

Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12–19 y: National Health and Nutrition Examination Survey

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BACKGROUND: Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are immunotoxic in laboratory studies. Human studies of immune effects are inconsistent. Using the US National Health and Nutrition Examination Survey (NHANES), we examined PFAS serum concentration and indicators of prevalent immune function among 12–19-y-old children.

METHODS: In this cross-sectional study, we examined PFAS serum concentration in relation to measles, mumps, and rubella antibody concentrations in NHANES 1999–2000 and 2003–2004 ($n = 1,191$) and to allergic conditions and allergic sensitization in NHANES 2005–2006 ($n = 640$).

RESULTS: In adjusted, survey-weighted models, a doubling of perfluorooctane sulfonate (PFOS) concentration among seropositive children was associated with a 13.3% (95% confidence interval (CI): –19.9, –6.2) decrease in rubella antibody concentration and a 5.9% decrease in mumps antibody concentration (95% CI: –9.9, –1.6). We observed no adverse association between exposure and current allergic conditions, including asthma. Children with higher PFOS concentration were less likely to be sensitized to any allergen (odds ratio (OR): 0.74; 95% CI: 0.58, 0.95).

CONCLUSION: Increased exposure to several PFAS was associated with lower levels to mumps and rubella antibody concentrations, especially among seropositive individuals. These lower antibody concentrations may indicate a less robust response to vaccination or greater waning of vaccine-derived immunity over time.

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been widely used since the 1950s as surfactants, surface treatment chemicals, and processing aids for many products, including oil, stain, grease, and water repellent coatings on carpet, textiles, leather, and paper (1). Human exposure typically occurs through transfer from food packaging and preparation materials, bioaccumulation in the food chain, and household dust (2). Some PFAS are persistent organic pollutants (3) and detected worldwide in wildlife and humans (4).

The US National Health and Nutrition Examination Survey (NHANES) began PFAS biomonitoring in the 1999–2000 survey (5). The two most commonly studied PFAS—perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA)—were detected in all serum samples with geometric mean concentrations of 5.21 and 30.4 ng/ml, respectively (5). Measurable levels of PFAS also have been found in amniotic fluid (6,7), maternal and umbilical cord blood (8,9), and breast milk (10,11). While efforts to reduce US production and use of these compounds (12,13) have led to declining serum concentrations, compounds such as perfluorononanoate (PFNA) have been increasing in both percent of the population with detectable levels and average serum concentration (14). Many PFAS have long biological half-lives. The serum elimination half-life is estimated at 2–4 y for PFOA (15,16), 5 y for PFOS (16), and 8.5 y for perfluorohexane sulfonate (PFHxS) (16). Given their long half-lives and worldwide dispersion, exposure even to the legacy compounds will likely persist for some time.

Toxicological studies underscore the immunotoxic potential of PFAS. PFOS and PFOA alter inflammatory responses, cytokine expression, and adaptive and innate immune responses in rodent, avian, and reptilian models as well as in mammalian and nonmammalian wildlife (17). Immune effects have been observed in laboratory models at serum concentrations close to those in highly exposed humans and wildlife (17). These immune effects appear to work through numerous pathways including activation of peroxisome proliferator-activated receptor- α , which can be anti-inflammatory (17) and activation of nuclear factor- κ B, which can suppress cytokine secretion by immune cells (18). Mice exposed to PFOA exhibited increased IgE response after exposure to environmental allergens (19). In a separate murine model, neither PFOA nor PFOS appeared to be risk factors for allergic asthma-like symptoms even though PFOA induced airway inflammation and altered airway function (20). In human cells, PFOA triggered mast cell allergic reactions by histamine release and expression of pro-inflammatory cytokines (21). A recent study of human cord blood gene expression provided support for immune effects

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mediated through peroxisome proliferator-activated receptor and nuclear factor- κ B (22).

Epidemiological evidence of PFAS exposure and immune perturbation is mixed. Several studies examined associations between PFAS concentrations and various markers and measures of immune function from birth through 10 y of age. BraMat ($n = 99$), a subcohort of the Norwegian Mother and Child Study, reported inverse associations between prenatal PFAS levels and serum antibody concentrations against rubella at age 3 (23). A Faroe Islands birth cohort ($n = 587$) reported inverse associations between prenatal PFAS concentration and tetanus and diphtheria toxoids at ages 5 and 7 (24). A separate Faroese cohort ($n = 38$) observed a negative association between prenatal PFOS and the autoantibody anti-actin IgG at age 7, although the clinical meaning of anti-actin IgG is unknown (25).

Three studies reported divergent associations between PFAS and serum IgE levels. In Japan ($n = 343$), prenatal PFOA was negatively associated with cord blood IgE among female infants (26). In Taiwan ($n = 244$), cord blood PFOS and PFOA were positively associated with cord blood IgE among male infants (27). A Taiwanese case-control study ($n = 456$) reported positive associations between higher PFAS exposure and serum IgE among 10–15-y-old asthmatics; nonasthmatic controls were not tested (28).

In examinations of clinical disease, the Japanese study found no relation between exposures and allergic disease or otitis media at age 18 mo despite the elevated IgE levels at birth (26). BraMat reported that higher maternal PFAS levels at delivery were associated with increased risk of common cold (PFOA, PFNA) and gastroenteritis (PFOA, PFHxS) in children up to age 3; no associations were found with allergy or asthma-related outcomes (23). A subset of the Danish National Birth Cohort ($n = 1,400$) observed no clear pattern between prenatal PFOS and PFOA exposure and risk for infectious disease hospitalizations in childhood (29). INUENDO reported a reduced risk of wheeze in unadjusted models among 5–9-y-old Ukrainian children ($n = 492$) with higher prenatal PFOS exposure (30). The Taiwanese case-control study reported increased risk of asthma among those with the highest PFAS serum concentrations, ranging from an odds ratio (OR) of 1.81 (95% confidence interval (CI): 1.02–3.23) for perfluorododecanoic acid (PFDoA) to an OR of 4.05 (95% CI: 2.21–7.42) for PFOA (28).

Recently, Humblet *et al.* (31), using four waves of NHANES for children aged 12–19 y ($n = 1,877$), also found a positive association between PFOA serum concentration and report of ever being diagnosed with asthma. In this cross-sectional study, a doubling of PFOA serum concentration yielded an OR of 1.18 (95% CI: 1.01–1.39). When the model accounted for NHANES survey weights, however, the magnitude of effect was diminished (OR: 1.11; 95% CI: 0.87–1.42). The measure of ever receiving an asthma diagnosis was selected because of the long half-life of many PFAS (31), but the validity of this measure in a cross-sectional study of 12–19-y olds depends on the hypothesized window of susceptibility.

To further explore possible links between PFAS exposure and perturbations of the immune system, we undertook a comprehensive examination of PFAS serum concentration and several indicators of immune function among children aged 12–19 y using multiple waves of NHANES. We examined PFAS serum concentration in relation to measles, mumps, and rubella antibody concentration levels in NHANES 1999–2000 and 2003–2004 and to allergic conditions and allergic sensitization in NHANES 2005–2006. In this cross-sectional study, we focused exclusively on prevalent outcomes, which is the time period most relevant to prevalent exposure measures.

RESULTS

Vaccine Study

The Vaccine Study included 1,191 children with PFAS serum concentrations ranging from geometric mean 0.76 ng/ml (95% CI: 0.65, 0.90) for PFNA to 20.8 ng/ml (95% CI: 19.1, 22.7) for PFOS (Table 1). Less than 1% of the PFAS were below the limit of detection (LOD) (PFOS 0%, PFOA 0.01%, PFHxS 0.14%, PFNA 0.17%). Pairwise correlations among PFAS were moderate to high (Supplementary Table S1 online) except for PFHxS and PFNA ($r = 0.14$, $P < 0.001$). In adjusted models, there was no association between any of the PFAS and measles antibody levels (Table 2). A doubling of PFOS was associated with a 7.4% (95% CI: –12.8, –1.7) decrease in mumps antibodies. This association was similar when restricted to seropositive children (–5.9%; 95% CI: –9.9, –1.6). In the seropositive model, there was also a 6.6% (95% CI: –11.7, –1.5) decrease in mumps antibodies with a doubling of PFOA. The largest decrease in antibody concentrations was for rubella, although only among the seropositive subset. A doubling of PFOS was associated with a 13.3% (95% CI: –19.9, –6.2) decrease in rubella antibodies. Decreases in rubella antibodies were also present for PFOA and PFHxS. Similar associations were observed in models with categorical treatment of PFAS (results not shown). In adjusted models, age was associated with antibody concentration level only for measles. In age-stratified models, the associations between PFAS and antibody concentration levels were not substantively different between 12–15-y olds and 16–19-y olds (results not shown).

Allergy Study

The allergy study included 640 children with serum concentrations ranging from geometric mean 0.93 ng/ml (95% CI: 0.78, 1.1) for PFNA to 15.0 ng/ml (95% CI: 14.3, 15.7) for PFOS (Table 3). Overall 11.3% of children reported current asthma, 20.4% reported current allergies, and 45.9% were sensitized to at least one of 19 measured allergens. Less than 1% of the PFAS were below the LOD (PFOS 0%, PFOA 0.01%, PFHxS 0.51%, PFNA 0.12%). Pairwise correlations among PFAS were moderate to high except for PFHxS and PFNA (Supplementary Table S2 online; $r = 0.15$, $P < 0.0001$). In adjusted models, the pattern of association between PFAS exposures and current allergic conditions appeared null for both continuous (Table 4) and categorical (results not shown) treatment of PFAS. The strongest finding, as well as the only statistically significant finding,

Table 1. Geometric mean (95% confidence interval) perfluoroalkyl and polyfluoroalkyl substances concentrations (ng/ml) by vaccine antibody study characteristics among children aged 12–19 y, 1999–2000, and 2003–2004 National Health and Nutrition Examination Surveys, *n* = 1,191

	<i>N</i>	PFOS ng/ml		PFOA ng/ml		PFHxS ng/ml		PFNA ng/ml	
		GeoMean	95% CI	GeoMean	95% CI	GeoMean	95% CI	GeoMean	95% CI
Overall	1,191	20.8	(19.1, 22.7)	4.13	(3.76, 4.53)	2.47	(2.15, 2.85)	0.765	(0.648, 0.903)
Survey year									
1999–2000	551	29.0	(26.3, 31.8)	5.41	(5.00, 5.85)	2.64	(2.14, 3.26)	0.471	(0.411, 0.54)
2003–2004	640	19.3	(17.6, 21.3)	3.89	(3.49, 4.33)	2.44	(2.06, 2.88)	0.852	(0.703, 1.03)
Age, years									
12–15	587	20.9	(18.8, 23.3)	4.08	(3.69, 4.51)	2.64	(2.21, 3.16)	0.734	(0.620, 0.868)
16–19	604	20.7	(18.9, 22.6)	4.18	(3.77, 4.63)	2.32	(1.98, 2.71)	0.798	(0.668, 0.953)
Sex									
Male	626	22.4	(20.2, 24.7)	4.69	(4.26, 5.16)	2.71	(2.25, 3.27)	0.852	(0.711, 1.02)
Female	565	19.3	(17.6, 21.0)	3.60	(3.24, 4.00)	2.24	(1.94, 2.60)	0.682	(0.583, 0.799)
Race/ethnicity									
Non-Hispanic white	278	22.0	(19.6, 24.7)	4.32	(3.88, 4.81)	2.77	(2.29, 3.35)	0.812	(0.703, 0.938)
Non-Hispanic black	364	21.0	(18.3, 24.1)	4.14	(3.75, 4.57)	2.61	(2.12, 3.21)	0.886	(0.652, 1.21)
Mexican American	447	16.5	(15.2, 18.0)	3.55	(3.28, 3.84)	1.73	(1.54, 1.95)	0.542	(0.477, 0.617)
Other	102	19.4	(16.2, 23.2)	3.79	(3.06, 4.69)	1.86	(1.48, 2.33)	0.668	(0.465, 0.959)
Measles seroconversion									
Positive	1,152	20.8	(19.1, 22.7)	4.15	(3.77, 4.56)	2.46	(2.13, 2.84)	0.768	(0.649, 0.909)
Negative	38	20.2	(15.6, 26.2)	3.56	(2.84, 4.46)	2.78	(1.55, 4.99)	0.674	(0.465, 0.977)
Mumps seroconversion									
Positive	1,101	20.7	(18.9, 22.6)	4.13	(3.73, 4.56)	2.46	(2.12, 2.86)	0.765	(0.644, 0.909)
Negative	85	22.1	(18.8, 25.9)	4.15	(3.79, 4.54)	2.59	(1.81, 3.70)	0.781	(0.646, 0.944)
Rubella seroconversion									
Positive	1,148	20.9	(19.2, 22.8)	4.16	(3.78, 4.57)	2.48	(2.16, 2.84)	0.768	(0.647, 0.912)
Negative	42	18.6	(14.0, 24.8)	3.64	(3.01, 4.40)	2.36	(1.32, 4.21)	0.701	(0.608, 0.807)

CI, confidence interval; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate.

Table 2. Adjusted percent change (95% confidence interval) in measles, mumps, and rubella antibody titer with a doubling in perfluoroalkyl and polyfluoroalkyl substances serum concentration among children aged 12–19 y, National Health and Nutrition Examination Survey, 1999–2000 and 2003–2004

	Measles		Mumps		Rubella	
	All (<i>n</i> = 1,188)	Seropositive (<i>n</i> = 1,152)	All (<i>n</i> = 1,186)	Seropositive (<i>n</i> = 1,101)	All (<i>n</i> = 1,190)	Seropositive (<i>n</i> = 1,148)
Perfluoroalkyl and polyfluoroalkyl substances						
PFOS	–3.5 (–18.3, 14.0)	–2.9 (–17.3, 13.9)	–7.4 (–12.8, –1.7)	–5.9 (–9.9, –1.6)	–8.4 (–17.9, 2.1)	–13.3 (–19.9, –6.2)
PFOA	–0.1 (–13.8, 15.6)	–3.4 (–16.7, 11.9)	–6.0 (–12.4, 0.9)	–6.6 (–11.7, –1.5)	–2.5 (–9.1, 5.3)	–8.9 (–14.6, –2.9)
PFHxS	–3.8 (–11.5, 4.6)	–2.8 (–10.1, 5.21)	–2.6 (–6.7, 1.7)	–2.3 (–5.5, 0.9)	–5.0 (–10.8, 1.2)	–6.0 (–9.6, –2.2)
PFNA	1.8 (–11.4, 17.1)	1.1 (–11.8, 15.9)	–2.7 (–7.2, 2.0)	–2.7 (–8.4, 3.4)	3.2 (–5.3, 12.6)	0.6 (–6.7, 8.5)

Adjusted for age (continuous), sex, race/ethnicity, survey year.

PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate.

was for increased PFOA and prevalent rhinitis (interquartile range (IQR) OR: 1.35; 95% CI: 1.10, 1.66).

Children with higher PFOS serum concentration were less likely to be sensitized to any allergen (IQR OR: 0.74; 95% CI: 0.58, 0.95; **Table 5**). Specifically, children with higher levels to some PFAS were less likely to be sensitized to plants (PFOS IQR OR: 0.71; 0.53, 0.97) and cockroach or shrimp (PFOS

IQR OR: 0.67; 95% CI: 0.48, 0.93; PFHxS IQR OR: 0.72; 95% CI: 0.56, 0.93). Children with higher PFOS levels, however, were more likely to be sensitized to mold (IQR OR: 1.33; 95% CI: 1.06, 1.69). Categorical treatment of PFAS endorsed the observed associations for any (PFOS *P*-trend = 0.02), cockroach or shrimp (PFOS *P*-trend = 0.003), and mold sensitizations (PFOS *P*-trend = 0.04; **Figures 1–3**). The association

Table 3. Geometric mean (95% confidence interval) perfluoroalkyl and polyfluoroalkyl substances concentrations (ng/ml) by allergic conditions and allergic sensitization study characteristics among children aged 12–19 y, 2005–2006 National Health and Nutrition Examination Survey, *n* = 640

	N	PFOS ng/ml		PFOA ng/ml		PFHxS ng/ml		PFNA ng/ml	
		GeoMean	95% CI	GeoMean	95% CI	GeoMean	95% CI	GeoMean	95% CI
Overall	640	15.0	(14.3, 15.7)	3.59	(3.26, 3.96)	2.09	(1.74, 2.52)	0.929	(0.782, 1.1)
Age, years									
12–15	313	13.7	(12.9, 14.6)	3.42	(3.10, 3.78)	1.85	(1.45, 2.37)	0.917	(0.740, 1.14)
16–19	321	16.4	(15.2, 17.6)	3.77	(3.36, 4.22)	2.36	(1.81, 3.08)	0.941	(0.774, 1.14)
Sex									
Male	328	16.5	(15.2, 17.9)	3.95	(3.57, 4.37)	2.15	(1.78, 2.61)	0.985	(0.847, 1.15)
Female	312	13.6	(12.1, 15.3)	3.26	(2.81, 3.77)	2.03	(1.54, 2.68)	0.875	(0.699, 1.10)
Race/ethnicity									
Non-Hispanic white	160	16.4	(15.0, 18.1)	3.85	(3.44, 4.3)	2.39	(1.96, 2.91)	0.937	(0.765, 1.15)
Non-Hispanic black	220	14.3	(12.8, 16.1)	3.23	(2.64, 3.94)	1.79	(1.25, 2.56)	0.966	(0.828, 1.13)
Mexican American	212	10.6	(9.54, 11.8)	2.95	(2.65, 3.27)	1.57	(1.10, 2.23)	0.820	(0.685, 0.981)
Other	48	13.3	(11.5, 15.4)	3.44	(2.96, 4.00)	1.6	(1.04, 2.47)	0.958	(0.687, 1.33)
Weight status									
Under or target weight	394	15.8	(14.7, 17.0)	3.7	(3.33, 4.09)	2.26	(1.83, 2.78)	0.947	(0.803, 1.12)
Overweight	93	13.2	(11.1, 15.8)	3.23	(2.60, 4.01)	1.99	(1.32, 2.99)	0.876	(0.690, 1.11)
Obese	150	13.5	(11.5, 15.9)	3.48	(3.07, 3.94)	1.68	(1.05, 2.69)	0.902	(0.693, 1.18)
Serum cotinine (ng/ml)									
Below LOD (<0.015)	93	13.3	(11.6, 15.3)	3.49	(3.00, 4.05)	1.56	(1.09, 2.22)	0.926	(0.724, 1.18)
Low (0.015–10)	460	14.6	(13.8, 15.5)	3.41	(3.05, 3.83)	2.08	(1.73, 2.50)	0.891	(0.747, 1.06)
High (>10)	87	18.5	(16.1, 21.3)	4.54	(3.79, 5.43)	2.84	(1.83, 4.40)	1.10	(0.891, 1.36)
Current allergic condition									
Asthma									
No	570	14.9	(14.2, 15.7)	3.56	(3.23, 3.92)	2.11	(1.72, 2.59)	0.917	(0.768, 1.10)
Yes	70	15.5	(13.4, 17.9)	3.86	(3.26, 4.56)	1.95	(0.883, 4.29)	1.03	(0.832, 1.26)
Wheeze									
No	568	15.1	(14.2, 16.0)	3.58	(3.25, 3.95)	2.08	(1.68, 2.58)	0.927	(0.789, 1.09)
Yes	72	14.2	(11.1, 18.1)	3.66	(2.77, 4.83)	2.21	(1.70, 2.88)	0.944	(0.665, 1.34)
Allergy									
No	537	14.8	(13.9, 15.8)	3.55	(3.22, 3.90)	2.11	(1.73, 2.57)	0.918	(0.758, 1.11)
Yes	102	15.5	(14.3, 16.8)	3.75	(3.24, 4.34)	1.99	(1.34, 2.96)	0.969	(0.846, 1.11)
Rhinitis									
No	476	14.6	(13.7, 15.5)	3.45	(3.09, 3.85)	2.16	(1.79, 2.61)	0.897	(0.736, 1.09)
Yes	164	16.0	(14.4, 17.8)	3.95	(3.60, 4.33)	1.94	(1.34, 2.81)	1.01	(0.881, 1.15)
Allergic sensitization									
Any									
No	297	15.8	(14.7, 17.0)	3.64	(3.23, 4.11)	2.20	(1.68, 2.87)	0.917	(0.733, 1.15)
Yes	343	14.0	(13.1, 15.1)	3.53	(3.21, 3.88)	1.97	(1.53, 2.54)	0.943	(0.818, 1.09)
Plants									
No	413	15.6	(14.6, 16.7)	3.66	(3.29, 4.07)	2.16	(1.73, 2.71)	0.936	(0.76, 1.15)
Yes	227	13.6	(12.4, 15.0)	3.44	(3.09, 3.83)	1.94	(1.34, 2.79)	0.913	(0.811, 1.03)
Dust mites									
No	462	15.0	(14.1, 15.9)	3.61	(3.29, 3.95)	2.10	(1.72, 2.56)	0.922	(0.765, 1.11)
Yes	178	15.0	(13.5, 16.8)	3.54	(3.09, 4.05)	2.08	(1.58, 2.74)	0.952	(0.774, 1.17)
Pets									
No	491	15.1	(14.3, 16.0)	3.52	(3.16, 3.93)	2.11	(1.73, 2.56)	0.902	(0.73, 1.11)
Yes	149	14.6	(12.7, 16.6)	3.84	(3.41, 4.32)	2.05	(1.46, 2.86)	1.03	(0.899, 1.18)
Cockroach or shrimp									
No	514	15.4	(14.5, 16.4)	3.65	(3.29, 4.05)	2.23	(1.86, 2.67)	0.937	(0.784, 1.12)
Yes	126	12.5	(11.0, 14.2)	3.25	(2.86, 3.70)	1.44	(0.924, 2.25)	0.881	(0.698, 1.11)
Rodents									
No	612	15.0	(14.2, 15.9)	3.56	(3.22, 3.95)	2.11	(1.75, 2.55)	0.915	(0.762, 1.1)
Yes	28	14.3	(8.43, 24.4)	4.34	(3.08, 6.10)	1.65	(0.966, 2.82)	1.35	(0.94, 1.95)
Mold									
No	535	14.6	(13.9, 15.4)	3.54	(3.20, 3.93)	2.09	(1.69, 2.58)	0.91	(0.745, 1.11)
Yes	105	17.0	(15.4, 18.8)	3.86	(3.33, 4.47)	2.11	(1.44, 3.11)	1.04	(0.863, 1.25)
Food									
No	583	15.1	(14.3, 16.0)	3.58	(3.23, 3.97)	2.09	(1.73, 2.53)	0.929	(0.777, 1.11)
Yes	57	13.4	(10.2, 17.6)	3.67	(3.00, 4.50)	2.10	(1.49, 2.95)	0.923	(0.708, 1.2)

CI, confidence interval; LOD, limit of detection; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate.

Table 4. Adjusted associations (OR (95% CI)) between perfluoroalkyl and polyfluoroalkyl substances serum concentration and current asthma and allergic conditions among children aged 12–19 y, National Health and Nutrition Examination Survey, 2005–2006, *n* = 638

Perfluoroalkyl and polyfluoroalkyl substances	Asthma	Wheeze	Allergy	Rhinitis
PFOS	1.20 (0.88, 1.63)	0.76 (0.45, 1.29)	1.05 (0.80, 1.37)	1.16 (0.90, 1.50)
PFOA	1.28 (0.81, 2.04)	0.94 (0.51, 1.73)	1.12 (0.85, 1.47)	1.35 (1.10, 1.66)
PFHxS	0.98 (0.51, 1.87)	0.99 (0.68, 1.44)	0.83 (0.59, 1.17)	0.81 (0.57, 1.16)
PFNA	1.26 (0.79, 2.01)	0.99 (0.58, 1.68)	1.12 (0.85, 1.47)	1.24 (0.97, 1.60)

Adjusted for age (continuous), gender, race, weight status, serum cotinine.

Effect estimates represent the odds ratio for the outcome with a shift from the 25th percentile to the 75th percentile in perfluoroalkyl and polyfluoroalkyl substances serum levels. (IQR [lnPFOS] = 0.76; IQR [lnPFOA] = 0.78; IQR [lnPFHxS] = 1.46; IQR [lnPFNA] = 0.84).

CI, confidence interval; IQR, interquartile range; OR, odds ratio; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate.

Table 5. Adjusted associations (OR (95% CI)) between perfluoroalkyl and polyfluoroalkyl substances serum concentration and allergic sensitization (slgE ≥ 0.35 kU/l) among children aged 12–19 y, National Health and Nutrition Examination Survey, 2005–2006, *n* = 638

Perfluoroalkyl and polyfluoroalkyl substances	Allergic sensitization (slgE ≥ 0.35 kU/l)							
	Any	Plants	Dust mites	Pets	Cockroach, shrimp	Rodents	Mold	Food
PFOS	0.74 (0.58, 0.95)	0.71 (0.53, 0.97)	1.00 (0.73, 1.38)	0.83 (0.56, 1.22)	0.67 (0.48, 0.93)	0.85 (0.29, 2.45)	1.33 (1.06, 1.69)	0.74 (0.39, 1.40)
PFOA	0.93 (0.72, 1.21)	0.88 (0.67, 1.15)	0.93 (0.75, 1.16)	1.17 (0.81, 1.68)	0.79 (0.55, 1.13)	1.65 (0.59, 4.60)	1.21 (0.85, 1.72)	1.02 (0.60, 1.73)
PFHxS	0.92 (0.66, 1.28)	0.93 (0.62, 1.39)	1.01 (0.84, 1.22)	0.96 (0.71, 1.30)	0.72 (0.56, 0.93)	0.81 (0.54, 1.21)	0.98 (0.65, 1.47)	1.03 (0.74, 1.42)
PFNA	1.04 (0.80, 1.35)	0.96 (0.74, 1.23)	1.05 (0.78, 1.41)	1.26 (0.85, 1.87)	0.86 (0.60, 1.24)	2.25 (0.83, 6.10)	1.31 (0.83, 2.06)	0.91 (0.55, 1.50)

Adjusted for age (continuous), gender, race, weight status, serum cotinine.

Effect estimates represent the odds ratio for the outcome with a shift from the 25th percentile to the 75th percentile in perfluoroalkyl and polyfluoroalkyl substances serum levels. (IQR [lnPFOS] = 0.76; IQR [lnPFOA] = 0.78; IQR [lnPFHxS] = 1.46; IQR [lnPFNA] = 0.84).

CI, confidence interval; IQR, interquartile range; OR, odds ratio; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate.

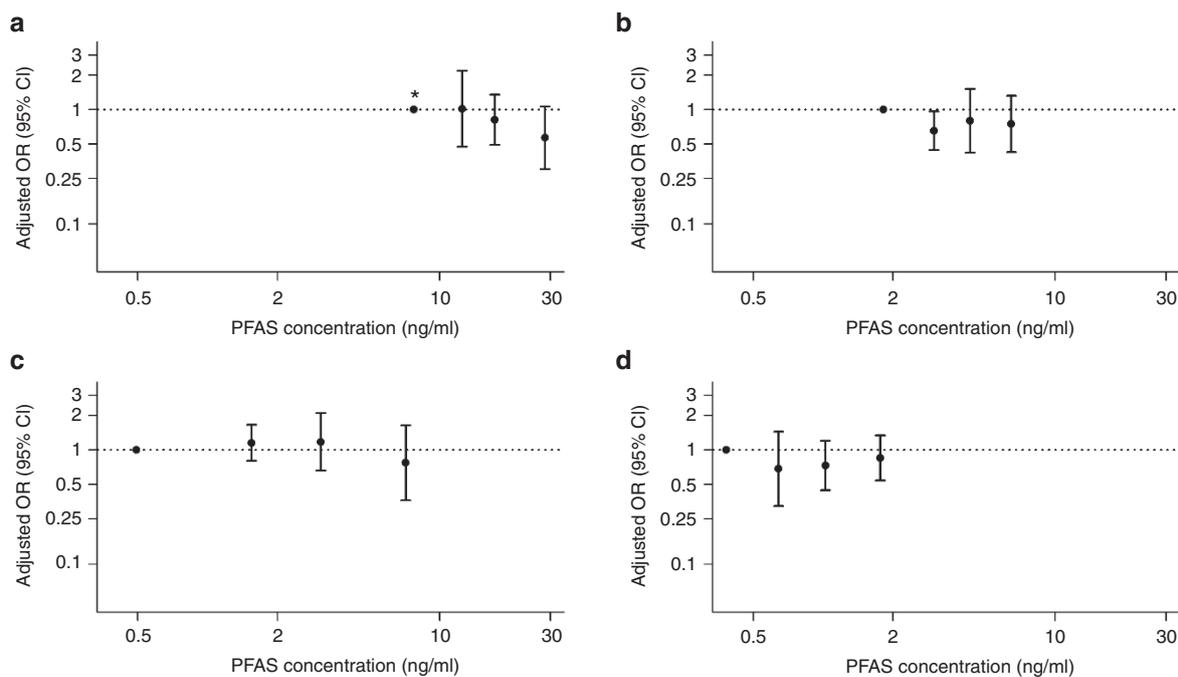


Figure 1. Adjusted odds ratio (95% confidence interval) for any IgE sensitization (IgE ≥ 0.35 kU/l) among children aged 12–19 y, National Health and Nutrition Examination Survey, 2005–2006 (*n* = 638). Perfluoroalkyl and polyfluoroalkyl substances biomarker concentrations plotted at the median by quartile of exposure. Adjusted for age, sex, race/ethnicity, BMI, cotinine, and survey weights. (a) Perfluorooctane sulfonate, **P* for trend < 0.05. (b) Perfluorooctanoate. (c) Perfluorohexane sulfonate. (d) Perfluorononanoate.

with mold appears to be driven by *Alternaria*; a doubling of PFOS was associated with a 7.5% (95% CI: 0.5, 15.1) increase in *Alternaria* IgE antibodies. Despite no other meaningful

associations with sensitization to any allergen-specific antibodies, children with higher PFOA and PFNA levels had higher total IgE antibodies (Table 6).

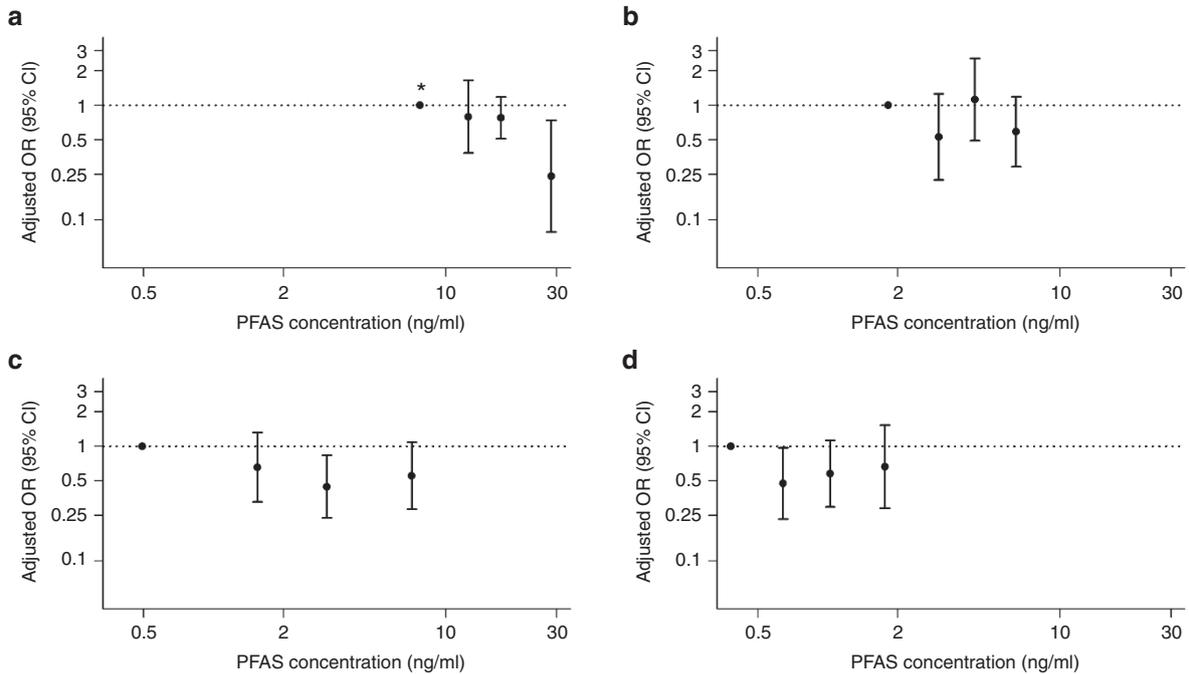


Figure 2. Adjusted odds ratio (95% confidence interval) for cockroach or shrimp IgE sensitization (IgE ≥0.35 kU/l) among children aged 12–19 y, National Health and Nutrition Examination Survey, 2005–2006 (n = 638). Perfluoroalkyl and polyfluoroalkyl substances biomarker concentrations plotted at the median by quartile of exposure. Adjusted for age, sex, race/ethnicity, BMI, cotinine, and survey weights. (a) Perfluorooctane sulfonate, *P for trend < 0.05. (b) Perfluorooctanoate. (c) Perfluorohexane sulfonate. (d) Perfluorononanoate.

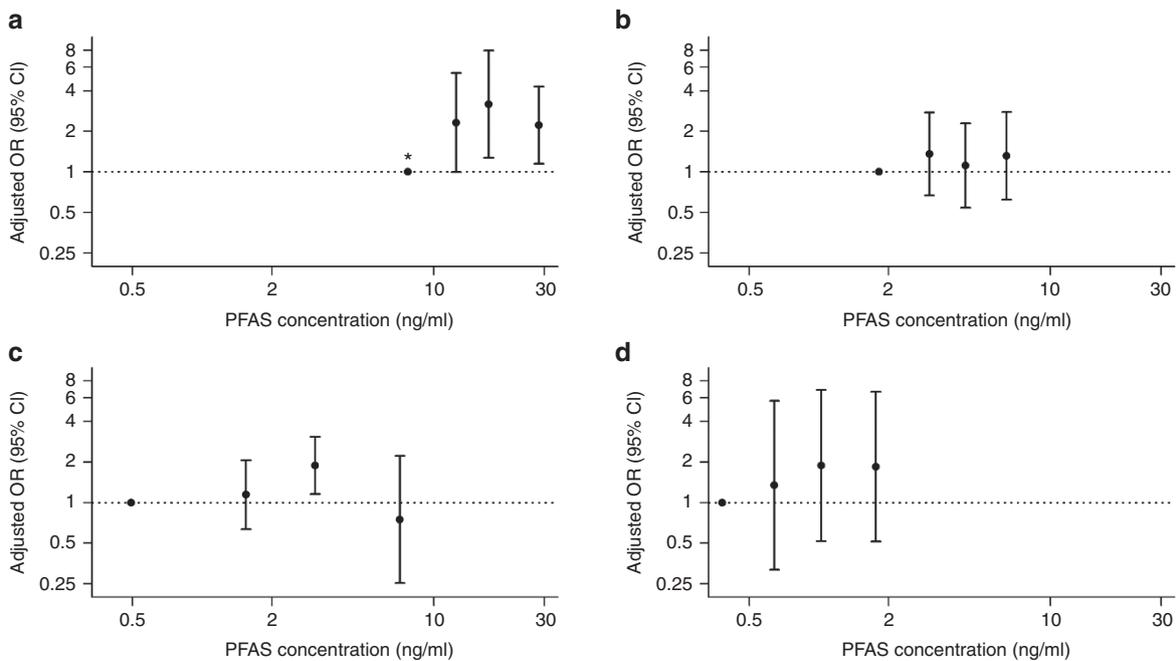


Figure 3. Adjusted odds ratio (95% confidence interval) for mold (*Alternaria* and *Aspergillus* species) IgE sensitization (IgE ≥ 0.35 kU/l) among children aged 12–19 y, National Health and Nutrition Examination Survey, 2005–2006 (n = 638). Perfluoroalkyl and polyfluoroalkyl substances biomarker concentrations plotted at the median by quartile of exposure. Adjusted for age, sex, race/ethnicity, BMI, cotinine, and survey weights. (a) Perfluorooctane sulfonate, *P for trend < 0.05. (b) Perfluorooctanoate. (c) Perfluorohexane sulfonate. (d) Perfluorononanoate.

DISCUSSION

We observed that PFAS serum concentration was related to allergen-specific IgE sensitization. To our knowledge, this is the first such report. Children with higher PFAS exposure

were less likely to have IgE sensitization to any of 19 allergens (PFOS) and specifically to plants (PFOS) and cockroaches or shrimp (PFOS, PFHxS). Children with higher PFOS exposure, however, were more likely to be sensitized to mold. Curiously,

although PFOA and PFNA were not related to most measured allergen-specific IgEs or to report of current asthma or allergies, a doubling of these exposures was associated with 10 and 19% increases in total IgE, respectively. This association between higher exposure and higher total IgE, but not between higher exposure and clinical disease, is interesting because higher total IgE levels are typically indicative of asthma and allergies (32). This seemingly paradoxical observation may reflect our reliance on self-reported allergic conditions, be due to unmeasured confounding, or be an artifact of our study's cross-sectional design. For instance, an analysis of the longitudinal Multi-centre Allergy Study showed that an increase in total IgE during childhood was temporally related to onset of respiratory allergies (33). In this cross-sectional study of serum PFAS concentrations and indicators of immune function among children aged 12–19, we observed no adverse association between exposure and current allergic conditions, including asthma.

In relation to vaccine antibody concentration levels, higher PFAS exposure was associated with lower antibody concentrations to mumps and rubella, but not measles. The validity of this finding was strengthened by the observation that the association between PFAS concentration and antibody concentration was comparable among 12–15- and 16–19-y olds. Lower antibody concentration levels, especially among seropositive individuals, may indicate a less robust response to vaccination or greater waning of vaccine-derived immunity over time.

Five studies have examined allergic conditions in relation to PFAS exposures, with results divided between protective/null (23,26,30) and adverse (28,31) effects. In a Taiwanese case-control study of children aged 10–15 y, Dong *et al.* (28) reported elevated ORs for asthma at the highest compared to lowest quartile of exposure for PFOS, PFOA, PFHxS, and PFNA. In this population, PFOS levels were higher and PFOA levels were lower as compared to our population. Additionally, asthma cases were recruited from hospitals and controls had no personal or family history of asthma. This recruitment strategy likely resulted in less outcome misclassification than the questionnaire data available for our study. Humblet *et al.* (31), however, reported a small, adverse association between PFOA concentration and lifetime asthma diagnosis in NHANES,

which became null when adjusting for NHANES complex survey design. Only lifetime asthma diagnosis—not current asthma—was statistically significant. In a cross-sectional study such as NHANES, it is uncertain that a contemporary measure of PFAS relates to a past diagnosis with asthma.

The Taiwanese case-control study also reported adverse associations between PFOS and PFOA and serum IgE among asthma cases (28), which suggests that PFAS may be related to allergic inflammation among asthmatic children. Two studies looked at the associations between maternal prenatal (26) and cord blood (27) PFAS concentrations and cord blood IgE levels. One study observed a protective association, but only among female neonates (26). The other study observed an adverse association, but only among male neonates (27). Divergent study populations, exposure levels, and analytical methods make comparing the magnitude of effects difficult. For instance, the former study examined asthmatic children and the latter studies examined disease-naïve neonates.

Higher PFOS levels appear to be associated with decreased IgE sensitization to plants and cockroaches or shrimp and increased IgE sensitization to mold. An interquartile increase in serum PFOS concentration was associated with a 26% reduction in odds of IgE sensitization to any of 19 specific measured allergens. These primarily inverse associations appear counter to toxicological studies showing increased IgE response in PFOA-dosed mice after exposure to environmental allergens (19), although PFOS and PFOA may act through different mechanisms.

Two birth cohorts reported adverse associations between prenatal PFAS concentration and vaccine antibody concentration levels to tetanus and diphtheria (24) and rubella (23), but no association with measles (23). Our study also observed an adverse association with rubella and no association with measles. Our study is the first to report associations with mumps antibody concentration levels.

The clinical relevance of lower vaccine antibody concentrations at the levels we report—a 13% decrease for rubella with a doubling of PFOS concentration—is uncertain, particularly because this finding was among a subset of children who had antibody levels high enough to be considered protected against disease. Rubella and mumps are strong immunogens and antibodies for these viral vaccines typically last decades (34). In comparison, diphtheria and tetanus need regular boosting to keep antibodies high enough for disease protection. It is noteworthy, then, that the greatest observed reduction was for rubella. Vaccination information was not available in NHANES, although beginning in 1990 most states have required a measles-mumps-rubella booster before starting school. Approximately 60% of the children included in our analyses are young enough to have entered school since 1990. While we do not know which children were vaccinated or on what schedule, our restriction to the seropositive population serves as a proxy for vaccination (35). If we see a 13% reduction in antibodies after what may be fewer than 15 y since vaccination it is possible that with longer follow-up time antibodies would continue to decline and eventually convert to seronegative.

Table 6. Adjusted percent change (95% confidence interval) in serum IgE antibody titer with a doubling in perfluoroalkyl and polyfluoroalkyl substances serum concentration among children aged 12–19 y, National Health and Nutrition Examination Survey, 2005–2006, $n = 634$

	IgE
perfluoroalkyl and polyfluoroalkyl substances	
PFOS	5.4 (–15.4, 31.4)
PFOA	10.5 (0.17, 22)
PFHxS	1.1 (–8.4, 11.7)
PFNA	19.4 (8.3, 31.7)

Adjusted for age (continuous), gender, race, weight status, serum cotinine. PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate.

The limitations of this study are typical of a cross-sectional study of existing data. We can make no claims of causality and were reliant on self-report for designation of allergic conditions. NHANES, however, does ensure a representative population, which is particularly important as serum concentrations of PFOS and PFOA decline and PFHxS and PFNA increase over time. There may also be less exposure misclassification than in a typical cross-sectional study because these compounds have long serum half lives. Additionally, NHANES 2005–2006 included specific IgE allergen testing, which is a unique feature of our investigation. NHANES also allowed us to examine several indicators of immune function, although lack of information on receipt and/or timing of measles-mumps-rubella vaccination is a weakness of the survey. With four PFAS and numerous outcomes, however, we subjected the data to multiple testing. Nonetheless, the observed strong, consistent association between increasing levels of PFOS and decreasing levels of rubella antibody concentration is concerning and deserves further investigation to better elucidate the relation and understand the mechanism of action.

Although the United States eliminated production and use of PFOS (12) and PFOA (13), these chemicals remain detectable in human sera and biomonitoring studies indicate that replacement chemicals are increasingly detectable (14). These replacement chemicals may have similar toxicological effects so our study of older (PFOS, PFOA) and newer (PFHxS, PFNA) compounds is relevant. Several findings may be important to public health. We observed that higher PFOS concentration was associated with decreased mumps and rubella antibody concentrations and that higher PFOA and PFNA concentrations were associated with increased total serum IgE. Overall the relationship between PFAS and allergic disease appears complicated with both increases and decreases in allergen-specific IgE with greater PFAS exposures. Our results suggest a complex interaction between PFAS and the human immune system that may influence vaccine-induced immunity as well as susceptibility to allergic disease. Additional investigation into the role of PFAS and response to vaccination is warranted.

METHODS

Study Population

NHANES is a nationally representative survey conducted by the US Centers for Disease Control and Prevention designed to monitor the health and nutrition status of the noninstitutionalized, civilian population (36). The survey includes interviews, examinations, and laboratory tests on approximately 5,000 persons each year; data are released in 2-y cycles. This investigation was conducted as two separate analyses because the information collected varies across NHANES cycles. We used NHANES 1999–2000 and 2003–2004 for examination of vaccine antibody concentrations (Vaccine Study) and NHANES 2005–2006 for examination of allergic conditions and allergic sensitization (Allergy Study). We included children aged 12–19 y (36) with serum PFAS measurements and data on the vaccine or allergy outcomes relevant to each study. NHANES is publicly available data and its use is not considered human subject research. As such, the Mount Sinai Program for the Protection of Human Subjects does not require IRB approval.

Exposure Assessment

PFAS were measured in sera from eligible participants aged 12 y and older. In 1999–2000, 11 PFAS were measured in surplus sera. In 2003–2004 and 2005–2006, 12 PFAS were measured in a one-third subsample of participants (36). Detailed descriptions of the analytic methods have been previously published (5,14). In brief, PFAS were detected and quantified in serum using a modification of online solid-phase extraction coupled to reversed-phase high-performance liquid chromatography–tandem mass spectrometry. PFAS concentrations below the LOD were substituted with LOD divided by the square root of two. We restricted our investigation to four highly detected PFAS (PFOS, PFOA, PFHxS, and PFNA). PFAS were natural-log transformed to correct for non-normal distribution and also ranked into quartiles. We obtained *P*-trend for quartiles by using the quartiles as ordinal variables.

Outcome Assessment

IgG antibodies to measles and rubella were detected using standard protocols for the California Viral and Rickettsial Disease Laboratory enzyme immunoassay (37). Optical density values were determined by enzyme-linked immunosorbent assay. For measles, optical density values ≥ 1.0 were considered seropositive. For rubella, International Unit values ≥ 10 were considered seropositive. IgG antibodies to mumps were measured using standard procedures for the Wampole IgG ELISA II test (38). For mumps, optical density values ≥ 1.1 were considered seropositive. Antibody concentrations were natural log transformed to correct for non-normal distributions.

Allergy study outcomes were derived from questionnaire data for conditions specific to the past 12 mo. Participants responding that they had ever been told by a doctor that they had asthma and either still had asthma or had an asthma attack in the past year were considered to have current asthma. Participants reporting wheezing or whistling in their chest in the past year were considered to have current wheeze. Participants reporting sneezing or a runny or blocked nose without a cold in the past year were considered to have current rhinitis. Participants responding Yes to the questions “Has a doctor or other health professional ever told you that you have allergies?” and “During the past 12 months have you had any allergy symptoms or an allergy attack?” were considered to have current allergies.

Additionally, allergen-specific and total IgE antibodies were measured in serum using the Pharmacia Diagnostics ImmunoCAP 1000 System (Kalamazoo, MI) (39). IgE levels were measured against 19 specific allergens. Following Salo *et al.* (40)’s work to group these allergens measured in NHANES into clusters sharing similar biological and statistical properties, we focused on seven IgE clusters: plants (Bermuda grass, rye grass, birch, oak, ragweed, Russian thistle, and peanut-specific IgE); dust mites (*D farina* and *D pteronyssinus*); pets (dog and cat); cockroach and shrimp; rodents (mouse and rat); molds (*Alternaria* and *Aspergillus* species); and foods (egg white and cow’s milk). All children with peanut allergies also had allergies to at least one other allergen in the plant cluster. Participants with allergen-specific IgE ≥ 0.35 kU/l were considered sensitized to that specific allergen and were considered sensitized to an IgE cluster if they were sensitized to any of the allergens within the cluster. We also examined a measure indicating sensitization to any of the 19 allergens and continuous, natural-log transformed measures of total and allergen-specific IgE antibodies.

Statistical Analysis

To account for NHANES study design, recommended sampling weights and design variables were included in all analyses and analyses were performed using SAS survey procedures (36). Descriptive statistics calculated geometric means (95% CI) for untransformed PFAS concentration by study characteristics and Pearson correlation coefficients for natural log transformed values. For both studies, we ran unadjusted and adjusted regression models. Adjusted regression models included age (continuous), sex, and race/ethnicity (non-Hispanic white; non-Hispanic black; Mexican American; other) as a priori factors. Next we identified a set of potential confounders based on the literature and then tested to determine whether these factors were associated with both exposure and outcome, necessary criteria for

confounders. For the vaccine study, no covariate met the criteria so the only addition to the adjusted model beyond the a priori covariates was a term for survey year. For the allergy study, serum cotinine (below LOD: <0.015 ng/ml; low: 0.015–10 ng/ml; high: >10 ng/ml) and weight status based on CDC guidelines for age- and sex-specific body mass index percentiles (<85th percentile; 85th to <95th percentile; ≥95th percentile) met the criteria and were included in all adjusted models.

For the vaccine study, we used linear regression to estimate the percent change (95% CI) in serum antibody concentration level for a doubling of serum PFAS concentration. Both PFAS and antibody concentrations were natural log-transformed; so, we applied the formula $((2^\beta)-1) \times 100$ to calculate percent change. We also used linear regression to estimate the change in natural log-transformed serum antibody concentration level (95% CI) by quartile of PFAS concentration. All models were run twice: once for the full population and once restricted to the seropositive population with the assumption that seropositive children have been exposed to the vaccine strain of these viruses (35). Receipt and/or timing of measles-mumps-rubella vaccination are not available in NHANES. Additionally, to explore the potential for residual confounding by age we ran age-stratified (12–15 y; 16–19 y) models.

For the allergy study, we used logistic regression to estimate ORs (95% CI) for a shift from the 25th to 75th percentile (ln-IQR) of PFAS serum concentration and by quartile of PFAS concentration. We used linear regression to estimate the percent change (95% CI) in total and allergen-specific IgE antibodies for a doubling of serum PFAS concentration. We also used linear regression to estimate the change in natural log-transformed total IgE antibody level (95% CI) by quartile of PFAS concentration.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/pr>

STATEMENT OF FINANCIAL SUPPORT

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