total IgE concentrations below 0.35 kU/L were defined as absent or undetectable. ELISA kits were used to measure ECP levels in serum samples according to the manufacturer's instructions (R&D Systems Europe, Abingdon, UK). The limit of quantitation for ECP concentrations was 0.125 ng/mL.

Asthma Control Test and asthma severity evaluation

The Asthma Control Test (ACT), a five-item questionnaire used to assess asthma control in the previous 4 weeks (Nathan et al. 2004), was administered to the asthmatic children. Questions pertaining to asthmatic symptoms, use of rescue medication, and limitation of daily activities are used to ascertain asthma management. The reliability, empirical validity, and discriminative properties in assessing the control of asthma by Chinese children are good (Chen et al. 2008). The sum of the scores of the five questions gives the total ACT score (range 5–25); the higher the score, the better controlled the disease. We also used a 13-item asthma severity score questionnaire to evaluate four overall components of asthma severity in the asthmatic children, including frequency of current asthma symptoms, use of systemic corticosteroids, use of other medications (besides systemic corticosteroids), and history of hospitalizations and intubations

(Eisner et al. 1998). Possible total scores range from 0 to 28, with higher scores reflecting more severe asthma.

PFC concentrations

PFCs were measured from 0.5 mL of serum using Agilent high-performance liquid chromatography (HPLC) – in tandem with an Agilent 6410 Triple Quadruple (QQQ) mass spectrometer (MS/MS) (Agilent, Palo Alto and Santa Clara, CA). Detailed information about standards and reagents, sample preparation and extraction, instrumental analysis, quality assurance and quality control, and recovery experiments in the present study is provided in Supplementary Material (see Supplementary Material, p.2-p.4) and is described elsewhere (Bao et al. 2011). Ten PFCs were analyzed in serum samples: PFOS, PFOA, perfluorobutane sulfonate (PFBS), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), perfluoroheptanoic acid (PFHpA), perfluorohexane acid (PFHxA), perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), and perfluorotetradecanoic acid (PFTA). The limit of quantification (LOQ) for PFOS, PFOA and PFNA was 0.03 ng/mL, for PFBS and PFHxS was 0.07 ng/mL, for PFDA and PFDoA was 0.1 ng/mL, for PFHpA and PFHxA was 0.05 ng/mL, and for PFTA was 0.02 ng/mL. All tests were duplicated and average of the two measures was calculated as the concentrations of PFC.

Statistical analysis

Statistical analysis was performed using SAS software (Version 9.2; SAS Institute Inc., Cary, North Carolina, USA). Concentrations of PFCs and biomarkers below the LOQ were assigned a value equal to the LOQ divided by the square root of 2 for statistical analyses. We calculated

univariate statistics, including medians, interquartile ranges (IQR), and ranges for each PFC. Because PFC concentrations were highly skewed, we utilized the Wilcoxon rank-sum test to compare PFC concentrations between children with and without asthma.

We used logistic regression to estimate odds ratio (ORs) and 95% confidence intervals (CIs) for each PFC quartile relative to the lowest quartile, with a priori adjustment for child age and gender. To determine the magnitude of other potential confounding, we examined the following variables using a backward deletion strategy: parental education, body mass index (BMI), environmental tobacco smoke (ETS) exposure and month of survey. If the estimated PFC effect changed by at least 10% when a covariate was included in the base model, the covariate was included in the final model. Multiple general linear models were used to estimate associations with continuous outcomes (IgE, AEC, and ECP) in PFCs quartiles, with the lowest PFCs quartile as reference group and adjusted for identified covariates. These models were applied separately for cases and controls. We modeled an ordinal variable assigned the median value for each corresponding quartile to estimate p-values for trend. A p-value of < 0.05 was considered statistically significant.

RESULTS

Compared to children without asthma, asthmatic children tended to be younger and less likely to report ETS exposure (Table 1). In addition, asthmatic children had significantly higher median plasma concentrations of IgE, AEC, and ECP.

Nearly all study participants had detectable serum concentrations of all PFCs (>94% of PFCs) except for PFDoA (84.4% in children with and without asthma) and PFHpA (53.3% in unasthmatic children, and 70.6% in asthmatic children) (Table 2). Because of the large numbers

of samples <LOQ, we did not conduct further analyses of PFHpA. Serum concentrations of PFCs were significantly higher in asthmatic children than in non-asthmatic children ($p < 0.05$), except for PFHxA concentrations, which were similar in both groups, and PFTA concentrations, which were significantly higher in non-asthmatic children.

Crude and adjusted ORs for asthma in association with the highest versus lowest quartile of exposure were significantly elevated for all PFCs except for PFHxA and PFTA (Table 3). In general, the data suggest increasing odds of asthma with increasing PFCs, with the strongest associations for exposures in the fourth quartile. Specifically, adjusted ORs for the highest versus lowest quartile were 2.63 (95%CI: 1.48, 4.69) for PFOS, 4.05 (95%CI: 2.21, 7.42) for PFOA, 1.90 (95%CI: 1.08, 3.37) for PFBS, 3.22 (95%CI: 1.75, 5.94) for PFDA, 1.81 (95%CI: 1.02, 3.23) for PFDoA, 3.83 (95%CI: 2.11, 6.93) for PFHxS, and 2.56 (95%CI: 1.41, 4.65) for PFNA.

None of the PFCs were significantly associated with serum levels of IgE or absolute eosinophil counts (AEC) among children without asthma, but serum ECP concentration was positively associated with PFDA and PFDoA (Table 4). In contrast, among children with asthma all three biomarkers were positively associated with PFOS and PFOA, with significant monotonic trends with increasing exposure (Table 5). For example, asthmatic children in the highest of PFOS quartile had mean IgE levels of 877.3 IU/dL (95%CI: 695.2, 1059.5), compared with 517.9 IU/dL (95% CI: 336.7, 699.2) for in the lowest quartile (Figure 1). In addition, with the exception of PFHxA, which was not associated with any of the biomarkers, all the remaining PFCs were associated with 2 of the 3 biomarkers evaluated.

Among asthmatic children, positive trends for associations with asthma severity scores were significant for PFOS, PFDA, PFDoA and PFTA, but none of the PFCs was associated with ACT scores (Table 6).

DISCUSSION

Serum concentrations of PFCs were significantly higher in asthmatic children compared with controls, and among children with asthma, all but one of the PFCs evaluated were positively associated with at least two of the three immunological biomarkers (IgE, AEC, and ECP). Although temporality cannot be determined due to the cross-sectional nature of the data, and non-causal associations due to uncontrolled confounding or other sources of bias cannot be ruled out, the robust associations of PFCs with asthma and asthma related biomarkers in children suggest that a causal relationship may be present. However, it should also be noted that concentrations of individual PFCs were positively correlated (see Supplemental Material, Table S1), and therefore it is not possible to determine if associations apply to multiple PFCs or to only a subset of individual PFCs.

There is little information in the literature about associations between environmental PFCs and asthma or asthma-related biomarkers in children. In a systematic Medline search, we identified only two studies of PFCs and atopic disease in humans. The first was a cross-sectional study of 566 residents with prolonged exposure to PFOA in their drinking water (Anderson-Mahoney et al. 2008). In that study, respiratory illness was evaluated by questionnaire, and standardized prevalence ratios (SPR) using national Health and Examination Survey (NHANES) data for comparison rates suggested an increased prevalence of asthma among the exposed participants than in the general U.S. population (SPR=1.82, 95%CI: 1.47, 2.25). The second was a cohort study of prenatal exposure to PFCs in association with IgE levels and atopic dermatitis in 244 newborns (Wang et al. 2011). In that study, prenatal PFOA and PFOS exposures were positively correlated with cord blood IgE levels, but were not significantly associated with atopic dermatitis.

In a murine model of asthma, Fairley et al. (2007) evaluated the effects of PFOA dermal exposure on the hypersensitivity response to ovalbumin (OVA). The authors reported that IgE increased to a greater extent in animals exposed to PFOA and OVA, and that the severity of the OVA-specific airway hyperreactivity response, and a pleiotropic cell response characterized by eosinophilia and mucin production, increased with increasing concentrations of PFOA. Grasty et al. (2005) evaluated the effect of prenatal PFOS exposure on maturation of the terminal airway epithelium in rats based on histological and morphometric examination, and reported that there were significant histologic and morphometric differences between control and PFOS-treated lungs in newborns, suggesting that PFOS may inhibit or delay perinatal lung development. Also, a recent study using an allergic murine model to evaluate effects of PFOS exposure on pulmonary function and airway responsiveness reported that PFOS exposure decreased baseline airway resistance, but significantly increased airway responsiveness in allergic mice (Basu et al. 2011). In our experimental studies, *in vivo* exposure to PFOS was linked decreased secretion of T_H1 -type cytokines (IL-2 and IFN- γ), and increased secretion of T_H2 -type cytokines (IL-4 and IL-10) and serum IgE, which suggested that PFOS exposure might shift immune responses toward a more T_H2 -like state (Dong et al. 2011). T_H1/T_H2 polarization towards T_H2 responses is a hallmark of atopy diseases (Colavita et al. 2000), and IgE plays a role in mediating type 1 hypersensitivity reactions, including asthma (Platts-Mills 2001). Therefore, we hypothesize that exposure to PFCs may augment the T_H2 response, resulting in airway hyper-reactivity to environmental allergens.

Potential mechanisms for effects of PFCs on immune response and asthma development in humans are uncertain. One possibility is an effect of PFCs on regulatory T cells that influence the development of immune-related diseases including asthma and allergy (Akbari et al. 2003).

Another possible mechanism for effects of PFCs on immune responses pertains to the peroxisome proliferator activated receptors (PPARs) signaling pathway. PFCs are known as agonists for peroxisome proliferator activated receptors (PPARs) (Vanden huevel et al. 2006). Both PPAR α and γ are expressed on cells of the monocyte / macrophage lineage, suggesting a possible role in immune function (Braissant and Wahli 1998). Although PPAR γ activation has been implicated as an important contributor to the pathogenesis of the toluene diisocyanate-induced asthma phenotype in a female BALB/c mice model (Lee et al. 2006), PFOC and PFOA have been shown to significantly increase activation of mouse or human PPAR α and β , but not of PPAR γ , in vitro (Takacs and Abbott, 2006). Lovett-Racke et al. (2004) reported that PPAR α agonists, including gemfibrozil, ciprofibrate, and fenofibrate, can increase the production of the Th2 cytokine IL-4, and suppress MBP AcI-11 induced proliferation by TCR transgenic T cells. In addition, gemfibrozil shifted cytokine secretion by inhibiting interferon- γ and promoting IL-4 secretion in human T-cell lines. These results suggest that PFCs may potentially augment the Th2 response and subsequent airway hyperreactivity to environmental allergens through a PPAR-mediated mechanism (Fairley et al. 2007).

In the present study, nearly all study participants had detectable serum concentrations of all PFCs, including both asthmatic children and controls. PFOS was the most abundant PFC in the serum measured in 2009 – 2010 in these Taiwanese children, with median concentrations (28.9 ng/mL in controls and 33.9 ng/mL in asthmatics) that were similar to levels reported for children age 12 – 19 years in the general U.S. population in 1999 – 2000 (29.4 ng/mL), but higher than the median concentration reported for U.S. children in 2007 – 2008 (11.3 ng/mL) (Kato et al. 2011) and concentrations reported for other populations of children sampled during the mid- to late- 2000s (see Supplemental Material, Table S2). In contrast, median concentrations of PFOA

in the present study population (0.2 ng/mL in controls and 1.2 ng/mL in asthmatics) were lower than reported for other populations of children during the late 2000s (e.g., 4.0 ng/mL based on NHANES data for 2007 – 2008), and substantially lower than median concentrations reported for children living in a U.S. community near a manufacturing facility (26.3 – 32.6 ng/mL) (Frisbee et al. 2010). Differences in PFC concentrations among populations may reflect changes in exposures over time, as well as differences in diet and other sources of exposure, and individual differences in rates or patterns of metabolism or excretion.

The limitations of these analyses should be noted. We based the PFCs measures on a single serum sample, and although PFCs have a half-life of 5.4 to 8.5 years (Olsen et al. 2007), samples taken at several time points might be more accurate than a single sample for classifying exposure. As previously noted, this is a cross-sectional study, and temporal relationships between exposures and outcomes cannot be established. In addition, associations with individual PFCs may be biased due to correlations with other PFCs. Finally, cases were recruited from two hospitals in Northern Taiwan, whereas controls were recruited from schools in the same region. Therefore, estimates also may have been influenced by selection bias or uncontrolled confounding.

In conclusion, we observed positive associations between serum PFCs and asthma, and positive associations between PFCs and IgE, AEC and ECP levels, and (to a lesser extent) asthma severity scores, in asthmatic children. These findings suggest that exposure to PFCs may not only be related to asthma outcomes but also to asthma severity. Although the production of some PFCs has been phased out in the United States and Europe, PFCs are still manufactured in Asia and elsewhere, and exposure may also result from the breakdown of similar compounds to PFCs in the environment (Organisation for Economic Co-operation and Development 2002; U.S.

EPA 2006). Therefore, continued exposure is of public health concern, and additional research on potential immunotoxic effects of PFCs is warranted.

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Table 1. Characteristics of children with and without asthma in the study population

Characteristic	Children without asthma (n=225)	Children with asthma (n=231)	P value
Age (year) ^a	13.6+/-0.7	12.9+/-1.7	<0.001
Height (cm) ^a	159.8+/-7.0	156.6+/-10.4	<0.001
Weight (kg) ^a	52.5+/-13.2	49.8+/-13.3	0.033
BMI (kg/m ²) ^a	20.4+/-4.1	20.1+/-3.9	0.379
Gender, n(%)			
Male	102 (45.3)	158 (68.4)	<0.001
Female	123 (54.7)	73 (31.6)	
Parental education, n (%)			
<High School	86 (38.2)	90 (39.0)	0.871
≥High School	139 (61.8)	141 (61.0)	
ETS exposure, n (%)			
No	93 (41.3)	138 (59.7)	<0.001
Ever	22 (9.8)	23 (10.0)	
Current	110 (48.9)	70 (30.3)	
Month of survey			
7~9	156 (69.3)	106 (45.9)	<0.001
11~12	69 (30.7)	125 (54.1)	
IgE (IU/dL) ^a	331.4+/-486.6	684.6+/-679.2	<0.001
AEC (×10 ⁹ /L) ^a	152.3+/-150.3	395.0+/-280.9	<0.001
ECP (μg/L) ^a	28.4+/-41.1	42.2+/-57.8	0.004

^aMean +/- SD.

AEC, absolute eosinophil count; ECP, eosinophilic cationic protein

Table 2. Serum PFC concentrations (ng/mL) in children with and without asthma

PFC	Children without asthma (n=225)				Children with asthma (n=231)				<i>P</i> value*
	Mean +/- SD	Median (IQR)	Range	% above LOQ	Mean +/- SD	Median (IQR)	Range	% above LOQ	
PFOS	33.4 +/- 26.4	28.9 (14.1, 43.0)	LOQ-148.1	97.3	45.5 +/- 37.3	33.9 (19.6, 61.1)	LOQ-149.6	97.0	0.002
PFOA	1.0 +/- 1.1	0.5 (0.4, 1.3)	LOQ-11.3	95.1	1.5 +/- 1.3	1.2 (0.5, 2.2)	LOQ-9.0	99.6	<0.001
PFBS	0.5 +/- 0.2	0.5 (0.4, 0.5)	LOQ-2.7	94.2	0.5 +/- 0.2	0.5 (0.4, 0.6)	LOQ-2.7	99.1	0.022
PFDA	1.0 +/- 0.5	1.0 (0.8, 1.2)	LOQ-5.0	95.1	1.2 +/- 0.5	1.1 (0.9, 1.5)	LOQ-3.5	97.4	<0.001
PFDoA	4.5 +/- 6.0	2.7 (0.8-6.0)	LOQ-43.1	84.4	5.8 +/- 6.0	3.8 (1.1, 8.4)	LOQ-36.1	84.4	0.014
PFHpA	0.2 +/- 0.3	0.2 (LOQ, 0.2)	LOQ-4.3	53.3	0.3 +/- 0.5	0.2 (LOQ, 0.3)	LOQ-5.0	70.6	<0.001
PFHxA	0.2 +/- 0.2	0.2 (0.1, 0.3)	LOQ-2.4	98.7	0.3 +/- 0.3	0.2 (0.1, 0.3)	LOQ-3.9	97.0	0.765
PFHxS	2.1 +/- 2.2	1.3 (0.6, 2.8)	LOQ-11.8	99.6	3.9 +/- 9.0	2.5 (1.3, 4.3)	LOQ-129.1	98.3	<0.001
PFNA	0.9 +/- 0.3	0.8 (0.6, 1.1)	0.26-2.5	100.0	1.1 +/- 0.5	1.0 (0.7, 1.3)	0.28-3.6	100.0	<0.001
PFTA	28.9 +/- 81.6	5.2 (0.4, 23.3)	LOQ-793.6	99.6	54.6 +/- 101.3	4.1 (0.2, 31.7)	LOQ-429.1	99.6	0.003

SD, Standard deviation; IQR, Interquartile range; LOQ, Limit of quantitation;

*Wilcoxon rank-sum test to compare the difference of PFCs levels between children without asthma and children with asthma.

Table 3. Association between PFCs and asthma among 456 participants in the Genetic and Biomarkers study for Childhood Asthma, Taiwan, 2009-2010

Exposure	No. Controls	No. Cases	Crude OR (95%CI)	Adjusted OR (95%CI) ^a
PFOS Quartile 1(lowest) ^b	67	47	1.00	1.00
Quartile 2	54	60	1.59 (0.94, 2.67)	1.96 (1.11, 3.47)
Quartile 3	64	50	1.11 (0.66, 1.88)	1.32 (0.75, 2.32)
Quartile 4 (highest)	40	74	2.64 (1.54, 4.51)	2.63 (1.48, 4.69)
<i>P</i> for trend ^c			0.003	0.003
PFOA Quartile 1(lowest) ^b	71	43	1.00	1.00
Quartile 2	64	50	1.29 (0.76, 2.19)	1.58 (0.89, 2.80)
Quartile 3	53	61	1.90 (1.12, 3.22)	2.67 (1.49, 4.79)
Quartile 4 (highest)	37	77	3.43 (1.99, 5.93)	4.05 (2.21, 7.42)
<i>P</i> for trend ^c			<0.001	<0.001
PFBS Quartile 1(lowest) ^b	63	51	1.00	1.00
Quartile 2	56	58	1.28 (0.76, 2.15)	1.31 (0.74, 2.31)
Quartile 3	58	56	1.19 (0.71, 2.01)	1.24 (0.70, 2.20)
Quartile 4 (highest)	48	66	1.70 (1.01, 2.87)	1.90 (1.08, 3.37)
<i>P</i> for trend ^c			0.072	0.021
PFDA Quartile 1(lowest) ^b	70	44	1.00	1.00
Quartile 2	68	46	1.08 (0.64, 1.83)	1.02 (0.58, 1.80)
Quartile 3	53	61	1.83 (1.08, 3.10)	1.30 (0.72, 2.33)
Quartile 4 (highest)	34	80	3.74 (2.16, 6.49)	3.22 (1.75, 5.94)
<i>P</i> for trend ^c			<0.001	<0.001
PFDoA Quartile 1(lowest) ^b	60	54	1.00	1.00
Quartile 2	61	53	0.97 (0.57, 1.62)	0.81 (0.46, 1.43)
Quartile 3	63	51	0.90 (0.53, 1.52)	0.71 (0.40, 1.26)
Quartile 4 (highest)	41	73	1.97 (1.16, 3.36)	1.81 (1.02, 3.23)
<i>P</i> for trend ^c			0.021	0.044
PFHxA Quartile 1(lowest) ^b	54	60	1.00	1.00
Quartile 2	56	58	0.93 (0.55, 1.57)	1.21 (0.69, 2.12)
Quartile 3	68	46	0.61 (0.36, 1.03)	0.90 (0.51, 1.60)
Quartile 4 (highest)	47	67	1.28 (0.76, 2.17)	1.60 (0.90, 2.86)
<i>P</i> for trend ^c			0.706	0.168
PFHxS Quartile 1(lowest) ^b	72	42	1.00	1.00
Quartile 2	69	45	1.12 (0.66, 1.91)	1.54 (0.85, 2.77)
Quartile 3	45	69	2.63 (1.54, 4.49)	2.94 (1.65, 5.25)
Quartile 4 (highest)	39	75	3.30 (1.92, 5.67)	3.83 (2.11, 6.93)
<i>P</i> for trend ^c			<0.001	<0.001
PFNA Quartile 1(lowest) ^b	69	45	1.00	1.00
Quartile 2	64	50	1.20 (0.71, 2.03)	1.19 (0.68, 2.09)
Quartile 3	53	61	1.76 (1.04, 2.99)	1.54 (0.86, 2.76)
Quartile 4 (highest)	39	75	2.95 (1.72, 5.06)	2.56 (1.41, 4.65)
<i>P</i> for trend ^c			<0.001	0.001
PFTA Quartile 1(lowest) ^b	52	62	1.00	1.00
Quartile 2	56	58	0.69 (0.41, 1.16)	0.62 (0.35, 1.09)
Quartile 3	63	51	0.61 (0.36, 1.02)	0.65 (0.37, 1.14)
Quartile 4 (highest)	54	60	0.84 (0.50, 1.41)	0.96 (0.55, 1.67)
<i>P</i> for trend ^c			0.410	0.899

^aAdjusted for age, gender, BMI, parental education, ETS exposure, and month of survey.

^bReference category.

^c*P* values were calculated using categories representing the median value of corresponding quartile.

Table 4. Estimated mean values (95% CI) serum IgE, AEC, and serum ECP according to serum PFCs concentration among children without asthma (n=225)^a

Exposure	IgE (IU/dL)	AEC ($\times 10^9$ /L)	ECP (μ g/L)
PFOS Quartile 1(lowest)	286.3 (157.0, 415.6)	138.9 (100.1, 177.8)	29.4 (18.5, 40.3)
Quartile 2	298.3 (164.6, 432.1)	141.6 (102.2, 181.1)	24.2 (13.0, 35.4)
Quartile 3	403.5 (274.1, 532.9)	167.8 (128.9, 206.6)	33.5 (22.5, 44.5)
Quartile 4 (highest)	336.3 (208.3, 464.2)	160.9 (122.2, 199.7)	26.6 (15.8, 37.4)
P for trend ^b	0.404	0.445	0.972
PFOA Quartile 1(lowest)	223.1 (76.8, 369.5)	118.5 (78.6, 158.5)	15.4 (3.2, 27.7)
Quartile 2	298.9 (170.9, 427.0)	110.1 (71.3, 148.8)	28.3 (17.6, 39.0)
Quartile 3	406.2 (274.6, 537.9)	198.3 (160.7, 235.9)	38.3 (27.3, 49.3)
Quartile 4 (highest)	393.9 (258.0, 529.8)	182.0 (139.3, 224.8)	31.2 (19.8, 42.6)
P for trend ^b	0.123	0.224	0.133
PFBS Quartile 1(lowest)	360.1 (229.6, 490.7)	156.0 (117.6, 194.3)	23.9 (13.0, 34.9)
Quartile 2	345.0 (214.4, 475.7)	108.2 (70.5, 145.9)	32.1 (21.2, 43.0)
Quartile 3	329.4 (198.2, 460.6)	151.7 (113.2, 190.3)	28.9 (17.9, 40.0)
Quartile 4 (highest)	291.8 (161.9, 421.8)	194.1 (155.6, 232.6)	28.7 (17.7, 39.8)
P for trend ^b	0.447	0.070	0.648
PFDA Quartile 1(lowest)	248.6 (122.2, 375.0)	118.2 (79.7, 156.7)	18.1 (7.2, 28.9)
Quartile 2	305.4 (179.9, 430.8)	148.0 (109.7, 186.3)	26.7 (15.8, 37.5)
Quartile 3	395.6 (267.4, 523.9)	178.7 (140.3, 217.1)	28.1 (17.4, 38.8)
Quartile 4 (highest)	379.4 (253.4, 505.5)	164.6 (125.6, 203.6)	40.7 (30.0, 51.4)*
P for trend ^b	0.092	0.073	0.004
PFDaA Quartile 1(lowest)	358.4 (230.9, 485.8)	89.9 (52.7, 127.1)	19.2 (8.6, 29.7)
Quartile 2	423.7 (293.2, 554.2)	190.6 (152.4, 228.7)	25.9 (15.1, 36.7)
Quartile 3	281.8 (151.8, 411.8)	172.0 (134.5, 209.6)	21.7 (11.0, 32.4)
Quartile 4 (highest)	261.9 (132.4, 391.4)	158.6 (120.8, 196.4)	46.5 (36.1, 57.0)*
P for trend ^b	0.145	0.067	0.001
PFHxA Quartile 1(lowest)	215.2 (83.7, 346.7)	127.4 (87.5, 167.4)	25.2 (13.9, 36.5)
Quartile 2	386.7 (257.7, 515.9)	158.3 (119.4, 197.0)	26.0 (15.1, 37.0)
Quartile 3	427.9 (299.6, 556.2)	178.7 (140.1, 217.2)	32.1 (21.2, 43.0)
Quartile 4 (highest)	296.7 (163.5, 429.9)	144.6 (104.8, 184.3)	30.4 (19.0, 41.7)
P for trend ^b	0.330	0.104	0.429
PFHxS Quartile 1(lowest)	257.1 (125.3, 389.0)	182.3 (141.9, 222.8)	24.7 (13.4, 36.1)
Quartile 2	390.6 (259.3, 521.9)	119.3 (80.5, 158.1)	39.6 (28.7, 50.5)
Quartile 3	363.6 (233.5, 493.8)	147.3 (108.1, 186.4)	25.4 (14.3, 36.5)
Quartile 4 (highest)	316.0 (180.4, 451.7)	159.0 (120.0, 198.0)	24.2 (13.2, 35.2)
P for trend ^b	0.581	0.321	0.537
PFNA Quartile 1(lowest)	278.4 (150.9, 405.9)	138.6 (100.5, 176.7)	28.2 (17.4, 39.0)
Quartile 2	331.3 (202.8, 459.9)	122.7 (83.7, 161.6)	20.1 (9.2, 31.1)
Quartile 3	237.5 (108.4, 366.6)	172.2 (134.1, 210.3)	28.7 (17.7, 39.7)
Quartile 4 (highest)	474.1 (347.8, 600.4)	175.5 (136.7, 214.3)	36.4 (25.7, 47.1)
P for trend ^b	0.084	0.086	0.167
PFTA Quartile 1(lowest)	275.6 (142.6, 408.6)	156.7 (117.9, 195.5)	29.7 (18.9, 40.6)
Quartile 2	330.5 (199.6, 461.5)	133.1 (93.6, 172.6)	34.9 (23.9, 46.0)
Quartile 3	344.3 (212.6, 476.0)	161.0 (121.8, 200.3)	28.6 (17.6, 39.6)
Quartile 4 (highest)	375.0 (245.6, 504.4)	158.5 (118.9, 198.0)	20.4 (9.3, 31.6)
P for trend ^b	0.293	0.954	0.196

^aModels were adjusted for age, gender, BMI, parental education, ETS exposure, and month of survey.

^b*P* values were calculated using categories representing the median value of corresponding quartile.

*Compared with the lowest of quartile: $p < 0.05$.

AEC, absolute eosinophil count; ECP, eosinophilic cationic protein

Table 5. Estimated mean values (95% CI) serum IgE, AEC, and serum ECP according to serum PFCs concentration among children with asthma (n=231)^a

	IgE (IU/dL)	AEC ($\times 10^9/L$)	ECP ($\mu g/L$)
PFOS Quartile 1 (lowest)	517.9 (336.7, 699.2)	329.4 (255.8, 403.0)	25.9 (10.4, 41.3)
Quartile 2	686.2 (501.3, 871.1)	368.6 (293.9, 443.3)	37.4 (21.9, 52.8)
Quartile 3	658.1 (475.2, 841.1)	431.3 (358.1, 504.6)	43.5 (27.5, 59.4)
Quartile 4 (highest)	877.3 (695.2, 1059.4)*	453.4 (379.4, 527.3)	62.4 (46.3, 78.4)*
P for trend ^b	0.008	0.009	0.001
PFOA Quartile 1 (lowest)	512.1 (329.4, 694.8)	325.9 (253.7, 398.1)	30.3 (14.3, 46.3)
Quartile 2	604.6 (422.1, 787.1)	339.7 (266.8, 412.6)	34.8 (18.9, 50.7)
Quartile 3	788.2 (607.1, 969.2)	422.1 (349.9, 494.2)	44.3 (28.4, 60.2)
Quartile 4 (highest)	836.4 (652.0, 1020.8)*	498.0 (423.7, 572.3)	57.8 (42.2, 73.4)
P for trend ^b	0.005	<0.001	0.010
PFBS Quartile 1 (lowest)	683.6 (497.0, 870.2)	343.0 (268.8, 417.2)	32.6 (16.3, 48.9)
Quartile 2	601.2 (416.7, 785.7)	374.0 (301.6, 446.5)	44.8 (29.1, 60.5)
Quartile 3	671.3 (485.9, 856.8)	380.4 (307.2, 453.5)	42.9 (27.0, 58.8)
Quartile 4 (highest)	780.6 (598.4, 962.7)	487.4 (413.4, 561.4)*	47.3 (31.1, 63.6)
P for trend ^b	0.496	0.009	0.210
PFDA Quartile 1 (lowest)	470.6 (289.7, 651.6)	333.6 (256.0, 407.2)	19.0 (3.6, 34.3)
Quartile 2	615.6 (436.0, 795.2)	351.7 (277.7, 425.8)	45.3 (29.9, 60.7)
Quartile 3	789.8 (608.2, 971.4)	422.2 (349.3, 495.0)	44.7 (28.9, 60.6)
Quartile 4 (highest)	862.2 (682.7, 1041.7)*	470.8 (398.6, 542.9)*	59.7 (44.0, 75.3)*
P for trend ^b	0.001	0.004	0.001
PFDoA Quartile 1 (lowest)	533.0 (348.1, 717.9)	344.1 (270.9, 417.2)	28.7 (13.3, 44.1)
Quartile 2	653.4 (470.7, 836.0)	385.2 (313.4, 457.0)	36.3 (20.7, 52.0)
Quartile 3	726.7 (546.6, 906.8)	356.3 (282.9, 429.8)	42.8 (27.3, 58.2)
Quartile 4 (highest)	823.5 (640.0, 1006.9)	496.9 (423.8, 570.0)*	62.0 (45.8, 78.2)*
P for trend ^b	0.016	0.011	0.003
PFHxA Quartile 1 (lowest)	539.7 (355.0, 724.4)	397.5 (323.2, 471.7)	36.9 (20.8, 53.0)
Quartile 2	744.7 (561.1, 928.2)	369.7 (294.7, 444.7)	31.5 (15.3, 47.8)
Quartile 3	661.7 (480.3, 843.1)	371.9 (298.4, 445.4)	49.5 (33.8, 65.2)
Quartile 4 (highest)	794.9 (610.9, 978.8)	443.4 (368.9, 517.9)	49.0 (33.3, 64.6)
P for trend ^b	0.075	0.407	0.148
PFHxS Quartile 1 (lowest)	682.4 (495.0, 869.7)	331.7 (256.3, 407.2)	25.8 (9.5, 42.0)
Quartile 2	643.0 (456.1, 830.0)	379.1 (305.2, 453.1)	39.6 (24.0, 55.2)
Quartile 3	679.9 (495.4, 864.5)	430.5 (356.8, 504.2)	41.0 (25.3, 56.6)
Quartile 4 (highest)	734.2 (549.1, 919.4)	439.7 (365.5, 513.6)	61.0 (45.4, 76.6)*
P for trend ^b	0.632	0.029	0.004
PFNA Quartile 1 (lowest)	410.9 (230.6, 591.2)	309.7 (236.4, 383.0)	28.8 (13.1, 44.4)
Quartile 2	704.5 (524.1, 884.9)	353.1 (280.3, 425.8)	34.8 (19.3, 50.3)
Quartile 3	828.8 (651.6, 1006.0)*	431.7 (359.5, 503.9)	43.5 (27.6, 59.5)
Quartile 4 (highest)	790.9 (610.1, 971.6)*	482.5 (411.1, 553.9)*	61.0 (45.3, 76.6)*
P for trend ^b	0.001	<0.001	0.003
PFTA Quartile 1 (lowest)	541.9 (356.4, 727.5)	328.8 (262.9, 394.8)	36.8 (20.9, 52.8)
Quartile 2	659.9 (493.4, 826.5)	351.4 (270.5, 432.3)	39.1 (23.3, 54.9)
Quartile 3	709.6 (507.5, 911.8)	405.0 (332.7, 477.2)	49.9 (31.9, 67.9)
Quartile 4 (highest)	833.1 (650.7, 1015.5)	502.3 (429.4, 575.1)*	43.6 (28.9, 58.2)
P for trend ^b	0.011	<0.001	0.409

^aModels were adjusted for age, gender, BMI, parental education, ETS exposure, and month of survey.

^b*P* values were calculated using categories representing the median value of corresponding quartile.

*Compared with the lowest of quartile: $p < 0.05$.

AEC, absolute eosinophil count; ECP, eosinophilic cationic protein

Table 6. Estimated mean scores (95% CI) for the relationship between the PFCs levels and asthma severity, asthma control test (ACT) among children with asthma (n=231)^a

Exposure	Asthma severity score	ACT score
PFOS Quartile 1(lowest)	3.33 (2.36, 4.31)	22.51 (21.71, 23.32)
Quartile 2	4.18 (3.19, 5.17)	22.72 (21.92, 23.52)
Quartile 3	4.49 (3.52, 5.45)	22.13 (21.30, 22.94)
Quartile 4 (highest)	4.57 (3.61, 5.54)	22.21 (21.41, 23.02)
P for trend ^b	0.045	0.450
PFOA Quartile 1(lowest)	3.63 (2.66, 4.60)	22.02 (21.22, 22.82)
Quartile 2	3.99 (3.02, 4.96)	22.14 (21.33, 22.96)
Quartile 3	4.39 (3.40, 5.38)	22.76 (21.96, 23.56)
Quartile 4 (highest)	4.57 (3.59, 5.55)	22.65 (21.84, 23.45)
P for trend ^b	0.119	0.168
PFBS Quartile 1(lowest)	3.48 (2.50, 4.47)	22.23 (21.41, 23.04)
Quartile 2	4.42 (3.46, 5.38)	22.39 (21.60, 23.19)
Quartile 3	3.82 (2.85, 4.79)	22.73 (21.92, 23.53)
Quartile 4 (highest)	4.85 (3.88, 5.82)	22.23 (21.42, 23.03)
P for trend ^b	0.092	0.836
PFDA Quartile 1(lowest)	3.43 (2.48, 4.37)	22.35 (21.54, 23.16)
Quartile 2	3.79 (2.83, 4.74)	22.55 (21.76, 23.35)
Quartile 3	4.07 (3.10, 5.04)	22.33 (21.53, 23.13)
Quartile 4 (highest)	5.32 (4.36, 6.29) [*]	22.34 (21.53, 23.15)
P for trend ^b	0.005	0.857
PFDoA Quartile 1(lowest)	3.68 (2.71, 4.65)	22.57 (21.77, 23.38)
Quartile 2	3.50 (2.55, 4.45)	22.54 (21.75, 23.33)
Quartile 3	4.50 (3.53, 5.46)	21.90 (21.10, 22.70)
Quartile 4 (highest)	4.91 (3.94, 5.88)	22.57 (21.77, 23.38)
P for trend ^b	0.024	0.709
PFHxA Quartile 1(lowest)	4.34 (3.36, 5.32)	22.21 (21.40, 23.01)
Quartile 2	4.10 (3.13, 5.07)	22.41 (21.62, 23.20)
Quartile 3	4.06 (3.08, 5.04)	21.92 (21.12, 22.72)
Quartile 4 (highest)	4.07 (3.09, 5.05)	23.03 (22.23, 23.83)
P for trend ^b	0.854	0.284
PFHxS Quartile 1(lowest)	3.96 (2.99, 4.94)	21.97 (21.17, 22.78)
Quartile 2	4.17 (3.19, 5.14)	22.39 (21.59, 23.19)
Quartile 3	4.44 (3.46, 5.42)	22.48 (21.68, 23.29)
Quartile 4 (highest)	4.01 (3.02, 5.00)	22.72 (21.91, 23.54)
P for trend ^b	0.722	0.251
PFNA Quartile 1(lowest)	4.05 (3.08, 5.02)	22.35 (21.55, 23.15)
Quartile 2	3.62 (2.64, 4.60)	22.79 (21.98, 23.60)
Quartile 3	4.25 (3.29, 5.21)	22.09 (21.30, 22.88)
Quartile 4 (highest)	4.65 (3.68, 5.63)	22.35 (21.55, 23.16)
P for trend ^b	0.217	0.695
PFTA Quartile 1(lowest)	3.40 (2.51, 4.28)	22.57 (21.84, 23.30)
Quartile 2	4.45 (3.39, 5.52)	22.33 (21.44, 23.22)
Quartile 3	4.05 (3.08, 5.01)	22.01 (21.21, 22.81)
Quartile 4 (highest)	4.89 (3.92, 5.86)	22.63 (21.82, 23.44)
P for trend ^b	0.050	0.917

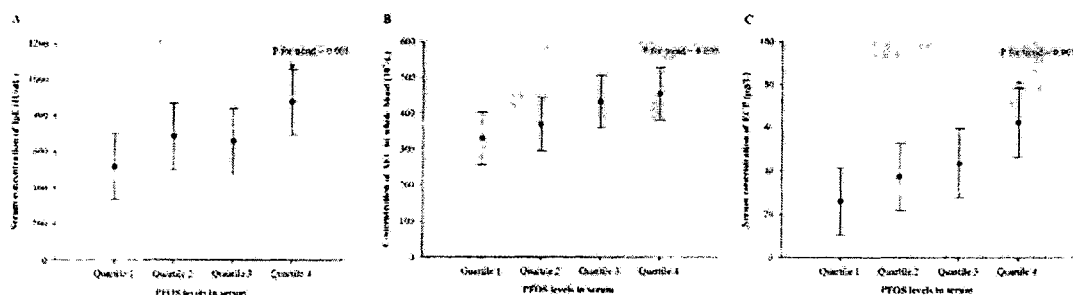
^aModels were adjusted for age, gender, BMI, parental education, ETS exposure, and month of survey.

^bCompared with the lowest of quartile: $p < 0.05$.

^{*}*P* values were calculated using categories representing the median value of corresponding quartile.

FIGURE LEGEND

Figure 1. Immunological markers (A) IgE, (B) AEC and (C) ECP among asthmatic children according to quartiles of PFOS exposure. The data are expressed as estimated mean with 95%CI adjusted for age, gender, BMI, parental education, ETS exposure, and month of survey. P values of trend are calculated using categories representing the median value of corresponding quartile (Quartile 1: < 19.64 ng/mL; Quartile 2: 19.64-33.85 ng/mL; Quartile 3: 33.85-61.08 ng/mL; Quartile 4: ≥ 61.08 ng/mL). *Compared with the lowest of quartile (Quartile 1): $P < 0.05$.



Supplemental Material

Serum Polyfluoroalkyl Concentrations, Asthma Outcomes, and Immunological Markers in a Case-Control Study of Taiwanese Children

Authors

Guang-Hui Dong, Kuan-Yen Tung, Ching-Hui Tsai, Miao-Miao Liu, Da Wang, Wei Liu, Yi-He Jin, Wu-Shiun Hsieh, Yungling Leo Lee, Pau-Chung Chen

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STANDARDS AND REAGENTS

The potassium salt of heptadecafluorooctane sulfonate (PFOS, 98%) was acquired from Fluka (Steinheim, Germany). The potassium salts of perfluorohexane sulfonate (PFHxS, 98%), perfluoroheptanoic acid (PFHpA, 98%), perfluorohexane acid (PFHxA, 98%) were acquired from Interchim (Montlucon, France). The potassium salt of nonafluoro-1-butanedisulfonate (PFBS, 98%) and heptadecafluoropelargonic acid (PFNA, 95%) were acquired from Tokyo Chemical Industry (Tokyo, Japan). Pentadecafluorooctanoic acid (PFOA, 95%) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Nonadecafluorodecanoic acid (PFDA, 96%) and perfluorododecanoic acid (PFDoA, 97%) were purchased from Acros Organics (Geel, Belgium). Perfluorotetradecanoic acid (PFTA, 97%) was purchased from Aldrich (Steinheim, Germany). Tetrabutylammonium hydrogensulfate (TBAHS) of HPLC grade and anhydrous extra pure sodium carbonate (Na_2CO_3 , 99.5%) were obtained from Acros Organics (Geel, Belgium). HPLC grade ammonium acetate was obtained from Dikma Technology (Richmond, VA). HPLC grade methyl tert-butyl ether (MTBE), methanol, and acetonitrile were obtained from Tedia (Fairfield, OH). Milli-Q water was cleaned using Waters Oasis HLB Plus (225 mg) cartridges (Milford, MA) to remove the potential residue of PFCs. Mixed stock PFC standard solution was prepared in methanol. All reagents were used as received.

SAMPLE PREPARATION AND EXTRACTION

Serum samples were extracted following a method developed by Hansen et al. (2001). Two millilitres of 0.25 M Na_2CO_3 and one liter of 0.5 M TBAHS were added to 0.5 mL of serum and then extracted twice with MTBE. The combined MTBE extracts were brought to dryness under a gentle stream of high purity nitrogen, and reconstituted in 1 mL mixture of methanol and 10 mM ammonium acetate (2:3, v/v) before final filtration with a 0.22 μm nylon filter.

INSTRUMENTAL ANALYSIS

Extracts of serum samples were analyzed via high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). Chromatography was performed by an Agilent 1200 HPLC system (Palo Alto, CA). A 25 μ L aliquot of extract was injected onto a 2.1 $\times$ 100 mm (3.5 μ m) Agilent Eclipse Plus C18 column (Palo Alto, CA) with 10 mM ammonium acetate and acetonitrile as mobile phases starting with 40% acetonitrile at a flow rate of 250 μ L/min and column temperature of 40 °C. The gradient was increased to 90% acetonitrile at 9 min and then held for 2 min. In addition, an 8 min re-equilibration interval was run before each following sample. The HPLC system was interfaced to an Agilent 6410 Triple Quadrupole (QQQ) mass spectrometer (Santa Clara, CA) operated with electrospray ionization (ESI) in negative mode. Instrumental parameters were optimized to transmit the $[M-K]^-$ ion before fragmentation to one or more product ions. Declustering potential and collision energies were optimized for each analyte and ranged from 35 to 90V and 10 to 35eV, respectively. Data were acquired by tandem mass spectrometry using multiple reaction monitoring (MRM) at transitions, 499 > 99 for PFOS, 413 > 369 for PFOA, 299 > 99 for PFBS, 399 > 99 for PFHxS, 363 > 319 for PFHpA, 313 > 269 for PFHxA, 463 > 419 for PFNA, 513 > 469 for PFDA, 613 > 569 for PFDoA, 713 > 669 for PFTA. Moreover, multiple daughter ions were monitored for confirmation, but quantitation was based on a single production. In all cases, the capillary was held at -4 kV and the desolvation temperature was kept at 350 °C.

QUALITY ASSURANCE AND QUALITY CONTROL

Procedural blanks were prepared at an interval of every ten samples to check if contamination had occurred during the extraction of samples. Solvent blanks containing acetonitrile and Milli-Q water (2:3, v/v) were run after every twenty samples to monitor for background contamination. Duplicate injections and calibration check standards were run after every twenty samples to assure the precision and accuracy of each run. Matrix spike recoveries were tested by spiking native

standards of all 10 target compounds into 12 randomly selected samples, at levels of 10 ng and 20 ng for each of the target compounds. All the matrix spike samples were analyzed in duplicate. Recoveries of native standards spiked in serum matrix were $98 \pm 5\%$, $101 \pm 6\%$, $95 \pm 3\%$, and $96 \pm 5\%$, for PFOS, PFOA, PFHxS, and PFNA, respectively. The average recoveries for other perfluorochemicals ranged from 82% to 96%. The relative standard deviations (RSD) of duplicate analyses were less than 5% for PFOS, PFOA, PFBS, PFDA, PFHpA, PFHxA, PFHxS, PFNA, PFTA, and less than 10% for PFDoA. The concentrations of serum extracts were quantified via nine-point matrix-matched calibration curves ranging from 0.01 to 100 ng/mL, which were performed by adding mixed PFC standard solution into blank and newborn bovine serum, respectively. The regression coefficients (r^2) of calibration curves for all the target analytes in different matrixes were higher than 0.99. The limit of detection (LOD) was defined as the peak of analyte that needed to yield a signal-to-noise (S/N) ratio of 3:1, and the limit of quantification (LOQ) was defined as the lowest point on the standard curve, above the LOD, with a RSD less than 10%.

Supplemental Material, Table S1. Spearman's rank correlation coefficients (rho) among different PFCs in blood samples (n=456)

PFC	PFOS	PFOA	PFBS	PFDA	PFDaA	PFHpA	PFHxA	PFHxS	PFNA	PFTA
PFOS	1.00	**0.64	**0.27	**0.34	**0.53	**0.27	**0.21	**0.37	**0.35	**0.24
PFOA		1.00	**0.42	**0.41	**0.34	**0.43	**0.26	**0.59	**0.46	*0.11
PFBS			1.00	**0.30	**0.28	**0.29	**0.19	**0.21	**0.18	**0.22
PFDA				1.00	**0.53	**0.31	0.09	**0.42	**0.79	**0.17
PFDaA					1.00	**0.33	*0.10	**0.28	**0.41	**0.71
PFHpA						1.00	**0.29	**0.27	**0.22	**0.24
PFHxA							1.00	**0.33	0.06	0.02
PFHxS								1.00	**0.50	0.05
PFNA									1.00	*0.16
PFTA										1.00

**p<0.01; *p<0.05

Supplemental Material, Table S2. Median serum PFOS and PFOA concentrations (ng/mL) reported for other populations

Reference	Studies/Country	Time	Sample	Age (years)	Median	
					PFOS	PFOA
Kato et al. (2011)	NHANES (USA)	1999-2000	543	12-19	29.4	5.60
	NHANES (USA)	2003-2004	640	12-19	19.9	4.00
	NHANES (USA)	2005-2006	640	12-19	14.9	3.80
	NHANES (USA)	2007-2008	357	12-19	11.3	4.00
Frisbee et al. (2010)	C8 Health Project (USA)	2005-2006	6536	1-11.9	20.7	32.6
	C8 Health Project (USA)	2005-2006	5934	12-17.9	19.3	26.3
OECD (2002)	USA	1995	599	2-12	37.5 ^a	----
Holzer et al. (2008)	Siegen, German	2006	80	5-6	5.2 ^a	5.2 ^a
	Amsberg, German	2006	90	5-6	5.4 ^a	24.6 ^a
Turgeon et al. (2012)	Canada	2006-2008	86	1-4.5	3.4 ^a	1.7 ^a
Toms et al. (2009)	Australia	2006-2007	----	6-9	18.3	8.2
			----	9-12	17.7	7.0
Zhang et al. (2010)	China	2009	85	5-10	5.6	2.2
Fei and Olsen (2011)	Danish	1998-2002	787	Pregnancy	34.4	5.4
OECD (2002)	USA	2000	645	20-69	34.9 ^a	----
	Sagamihara, Japan	1999	32	Adults	40.3 ^b	----
	Tokyo, Japan	1999	30	Adults	52.3 ^b	----

^a Geometric mean; ^b Arithmetic mean;

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Perfluorooctanoic Acid Exposure and Cancer Outcomes in a Contaminated Community: A Geographic Analysis

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Perfluorooctanoic Acid Exposure and Cancer Outcomes in a Contaminated Community: A Geographic Analysis

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Running title: PFOA exposure and cancer

Key Words: C8, GIS, kidney cancer, PFOA, testicular cancer

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Competing interests: The authors declare they have no competing interests.

Abbreviations:

CI: Confidence Interval

GIS: Geographic Information Systems

LACS: Locatable Address Conversion System

OCISS: Ohio Cancer Incidence Surveillance System

OH: Ohio

OR: Odds Ratio

PFOA, C8: Perfluorooctanoic acid

SES: Socioeconomic Status

SIR: Standardized Incidence Ratio

WD: Water District

WV: West Virginia

Abstract

Background: Perfluorooctanoic acid (PFOA) has been linked to cancer in occupational mortality studies and animal toxicological research.

Objective: We investigated the relationship between PFOA exposure and cancer among residents living near the DuPont plant in Parkersburg, West Virginia (WV).

Methods: Our analyses included incident cases of 18 cancers diagnosed from 1996-2005 in five Ohio (OH) counties and eight WV counties. For analyses of each cancer outcome, controls comprised all other cancers in the study dataset except kidney, pancreatic, testicular, and liver cancers, which have been associated with PFOA in animal or human studies. We applied logistic regression models to individual-level data to calculate odds ratios (OR) and confidence intervals (CI). For the combined analysis of WV and OH data, the exposure of interest was resident water district. Within OH, geocoded addresses were integrated with a PFOA exposure model to examine the relationship between cancer odds and categories of estimated PFOA serum.

Results: Our final dataset included 7,869 OH cases and 17,238 WV cases. There was a positive association between kidney cancer and the very high and high serum exposure categories (OR: 2.0, 95% CI: 1.0, 3.9; n=9 and OR: 2.0, 95% CI: 1.3, 3.2; n=22, respectively) and a null association with the other exposure categories compared to the unexposed. The largest OR was for testicular cancer with the very high exposure category (OR: 2.8, 95% CI: 0.8, 9.2; n=6) but there was an inverse association with the lower exposure groups, and all estimates are imprecise because of small case numbers.

Conclusions: This study suggests that higher PFOA serum levels may be associated with testicular, kidney, prostate, and ovarian cancers and non-Hodgkin's lymphoma. Strengths of this

study include near-complete case ascertainment for state residents, and well characterized contrasts in predicted PFOA serum levels from 6 contaminated water supplies.

Introduction

The current study uses geographic methods to investigate the relationship between exposure to PFOA (perfluorooctanoic acid, C8) and patterns of cancer risk in the mid-Ohio River Valley using data from the West Virginia (WV) and Ohio (OH) cancer registries. This work is one of a series of studies investigating health effects of PFOA exposure among residents living near the Washington Works DuPont Teflon-manufacturing plant in Parkersburg, WV (Steenland et al. 2009). PFOA was released into the environment via aerial emissions and discharged into the Ohio River since the 1950s, resulting in the contamination of the local drinking water (Paustenbach et al. 2007; Shin et al. 2011a). In addition to hundreds of impacted private drinking water wells, six nearby public water districts in WV and OH were also contaminated (Figure 1), and monitoring data show that even after a drastic reduction in releases, PFOA contamination of drinking water persisted and continued to increase in some water districts near the plant (Shin et al. 2012).

As part of a settlement from a large class action lawsuit against DuPont, the C8 Science Panel (2012) was established to investigate potential health effects resulting from PFOA exposure, and a one-year cross sectional survey (2005-2006), known as the C8 Health Project, was conducted among over 69,000 residents with a minimum of one year residency in public water districts contaminated by PFOA (Frisbee et al. 2009). Measured mean PFOA levels in public drinking water supplies at the time of the survey ranged from 0.03 µg/L in Mason, WV to 3.49 µg/L in Little Hocking, OH, and private drinking water was measured at levels as high as 22.1 µg/L (Shin et al. 2011a). The median serum PFOA level in this cross-sectional study population was 28.2 µg/L with a range of 0.2 to 22,412 µg/L (Steenland et al. 2009). PFOA is

also detected in the serum of the general U.S. population, albeit at a much lower median level of 3.9 µg/L (Calafat et al. 2007).

The stain-resistant and water-repellant properties of PFOA make it widely used, and given its persistence it is ubiquitous in many indoor environments, including homes and work places (Fraser et al. 2012; Haug et al. 2011). Animal toxicologic data links PFOA to pancreatic cancer (acinar cells), testicular cancer (Leydig cells), and liver cancer (Lau et al. 2007). A recent review of the epidemiologic data concluded that more studies were needed to determine if any potential health effects exist, and, specifically, that the evidence for cancer is not conclusive (Steenland et al. 2010). Human data for cancer from two occupational cohorts are limited to mortality and are based on small numbers. One of the two cohorts showed excesses of kidney cancer (Leonard et al. 2008), while the other showed positive exposure-response trends for pancreatic and prostate cancer which were not statistically significant (Lundin et al. 2009). In a prospective Danish cohort study, plasma concentrations of background PFOA exposures were not associated with prostate, bladder, pancreatic, or liver cancer (Eriksen et al. 2009). A case-control study of Greenland Inuit women found a positive but not statistically significant association between PFOA exposure and breast cancer (Bonefeld-Jorgensen et al. 2011). The positive associations were generally not consistent among cancer sites between studies and for the remaining cancer sites reported, no associations were observed.

The objective of this study is to investigate the association between PFOA exposure and the odds of cancer using data from the WV and OH cancer registries. The current study includes residents of exposed water districts and unexposed geographic areas outside of the C8 Health Project area, enabling a comparison between populations exposed to varying degrees and unexposed populations while controlling for individual-level risk factors. Results of this

geographic study complement other studies being done within the C8 Health Project population by including a more complete ascertainment of cases, including those who died prior to the 2005-6 survey. The weight of evidence from the combination of studies within this population was heavily considered in the determination by the C8 Science Panel that there was a probable link between PFOA exposure and testicular and kidney cancers.

Methods

Study Population and Data

The study area encompasses 6 contaminated public water districts (WD) and 13 counties in WV and OH that surround the Washington Works DuPont facility (Figure 1). Incident cancer cases diagnosed from 1996-2005 in the OH counties of Athens, Meigs, Gallia, Washington, and Morgan and the WV counties of Wood, Mason, Wirt, Putnam, Jackson, Pleasants, Ritchie, and Cabell, were obtained from the OH Cancer Incidence Surveillance System (OCISS) and from the WV Cancer Registry (WVCR), respectively. The WVCR provided an incident cancer dataset of all cancer types with a total of 19,716 individual cases. There were 10,044 (51%) male cases and 9,673 (49%) female cases. The OCISS provided us with registry data for 9,402 incident cases of all cancer types. For our analyses, we were able to geocode 8,650 (92%) of the OH addresses at diagnosis to the street level and the remaining 752 (8%) at the ZIP code level, with little variation in these proportions by cancer type. There were 4,396 (51%) male cases and 4,254 (49%) female cases. The median age for both datasets was 67 years. We excluded 745 OH cases and 2,411 WV cases of cancer types including oral cavity, pharynx, esophagus, larynx, stomach, and Hodgkin's lymphoma with too few cases (<100 OH cases, the smaller of the two analyses) for meaningful analysis, or that have not been previously investigated in relation to PFOA in

animal toxicologic studies or occupational mortality studies (Lau et al. 2007; Leonard et al. 2008; Steenland and Woskie 2012). We also excluded 36 OH cases and 67 WV cases that were diagnosed at less than 15 years of age. Our final dataset included 7,869 geocoded OH cases and 17,238 WV cases of 18 cancer categories (bladder, brain, female breast, cervix, colon/rectum, kidney, leukemia, liver, lung, melanoma of the skin, multiple myeloma, non-Hodgkin's lymphoma, ovary, pancreas, prostate, testis, thyroid, and uterus).

Based on 2010 U.S. census population estimates, the population of the study area is over 500,000, with one third in OH and two thirds in WV. Using a PFOA exposure model and data collected from the C8 Health Project, the corresponding 1995 median PFOA serum concentrations in the 6 public WDs were previously estimated as follows: Little Hocking (Washington and Athens Counties, OH) = 125 µg/L; Lubeck (Wood County, WV) = 65.8 µg/L; Tupper Plains (Athens and Meigs Counties, OH) = 23.9 µg/L; Belpre (Washington County, OH) = 18.7 µg/L; Pomeroy (Meigs County, OH) = 10.7 µg/L; and Mason (Mason County, WV) = 5.3 µg/L (Shin et al. 2011b). The Institutional Review Boards at the Boston University Medical Campus, the London School of Hygiene and Tropical Medicine, the OH Department of Health, and the WV Bureau of Health Statistics have approved the research. This study was granted a waiver of informed consent.

Overview of Analyses

The final dataset included information for study area residents diagnosed with 18 different categories of cancer. We applied logistic regression to individual-level data using registry-based cancer controls to calculate adjusted odds ratios (ORs) and confidence intervals (CIs) for each cancer category, with the other cancer categories excluding kidney, pancreatic,

testicular, and liver cancers (which have been linked to PFOA exposure in animal and human studies) serving as controls. As a sensitivity analysis, we also performed analyses using a control group consisting of those with all other cancer diagnoses included in the dataset, without exclusions. We adjusted for age, gender, diagnosis year, smoking status (current, past, unknown, with never smoker as the reference) and insurance provider (government insured Medicaid, uninsured, unknown, with privately insured as the reference). We ran additional analyses stratified by gender for cancers with ≥ 100 cases of each gender; this included cancers of the bladder, colon-rectum, kidney, and lung, as well as melanoma of the skin and non-Hodgkin's lymphoma. To test the sensitivity of results to missing smoking and health insurance data, we generated 10 datasets with imputation of missing values using default predictive mean matching and logistic regression imputation via the 'mice' library in R (Van Buuren and Oudshoorn 2007). We obtained parameter estimates by averaging over all 10 datasets of parameter estimates, and variance estimates by combining the between- and within-imputation variances.

For exposure assessment purposes, OCISS provided addresses at diagnosis that we geocoded, while the WVCR provided an identifier for geographic unit, which allowed us to assign case addresses to contaminated water district areas or to the unexposed group. We conducted two different analyses to compare the robustness of our results across different exposure metrics. The first analysis used water district of residence as the exposure of interest and included both OH and WV data. The second analysis was restricted to OH and took advantage of the availability of geocoded OH addresses at time of diagnosis. We used an existing PFOA exposure model (Shin et al. 2011a; 2011b) to estimate serum levels at a finer geographic resolution for different latency assumptions. For OH-only analyses, we also adjusted for race, modeled as a binary variable for white or non-white, which was provided by OCISS but not

WVCR due to confidentiality concerns. The two analyses are described in detail below. All statistical analyses were conducted using R 2.10.1 (Vienna, Austria).

Water District Analysis in WV and OH

For the combined WV and OH data, we used residency within a contaminated WD area as our exposure of interest. In OH, we assigned cases to WDs using geocoding, the process by which measures of longitude and latitude are calculated for street addresses using reference street files. We first cleaned and standardized addresses using ZP4 address correction software with the LACS database (version expiring April 1 2011; www.semaphorecorp.com) and converted additional rural route boxes to street addresses using Enhanced 911 address conversion tables (Vieira et al. 2010). Geocoding was then performed using a geographic information system (GIS), ESRI ArcView version 9.3 (Redlands, CA) with the ESRI StreetMap Premium North America NAVTEQ 2010 enhanced street dataset as the reference address locator. Using geocoding, we were able to identify cases living within a contaminated water district area. Cases not in contaminated water districts were assigned to the unexposed group.

For WV cases, data release restrictions prohibited identifiable geographic location from being included with the cancer data. Instead, a variable was provided to indicate whether cases were located in Lubeck WD, Mason County WD, or unexposed areas. Only addresses in Wood County were geocoded to the WD distribution system at the WVCR to determine if the case was living at a street address serviced by the Lubeck WD. Wood County cases not on the Lubeck WD distribution system were considered unexposed. All cases in Mason County were assigned exposure to the Mason County WD. Mason County addresses were not geocoded because the median PFOA serum levels were close to background.

We calculated adjusted ORs and CIs for each of the 18 cancer categories in association with one of the six contaminated WDs versus an unexposed WD. We also calculated the adjusted ORs for living in any exposed WD relative to unexposed WDs.

Estimated PFOA Serum Level Analysis in OH

To take advantage of the availability of geocoded street addresses in the OH data, we also used modeled serum PFOA concentration as an exposure metric. All OH addresses at time of diagnosis were geocoded to determine if the case was serviced by one of the contaminated public WDs, a contaminated private well, or unexposed. This geocoding allowed us to be even more specific about exposure as cases living within a water district area, but not on a street serviced by a distribution pipe (or before the year of pipe installation), would likely be accessing drinking water from a private residential well. The methods for estimating individual serum PFOA levels from linked environmental, exposure, and pharmacokinetic models are described in detail elsewhere (Shin et al. 2011a; 2011b). Briefly, the environmental models integrate facility emissions data, fate and transport characteristics of PFOA, and hydrogeological properties of the study area to estimate PFOA air and water concentrations from 1951-2008. Using GIS, we were also able to determine what year the pipe that serviced the case was installed. For each case, annual PFOA serum levels were calculated from 1951 to date of diagnosis by linking historical air and groundwater concentrations to residential information at time of diagnosis and applying standard assumptions about water intake, body weights, and a PFOA half-life in the exposure and pharmacokinetic models (Shin et al. 2011b). Because only the residence at diagnosis was available, annual serum levels were estimated assuming cases lived at that address for 10 years. As a sensitivity analysis, we also estimated serum levels with and without a 10-year latency

period prior to date of diagnosis assuming a lifetime residency at that address. We then extracted two exposure metrics for each latency and residency assumption: the estimated annual serum level corresponding to the year of diagnosis or 10 years prior for latency analyses and a cumulative measure summed over the corresponding years of exposure. The estimated annual serum level is equivalent to what would be measured in a serum sample taken during that year whereas the cumulative measure is the area under the serum level profile curve.

We first categorized individual-level exposure as very high, high, medium, low, and unexposed using cutoffs based on the distribution of the annual PFOA serum concentrations among the exposed study population assuming a 10-year residency. The distribution of estimated annual PFOA serum levels among the exposed study population ranged from 3.7-655 $\mu\text{g/L}$ for 10-yr residency assuming 10-yr latency (see Supplemental Material, Figure S1). The tertile breaks of the distribution defined the cutoffs for low, medium, and high. We used the tertile breaks of 12.9 and 30.8 to define high (30.8-109 $\mu\text{g/L}$), medium (12.9-30.7 $\mu\text{g/L}$), and low exposure categories (3.7-12.9), with unexposed serving as the reference category. There is a large break in the distribution at 110 $\mu\text{g/L}$ so a very high group was created based on this break value which included the upper 10% of our exposed population (see Supplemental Material, Figure S1). Cumulative exposure categories were based on the distribution among the exposed cases and were divided into the following groups: very high=600-4,679 $\mu\text{g/L-years}$; high= 198-599 $\mu\text{g/L-years}$; medium=89-197 $\mu\text{g/L-years}$; and low=3.9-88 $\mu\text{g/L-years}$. We analyzed the individual-level OH data using logistic regression to calculate adjusted ORs and CIs for exposure categories with unexposed serving as the referent. For comparison, separate analyses were conducted for the annual and cumulative exposure measures calculated for the different latency and residency assumptions.

Results

WV and OH Water District Analyses

Table 1 shows the distribution of cases in the contaminated WD areas and surrounding unexposed geographic area. The Little Hocking WD is the highest exposed district, followed by Lubeck, Tupper Plains, Belpre, Pomeroy, and Mason County. The odds of testicular cancer was increased in Little Hocking (OR: 5.1, 95% CI: 1.6, 15.6; n=8), and the odds of kidney cancer was elevated in Little Hocking (OR: 1.7, 95% CI: 0.4, 3.3; n=10) and Tupper Plains (OR: 2.0, 95% CI: 1.3, 3.1; n=23). Residents of Little Hocking also had increased odds of non-Hodgkin lymphoma (OR: 1.6, 95% CI: 0.9, 2.8; n=14) and prostate cancer (OR: 1.4, 95% CI: 0.9, 2.3; n=36).

OH Serum Level Analyses

Adjusted odds ratios suggested associations between the very high PFOA exposure category and several cancers, but ORs for lower exposure categories generally did not support a positive dose-response relation (Table 2). Kidney cancer was positively associated with very high and high exposure categories (OR: 2.0, 95% CI: 1.0, 3.9; n=9 and OR: 2.0, 95% CI: 1.3, 3.2; n=22, respectively) while ORs for medium and low exposure categories were close to the null compared to the unexposed. The largest OR was for testicular cancer with the very high exposure category (OR: 2.8, 95% CI: 0.8, 9.2; n=6) but the estimate was imprecise due to small numbers, and ORs for high, medium or low exposure categories, which were based on only 1, 3 and 1 cases, respectively, were all <1.0. Ovarian cancer was also positively associated with the very high exposure category (OR: 2.1, 95% CI: 0.8, 5.5; n=5) but again imprecise due to small numbers, and weaker associations for the high and medium exposure categories with a negative

association in the lowest exposure category. ORs for the association between the very high and medium exposure categories and non-Hodgkin's lymphoma were moderate (OR: 1.8, 95% CI: 1.0, 3.4; $n=11$ and OR: 1.5, 95% CI: 1.0, 2.2; $n=28$, respectively), while ORs for high and low exposure categories were close to the null compared to the unexposed. Prostate cancer showed a weak but relatively precise positive association with very high exposure (OR: 1.5, 95% CI: 0.9, 2.5; $n=31$) and no association with lower levels of exposure. Results were very similar for associations with the cumulative exposure measure (see Supplemental Material, Table S1), and for exposure estimates that did not account for latency (see Supplemental Material, Table S2) which were highly correlated with estimated exposures that assumed a 10-year latency (Spearman's rank correlation $\rho=0.997$, $p\text{-value}<0.001$). In addition, associations were similar when the alternative control group (that included kidney, liver, pancreas and testis cancer cases) was used (see Supplemental Material, Table S3).

To test the sensitivity of our analyses to missing smoking ($n=2,452$) and health insurance data ($n=1824$), we ran multiple imputations for cancers of the bladder, colon/rectum, female breast, kidney, lung, prostate, uterus, melanoma of the skin, and non-Hodgkin's lymphoma with sufficient numbers (≥ 100 cases with complete covariate data) and we observed similar results (see Supplemental Material, Table S4).

For cancers of the bladder, colon/rectum, kidney, and lung, melanoma of the skin, and non-Hodgkin's lymphoma with sufficient numbers to stratify by gender (>100 cases in men and women, respectively), we observed generally similar results with regards to PFOA exposure categories (data not shown). An exception was kidney cancer, which was positively associated with very high exposure in women ($n=108$) (OR: 3.5, 95% CI: 1.4, 8.3; $n=6$) but not men ($n=138$) (OR: 1.0, 95% CI: 0.3, 3.4; $n=3$) relative to the unexposed.

Discussion

Testicular cancer was positively associated with the highest PFOA water district (OR: 5.1, 95% CI: 1.6, 15.6; n=8) and the highest serum exposure category (OR: 2.8, 95% CI: 0.8, 9.2; n=6) compared to cases living in unexposed areas. However, we also observed an inverse association between testicular cancer and the lower exposure groups, and all of the estimates are imprecise because of small numbers of cases. Evidence of effects of PFOA on testicular Leydig cell tumors in animal models has been reported (Lau et al. 2007). Kidney cancer was increased in association with both high and very high PFOA exposure, based on larger numbers of cases. We also observed elevated adjusted odds ratios for very high PFOA exposure and ovarian (OR: 2.0, 95% CI: 0.8, 5.1; n=5) and prostate (OR: 1.5, 95% CI: 1.0, 2.5; n=31) cancers and non-Hodgkin lymphoma (OR: 1.8, 95% CI: 0.9, 3.3; n=11).

A limitation of our study is that we used other types of cancer as our controls (referents). Our analysis assumes that referent cancers are not associated with exposure to PFOA. For our main analyses we excluded kidney, pancreatic, testicular, and liver cancers from controls as these cancers have been linked to PFOA exposure in animal or human studies, but analyses using all other cancers as referents were comparable. We further assume that different types of cancer are ascertained by the registry in the same way, and that they are sampled from the same source population.

Despite the large overall sample size of our study, the water district analyses and the analyses of the very high exposure group were limited by small numbers of many individual cancers. There is also little consistency in the results across exposure categories, with no evidence of a positive dose response. We were further limited by the covariates we could adjust

for, which included only age, gender, race (white or non-white, OH only), smoking status, and health insurance provider. We were therefore unable to adjust for other risk factors of potential interest—e.g., prenatal exposure to xenoestrogens in relation to testicular cancer—although such factors would also have to be associated with exposure to cause confounding. Chance is also a concern as we are investigating multiple cancer sites.

As expected under the assumption that a positive association is truly present, we observed similar but weaker associations for most outcomes when no latency was assumed. Because exposure in our study is dependent on location and the ranking of exposure between districts generally remained stable over time, there is very little movement of cases across exposure categories with respect to latency assumptions. As a sensitivity analysis, we also modeled exposures assuming the cases lived at their residence their entire lifetime and observed similar results (see Supplemental Materials, Table S4). However both the latency and residential history measures were highly correlated (Spearman's rank correlations: $\rho > 0.99$, $p\text{-value} < 0.001$). Moving within the same public water district would also have little or no impact on the estimated serum values, but moving across water districts, or especially from more distant locations, could lead to exposure misclassification. Based on data from the C8 Health Project for residents older than 50 years of age, the median residency duration for their current residence in 2005-6 was 17 years. Therefore, we felt 10-year residency duration with a 10-year latency was a reasonable assumption. Any resulting exposure misclassification is likely non-differential, so the bias in the highest exposure category should on average be towards the null.

Strengths of our study include a relatively large overall sample size, ascertainment of cases from cancer registries using controls from the same population as the cases, good success in geocoding of OH residences, and the use of a validated exposure model for predicting serum

levels. Previous work has shown that the correlation between measured and predicted serum in 2005-6 using this exposure model was 0.82 (Shin et al. 2011b). Water and serum concentrations in the more exposed areas are well above background, providing a larger exposure contrast compared to other cancer studies of PFOA in general populations. We found qualitatively similar results for testicular, kidney, ovarian, prostate cancers, and non-Hodgkin lymphoma using the two different analyses; the robustness of these results is another strength.

Both analyses used individual-level outcome and risk factor information, but the first analysis used a group-level water district exposure measure so that both WV and OH data could be analyzed together. The second analysis used estimates of serum PFOA as the exposure of interest, allowing us to use geocoded residences to estimate exposure metrics based on points in time or cumulative measures, but for OH cases only. The second analysis has the advantages of being a fully individual-level design, eliminating the possible semi-ecologic bias in the other analysis (Webster 2007), and using an exposure model that has been validated as a predictor of serum levels in this context. However, there is still a potential for exposure misclassification using residence at diagnosis. A disadvantage is that we were only able to analyze OH data because geocoded residences were not available for WV.

Associations between PFOA exposures and the same cancers have been reported in other unpublished C8 Science Panel (2012) studies of the same community. Interviews of 32,254 adult community residents and DuPont workers were conducted in 2008-11, and medical records were sought. Cox regression of hazard ratios of medically validated cancers in relation to modeled cumulative PFOA serum levels at dates of diagnoses indicated increasing kidney cancer risk with increasing exposure when latency was not considered. When 10-year latency was included in the exposure metric, the association was less evident. The relative risks (RR) for testicular cancer in

relation to increasing exposure quartiles with 10-year latency were 1.0, 1.2, 1.7, and 3.0. Cross-sectional analysis of prevalent cancers among 49,082 adult community members interviewed in 2005-6 in relation to measured PFOA indicated increased RRs with increased serum PFOA quartiles compared to the lowest quartile (RRs =1.0, 1.5, 1.7, and 1.7, respectively).

Conclusions

The geographic analyses of cancer registry data provide some evidence that higher PFOA serum levels may be associated with certain cancers. The association in the highest PFOA exposure group was largest but very imprecise for testicular cancer, and smaller but more precise for kidney cancer. Non-Hodgkin's lymphoma, ovarian and prostate cancers were associated with very high exposure based on some models, but there was little or no evidence of associations with other cancers. Analyses were limited by a case-only design with minimal control of confounders and small case numbers, despite having ten years of data. In addition, residential history information was not available to account for latency, migration, and other issues regarding timing of exposure relative to cancer. However, the registries cover all residents in the study area, which comprises water districts with large and known contrasts in contamination. Thus geographic analyses using cancer registry data contributed to the evidence for the C8 Science Panel (2012) conclusion that there is a probable link between PFOA exposure and testicular and kidney cancers.

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Table 1. WV and OH water district results: number (n), adjusted odds ratios^a (AOR), and 95% confidence intervals (CI) for exposure to contaminated water districts^b

Outcome	Total n	Total Exposed n	AOR (CI)	Little Hocking n	AOR (CI)	Lubeck n	AOR (CI)	Tuppers Plains n	AOR (CI)	Belpre n	AOR (CI)	Pomeroy n	AOR (CI)	Mason n	AOR (CI)
All 18 Cancer Types	25107	2932		208		430		405		454		100		1326	
Bladder	1350	137	0.8 (0.7, 1.0)	7	0.6 (0.3, 1.4)	24	1.0 (0.6, 1.5)	20	0.9 (0.6, 1.5)	24	1.1 (0.7, 1.6)	4	0.8 (0.3, 2.1)	58	0.7 (0.6, 1.0)
Brain	506	60	1.0 (0.8, 1.3)	1	0.2 (0.0, 1.5)	7	0.8 (0.4, 1.8)	9	1.1 (0.5, 2.1)	11	1.2 (0.6, 2.2)	3	1.7 (0.5, 5.4)	29	1.1 (0.7, 1.6)
Female Breast	4057	436	1.0 (0.9, 1.1)	33	1.2 (0.8, 2.0)	69	1.2 (0.9, 1.7)	50	0.7 (0.5, 1.1)	73	1.1 (0.8, 1.5)	18	0.8 (0.5, 1.5)	193	1.0 (0.8, 1.2)
Cervix	338	35	0.8 (0.6, 1.2)	4	0.9 (0.3, 2.9)	5	0.7 (0.3, 1.7)	8	1.8 (0.8, 3.8)	5	0.6 (0.2, 1.6)	2	0.9 (0.2, 4.1)	11	0.7 (0.4, 1.3)
Colon/Rectum	3543	383	0.9 (0.8, 1.0)	20	0.7 (0.5, 1.2)	44	0.7 (0.5, 1.0)	66	1.2 (0.9, 1.6)	55	0.9 (0.7, 1.2)	18	1.2 (0.7, 2.1)	180	0.9 (0.8, 1.1)
Kidney	751	94	1.1 (0.9, 1.4)	10	1.7 (0.9, 3.3)	9	0.7 (0.4, 1.3)	23	2.0 (1.3, 3.1)	17	1.4 (0.8, 2.3)	0	---	35	0.9 (0.6, 1.3)
Leukemia	674	72	0.9 (0.7, 1.1)	5	1.0 (0.4, 2.3)	11	0.9 (0.5, 1.6)	9	0.8 (0.4, 1.7)	12	1.0 (0.6, 1.9)	1	0.4 (0.1, 2.8)	34	0.9 (0.6, 1.3)
Liver	179	23	1.1 (0.7, 1.6)	1	0.8 (0.1, 5.6)	4	1.3 (0.5, 3.5)	3	1.0 (0.3, 3.3)	3	1.0 (0.3, 3.1)	1	1.4 (0.2, 10.5)	11	1.0 (0.5, 1.9)
Lung	4926	632	1.2 (1.1, 1.3)	37	1.0 (0.7, 1.5)	85	1.1 (0.8, 1.4)	84	1.3 (1.0, 1.7)	90	1.1 (0.9, 1.4)	23	1.1 (0.7, 1.8)	313	1.3 (1.1, 1.5)
Melanoma of the Skin	1428	168	0.9 (0.8, 1.1)	12	1.0 (0.6, 1.9)	32	1.2 (0.8, 1.7)	21	0.9 (0.6, 1.4)	38	1.4 (1.0, 2.0)	4	0.9 (0.3, 2.5)	61	0.7 (0.5, 0.9)
Multiple Myeloma	285	36	1.1 (0.8, 1.6)	1	0.5 (0.1, 3.6)	4	0.9 (0.3, 2.3)	3	0.7 (0.2, 2.2)	7	1.5 (0.7, 3.2)	1	0.9 (0.1, 6.6)	20	1.4 (0.9, 2.2)
Non-Hodgkin's Lymphoma	1124	152	1.2 (1.0, 1.5)	14	1.6 (0.9, 2.8)	20	1.1 (0.7, 1.7)	21	1.2 (0.8, 1.9)	24	1.3 (0.9, 2.0)	5	1.1 (0.4, 2.7)	68	1.2 (0.9, 1.5)
Ovary	417	48	1.0 (0.8, 1.4)	5	1.8 (0.7, 4.4)	5	0.7 (0.3, 1.7)	6	1.1 (0.5, 2.4)	11	1.6 (0.9, 3.0)	2	1.1 (0.3, 4.4)	19	0.9 (0.5, 1.4)
Pancreas	495	58	1.0 (0.8, 1.3)	4	1.1 (0.4, 3.0)	9	1.1 (0.6, 2.1)	10	1.3 (0.7, 2.5)	8	0.9 (0.4, 1.8)	2	1.0 (0.2, 4.1)	25	0.9 (0.6, 1.4)
Prostate	3678	434	0.9 (0.8, 1.1)	36	1.4 (0.9, 2.3)	78	1.2 (0.9, 1.6)	56	0.8 (0.6, 1.1)	56	0.8 (0.6, 1.1)	12	1.3 (0.6, 2.6)	196	0.9 (0.7, 1.0)
Testis	134	18	1.0 (0.6, 1.8)	8	5.1 (1.6, 15.6)	2	0.9 (0.2, 4.5)	2	0.4 (0.1, 2.0)	1	0.6 (0.1, 5.0)	0	---	5	0.5 (0.2, 1.5)
Thyroid	343	40	1.1 (0.7, 1.5)	3	0.8 (0.3, 2.7)	7	1.2 (0.6, 2.6)	2	0.3 (0.1, 1.4)	5	0.9 (0.4, 2.2)	0	---	23	1.4 (0.9, 2.2)
Uterus	879	97	1.0 (0.8, 1.3)	7	1.1 (0.5, 2.4)	15	1.1 (0.6, 1.9)	12	0.9 (0.5, 1.6)	14	0.9 (0.5, 1.6)	4	0.9 (0.3, 2.4)	45	1.1 (0.8, 1.5)

a. Adjusted for age, gender, diagnosis year, insurance provider, and smoking status. Controls were other listed cancers excluding kidney, liver, pancreas and testis cancers.

b. The estimated 1995 median PFOA serum concentrations in the WDs are: Little Hocking = 125 µg/L; Lubeck = 65.8 µg/L; Tupper Plains = 23.9 µg/L; Belpre = 18.7 µg/L; Pomeroy = 10.7 µg/L; and Mason = 5.3 µg/L. Unexposed was the reference.

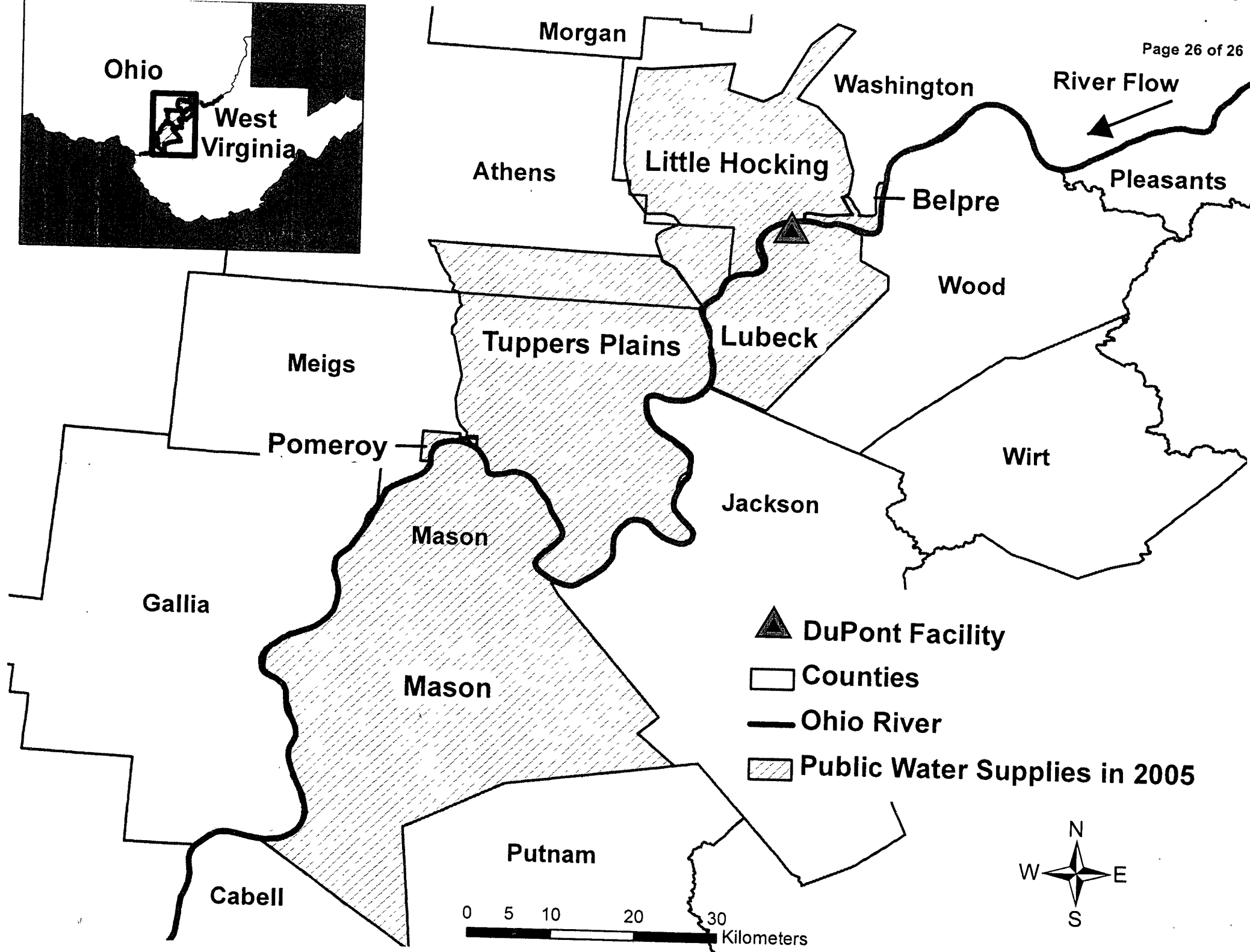
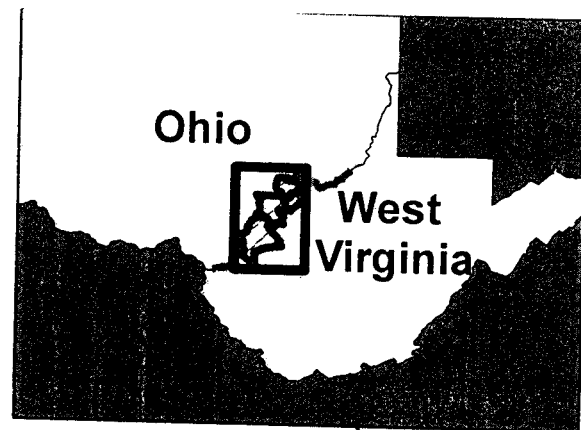
Table 2. OH serum-level results: number (n), adjusted odds ratios^a (AOR), and 95% confidence intervals (CI) for individual-level annual PFOA serum exposure categories^b assuming 10-yr residency and latency

Outcome	Total	Total Exposed	Very High		High		Medium		Low	
	n	n	n	AOR (CI)	n	AOR (CI)	n	AOR (CI)	n	AOR (CI)
All 18 Cancer Types	7869	1496	159		374		489		474	
Bladder	395	69	4	0.6 (0.2, 1.5)	21	1.2 (0.8, 2.0)	21	0.9 (0.6, 1.4)	23	0.9 (0.6, 1.4)
Brain	150	32	0	---	4	0.6 (0.2, 1.6)	16	1.8 (1.1, 3.2)	12	1.5 (0.8, 2.7)
Female Breast	1260	223	29	1.4 (0.9, 2.3)	45	0.7 (0.5, 1.0)	77	1.1 (0.8, 1.5)	72	0.9 (0.7, 1.2)
Cervix	144	25	2	0.6 (0.1, 2.6)	8	1.7 (0.8, 3.8)	4	0.5 (0.2, 1.5)	11	1.1 (0.6, 2.2)
Colon/Rectum	1149	212	13	0.6 (0.3, 1.0)	63	1.3 (1.0, 1.7)	64	0.9 (0.7, 1.2)	72	1.0 (0.8, 1.3)
Kidney	246	59	9	2.0 (1.0, 3.9)	22	2.0 (1.3, 3.2)	17	1.2 (0.7, 2.0)	11	0.8 (0.4, 1.5)
Leukemia	191	36	2	0.6 (0.1, 2.3)	8	0.9 (0.4, 1.8)	12	1.0 (0.6, 1.9)	14	1.2 (0.7, 2.1)
Liver	61	11	0	---	3	1.0 (0.3, 3.1)	4	0.9 (0.3, 2.5)	4	1.1 (0.4, 3.1)
Lung	1526	293	29	1.0 (0.7, 1.6)	78	1.2 (0.9, 1.6)	95	1.0 (0.8, 1.3)	91	1.0 (0.7, 1.2)
Melanoma of the Skin	429	95	9	0.9 (0.5, 1.9)	21	1.0 (0.6, 1.5)	38	1.3 (0.9, 1.8)	27	1.2 (0.8, 1.8)
Multiple Myeloma	83	18	1	0.6 (0.1, 4.7)	4	1.0 (0.3, 2.7)	6	1.1 (0.5, 2.6)	7	1.4 (0.7, 3.2)
Non-Hodgkin's Lymphoma	347	76	11	1.8 (1.0, 3.4)	17	1.1 (0.7, 1.9)	28	1.5 (1.0, 2.2)	20	1.0 (0.6, 1.6)
Ovary	128	27	5	2.1 (0.8, 5.5)	8	1.4 (0.7, 2.9)	10	1.4 (0.7, 2.7)	4	0.5 (0.2, 1.4)
Pancreas	162	33	2	0.6 (0.1, 2.5)	9	1.1 (0.6, 2.3)	10	0.9 (0.5, 1.7)	12	1.3 (0.7, 2.3)
Prostate	1155	214	31	1.5 (0.9, 2.5)	47	0.8 (0.5, 1.1)	65	0.8 (0.6, 1.0)	71	1.1 (0.8, 1.5)
Testis	61	11	6	2.8 (0.8, 9.2)	1	0.3 (0.0, 2.7)	3	0.6 (0.2, 2.2)	1	0.2 (0.0, 1.6)
Thyroid	94	15	2	0.8 (0.2, 3.5)	3	0.7 (0.2, 2.1)	5	0.9 (0.4, 2.3)	5	0.9 (0.4, 2.3)
Uterus	288	47	4	0.7 (0.3, 1.5)	12	1.7 (1.2, 2.5)	14	0.9 (0.6, 1.3)	17	1.2 (0.8, 1.7)

- a. Adjusted for age, race, gender, diagnosis year, insurance provider, and smoking status. Controls were other listed cancers excluding kidney, liver, pancreas and testis cancers.
- b. Categories of modeled PFOA serum concentrations ($\mu\text{g/L}$): Very High=110-655 $\mu\text{g/L}$; High=30.8-109 $\mu\text{g/L}$; Medium=12.9-30.7 $\mu\text{g/L}$; Low=3.7-12.8 $\mu\text{g/L}$; reference = unexposed.

Figure Legend

Figure 1. Study area of 13 counties encompassing 6 contaminated water districts



Supplemental Material

Perfluorooctanoic Acid Exposure and Cancer Outcomes in a Contaminated Community: A Geographic Analysis

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Supplemental Material, Figure S1, page 6. Histogram of serum estimates assuming a 10-yr residency and latency among exposed OH study population

Supplemental Material, Table S1. OH serum-level results: adjusted odds ratios^a and 95% confidence intervals for cumulative PFOA serum exposure^b assuming 10-yr residency and latency

Category	Very High	High	Medium	Low
Bladder	0.6 (0.2, 1.7)	1.0 (0.6, 1.7)	1.1 (0.7, 1.6)	0.9 (0.6, 1.4)
Brain	--- ^c	0.7 (0.3, 1.8)	1.7 (1.0, 2.9)	1.5 (0.8, 2.7)
Female Breast	1.4 (0.8, 2.3)	0.7 (0.5, 0.9)	1.1 (0.8, 1.5)	0.9 (0.7, 1.2)
Cervix	0.6 (0.1, 2.7)	1.7 (0.7, 3.9)	0.6 (0.2, 1.6)	1.1 (0.6, 2.2)
Colon/Rectum	0.6 (0.3, 1.1)	1.2 (0.9, 1.6)	1.0 (0.7, 1.3)	1.0 (0.8, 1.3)
Kidney	2.1 (1.1, 4.2)	2.0 (1.3, 3.2)	1.2 (0.7, 2.0)	0.8 (0.4, 1.5)
Leukemia	0.6 (0.1, 2.4)	1.4 (0.8, 2.6)	0.7 (0.4, 1.5)	1.1 (0.6, 2.0)
Liver	--- ^c	1.0 (0.3, 3.2)	1.2 (0.5, 3.0)	0.9 (0.3, 2.8)
Lung	0.9 (0.6, 1.5)	1.2 (0.9, 1.6)	1.0 (0.8, 1.3)	1.0 (0.7, 1.2)
Melanoma of the skin	0.9 (0.4, 1.8)	1.2 (0.8, 1.9)	1.2 (0.8, 1.7)	1.1 (0.8, 1.7)
Multiple Myeloma	0.7 (0.1, 5.0)	0.7 (0.2, 2.3)	1.1 (0.5, 2.6)	1.7 (0.8, 3.6)
Non-Hodgkin's Lymphoma	2.0 (1.0, 3.7)	1.0 (0.6, 1.7)	1.5 (1.0, 2.2)	1.0 (0.6, 1.6)
Ovary	2.2 (0.9, 5.7)	1.7 (0.9, 3.4)	0.9 (0.4, 2.1)	0.7 (0.3, 1.6)
Pancreas	0.6 (0.2, 2.7)	1.3 (0.7, 2.5)	0.7 (0.3, 1.4)	1.4 (0.8, 2.4)
Prostate	1.5 (0.9, 2.5)	0.8 (0.6, 1.1)	0.8 (0.6, 1.0)	1.1 (0.8, 1.5)
Testis	2.8 (0.8, 9.6)	0.4 (0.0, 2.9)	0.4 (0.1, 1.8)	0.4 (0.1, 1.9)
Thyroid	0.9 (0.2, 3.7)	0.9 (0.3, 2.5)	0.7 (0.3, 2.0)	0.9 (0.4, 2.3)
Uterus	0.7 (0.3, 1.7)	1.6 (1.1, 2.3)	0.9 (0.6, 1.3)	1.2 (0.8, 1.7)

a. Adjusted for age, race, gender, insurance provider, and smoking status. Controls were other listed cancers excluding kidney, liver, pancreas and testis cancers.

a. Categories of modeled PFOA serum concentrations (µg/L-year): Very High=600-4679 µg/L-year; High: 198-599 µg/L-year; Medium=89-197 µg/L-year; Low=3.8-88 µg/L-year; reference: unexposed

b. No cases in exposure category

Supplemental Material, Table S2. OH serum-level results: adjusted odds ratios^a and 95% confidence intervals for annual PFOA serum exposure^b assuming 10 year residency and no latency

Category	Very High	High	Medium	Low
Bladder	0.7 (0.3, 1.6)	1.1 (0.7, 1.7)	1.0 (0.6, 1.6)	0.8 (0.5, 1.4)
Brain	--- ^c	1.1 (0.6, 2.1)	1.7 (0.9, 3.1)	1.3 (0.6, 2.7)
Female Breast	1.2 (0.7, 1.9)	0.7 (0.5, 0.9)	1.1 (0.9, 1.5)	1.0 (0.7, 1.4)
Cervix	1.0 (0.3, 2.8)	1.8 (0.9, 3.5)	0.5 (0.2, 1.3)	0.9 (0.4, 2.2)
Colon/Rectum	0.7 (0.4, 1.1)	1.2 (0.9, 1.5)	1.0 (0.7, 1.3)	1.0 (0.7, 1.3)
Kidney	1.7 (0.9, 3.3)	1.8 (1.2, 2.8)	1.1 (0.6, 1.9)	0.9 (0.5, 1.8)
Leukemia	1.1 (0.4, 2.7)	0.9 (0.5, 1.7)	0.9 (0.5, 1.7)	1.2 (0.6, 2.4)
Liver	--- ^c	0.9 (0.3, 2.6)	0.8 (0.2, 2.5)	1.3 (0.4, 4.2)
Lung	1.0 (0.7, 1.4)	1.2 (0.9, 1.5)	1.1 (0.8, 1.4)	0.9 (0.6, 1.2)
Melanoma of the skin	1.0 (0.5, 1.9)	1.0 (0.6, 1.4)	1.3 (0.9, 1.9)	1.2 (0.8, 2.0)
Multiple Myeloma	0.5 (0.1, 3.7)	0.9 (0.4, 2.3)	1.1 (0.5, 2.6)	1.9 (0.8, 4.3)
Non-Hodgkin's Lymphoma	1.8 (1.0, 3.1)	1.3 (0.9, 2.0)	1.3 (0.8, 2.0)	0.9 (0.5, 1.6)
Ovary	1.7 (0.7, 4.2)	1.7 (0.9, 3.1)	0.9 (0.4, 2.1)	0.6 (0.2, 1.8)
Pancreas	0.9 (0.3, 2.6)	1.1 (0.6, 2.1)	0.9 (0.4, 1.7)	1.2 (0.6, 2.5)
Prostate	1.3 (0.8, 2.0)	0.8 (0.6, 1.1)	0.7 (0.5, 0.9)	1.3 (0.9, 1.8)
Testis	2.2 (0.7, 6.6)	0.6 (0.2, 2.0)	0.3 (0.0, 2.9)	--- ^c
Thyroid	1.0 (0.3, 3.4)	0.5 (0.2, 1.6)	0.8 (0.3, 2.3)	1.2 (0.5, 3.0)
Uterus	0.7 (0.3, 1.5)	1.6 (1.1, 2.3)	0.9 (0.6, 1.4)	1.1 (0.7, 1.7)

b. Adjusted for age, race, gender, diagnosis year, insurance provider, and smoking status.

Controls were other listed cancers excluding kidney, liver, pancreas and testis cancers.

a. Categories of modeled PFOA serum concentrations ($\mu\text{g/L}$): Very High=110-655 $\mu\text{g/L}$; High=30.8-109 $\mu\text{g/L}$; Medium=12.9-30.7 $\mu\text{g/L}$; Low=3.7-12.8 $\mu\text{g/L}$; reference = unexposed

b. No cases in exposure category

Supplemental Material, Table S3. OH serum-level results: adjusted odds ratios^a and 95% confidence intervals for annual PFOA serum exposure^b assuming 10-yr residency and latency with alternative control group

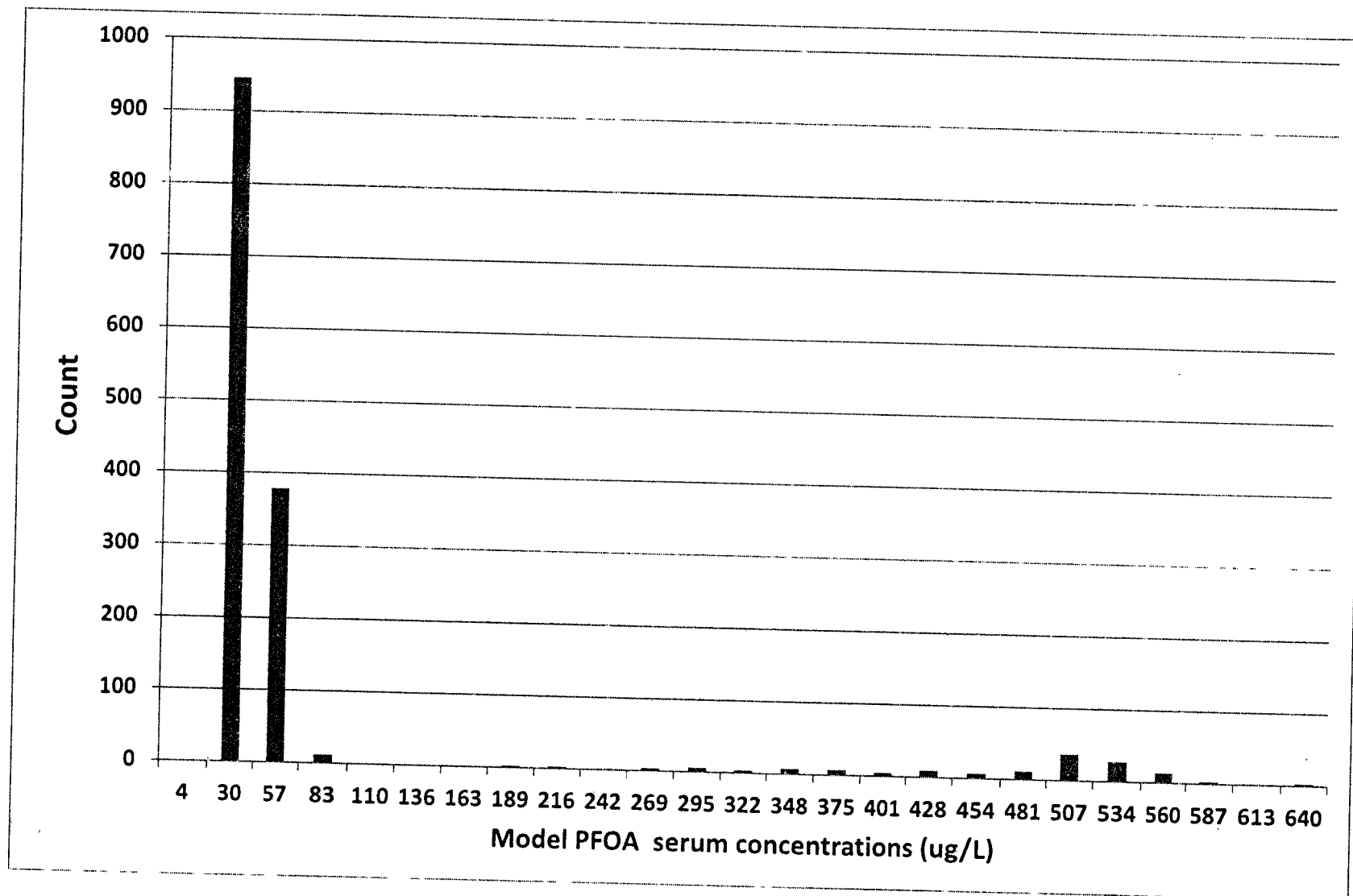
Category	Very High	High	Medium	Low
Bladder	0.6 (0.2, 1.5)	1.2 (0.8, 1.9)	0.9 (0.6, 1.4)	0.9 (0.6, 1.4)
Brain	--- ^c	0.6 (0.2, 1.6)	1.8 (1.1, 3.2)	1.5 (0.8, 2.7)
Female Breast	1.3 (0.8, 2.1)	0.7 (0.5, 0.9)	1.1 (0.8, 1.5)	0.9 (0.7, 1.2)
Cervix	0.6 (0.1, 2.6)	1.7 (0.8, 3.7)	0.5 (0.2, 1.5)	1.1 (0.6, 2.2)
Colon/Rectum	0.6 (0.3, 1.0)	1.2 (0.9, 1.6)	0.9 (0.7, 1.2)	1.0 (0.8, 1.3)
Kidney	1.9 (1.0, 3.9)	2.0 (1.3, 3.2)	1.2 (0.7, 2.0)	0.8 (0.4, 1.5)
Leukemia	0.5 (0.1, 2.2)	0.9 (0.4, 1.8)	1.0 (0.6, 1.9)	1.2 (0.7, 2.1)
Liver	--- ^c	0.9 (0.3, 3.1)	0.9 (0.3, 2.6)	1.2 (0.4, 3.3)
Lung	1.0 (0.6, 1.5)	1.2 (0.9, 1.5)	1.0 (0.8, 1.2)	1.0 (0.8, 1.3)
Melanoma of the skin	0.8 (0.4, 1.6)	0.9 (0.6, 1.5)	1.3 (0.9, 1.8)	1.2 (0.8, 1.8)
Multiple Myeloma	0.6 (0.1, 4.6)	0.9 (0.3, 2.6)	1.1 (0.5, 2.6)	1.5 (0.7, 3.2)
Non-Hodgkin's Lymphoma	1.8 (0.9, 3.3)	1.1 (0.7, 1.8)	1.5 (1.0, 2.2)	1.0 (0.6, 1.6)
Ovary	2.0 (0.8, 5.1)	1.3 (0.6, 2.8)	1.4 (0.7, 2.7)	0.5 (0.2, 1.4)
Pancreas	0.6 (0.1, 2.3)	1.1 (0.6, 2.2)	0.9 (0.5, 1.7)	1.3 (0.7, 2.3)
Prostate	1.5 (1.0, 2.5)	0.7 (0.5, 1.0)	0.8 (0.6, 1.0)	1.1 (0.8, 1.5)
Testis	2.4 (0.7, 7.5)	0.3 (0.0, 2.9)	0.6 (0.2, 2.3)	0.2 (0.0, 1.5)
Thyroid	0.8 (0.2, 3.3)	0.7 (0.2, 2.1)	0.9 (0.4, 2.3)	0.9 (0.4, 2.3)
Uterus	0.6 (0.3, 1.5)	1.6 (1.1, 2.4)	0.9 (0.6, 1.3)	1.2 (0.8, 1.7)

- Adjusted for age, gender, race, diagnosis year, insurance provider, and smoking status. Cases of the other 17 cancer types were used as controls.
- Categories of modeled PFOA serum concentrations ($\mu\text{g/L}$): Very High=110-655 $\mu\text{g/L}$; High=30.8-109 $\mu\text{g/L}$; Medium=12.9-30.7 $\mu\text{g/L}$; Low=3.7-12.8 $\mu\text{g/L}$; reference = unexposed
- No cases in exposure category

Supplemental Material, Table S4. OH serum-level results: adjusted odds ratios^a and 95% confidence intervals for annual PFOA serum exposure^b assuming 10-yr residency and latency with multiple imputations^c for missing data

Category	Very High	High	Medium	Low
Bladder	0.4 (0.1, 1.7)	1.3 (0.7, 2.4)	0.8 (0.4, 1.5)	0.9 (0.5, 1.5)
Female Breast	1.2 (0.6, 2.3)	0.9 (0.5, 1.4)	0.9 (0.6, 1.4)	0.9 (0.6, 1.3)
Colon/Rectum	0.7 (0.3, 1.3)	1.1 (0.8, 1.7)	0.6 (0.4, 1.0)	1.1 (0.8, 1.5)
Kidney	2.0 (0.3, 3.3)	2.3 (1.3, 4.1)	0.9 (0.4, 2.0)	0.6 (0.3, 1.4)
Lung	1.2 (0.6, 2.2)	1.6 (1.1, 2.4)	1.3 (0.9, 1.9)	0.9 (0.7, 1.3)
Melanoma of the Skin	0.9 (0.6, 1.3)	1.0 (0.8, 1.3)	1.5 (1.3, 1.8)	1.1 (0.9, 1.4)
Non-Hodgkin's Lymphoma	2.1 (1.0, 4.5)	0.8 (0.4, 1.8)	1.6 (0.9, 2.8)	1.1 (0.7, 1.9)
Prostate	1.9 (1.0, 3.6)	0.6 (0.4, 1.1)	1.0 (0.7, 1.7)	1.3 (0.9, 1.9)
Uterus	0.9 (0.3, 3.0)	1.0 (0.5, 2.1)	0.6 (0.2, 1.6)	0.9 (0.5, 1.7)

- c. Adjusted for age, race, gender, diagnosis year, insurance provider, and smoking status. Controls were other listed cancers excluding kidney, liver, pancreas and testis cancers.
- d. Categories of modeled PFOA serum concentrations ($\mu\text{g/L}$): Very High=110-655 $\mu\text{g/L}$; High=30.8-109 $\mu\text{g/L}$; Medium=12.9-30.7 $\mu\text{g/L}$; Low=3.7-12.8 $\mu\text{g/L}$; reference = unexposed
- e. Multiple imputations were only run for cancers with sufficient number of cases (≥ 100 cases with complete covariate information)



Supplemental Material, Figure S1. Histogram of serum estimates assuming a 10-yr residency and latency among the exposed OH study population

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