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Perfluoroalkyl Substance Serum Concentrations and Immune Response to FluMist Vaccination among Healthy Adults

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Abstract

Perfluoroalkyl substances (PFAS) were shown to be immunotoxic in laboratory animals. There is some epidemiological evidence that PFAS exposure is inversely associated with vaccine-induced antibody concentration. We examined immune response to vaccination with FluMist intranasal live attenuated influenza vaccine in relation to four PFAS (perfluorooctanoate, perfluorononanoate, perfluorooctane sulfonate, perfluorohexane sulfonate) serum concentrations among 78 healthy adults vaccinated during the 2010 – 2011 influenza season. We measured anti-A H1N1 antibody response and cytokine and chemokine concentrations in serum pre-vaccination, 3 days post-vaccination, and 30 days post-vaccination. We measured cytokine, chemokine, and mucosal IgA concentration in nasal secretions 3 days post-vaccination and 30 days post-vaccination. Adults with higher PFAS concentrations were more likely to seroconvert after FluMist vaccination as compared to adults with lower PFAS concentrations. The associations, however, were imprecise and few participants seroconverted as measured either by hemagglutination inhibition (9%) or

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immunohistochemical staining (25%). We observed no readily discernable or consistent pattern between PFAS concentration and baseline cytokine, chemokine, or mucosal IgA concentration, or between PFAS concentration and change in these immune markers between baseline and FluMist-response states. The resuts of this study do not support a reduced immune response to FluMist vaccination among healthy adults in relation to serum PFAS concentration. Given the study's many limitations, however, it does not rule out impaired vaccine response to other vaccines or vaccine components in either children or adults.

Keywords

fluorocarbons; influenza vaccines; epidemiology; seroconversion; adult

1. Introduction

Perfluoroalkyl 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 (EPA 2009). Several PFAS have been widely detected in human sera since biomonitoring began in 1999 in the United States National Health and Nutrition Examination Survey (NHANES) (Kato et al., 2011b). Human exposure typically occurs through transfer from food packaging and preparation materials, bioaccumulation in the food chain, and household dust (D'Eon J and Mabury, 2011). The serum elimination half-life is estimated at a median of 2.3 years (95% confidence interval (CI) 2.1 – 2.4) (Bartell et al., 2010) to a geometric mean of 3.5 years (95% CI, 3.0–4.1) (Olsen et al., 2007) for perfluorooctanoate (PFOA), geometric mean 4.8 years (95% CI, 4.0–5.8) for perfluorooctane sulfonate (PFOS) (Olsen et al., 2007), and geometric mean 7.3 years (95% CI, 5.8–9.2) for perfluorohexane sulfonate (PFHxS) (Olsen et al., 2007). Given these chemical's long half-lives and dispersion, exposure even to PFOS and PFOA, whose use and production in the United States has been curtailed (EPA 2000, EPA 2013), will likely persist for some time.

Toxicological studies suggest that some PFAS may be immunotoxic, although laboratory findings appear dependent on animal sex, strain, and species as well as route of exposure and specific outcome examined (reviewed in Chang et al., 2016). It is also challenging to differentiate between direct immunotoxic effects and the downstream effects of non-immune toxicity. Some laboratory studies show that PFOS and PFOA alter inflammatory responses, cytokine expression, and adaptive and innate immune responses in multiple animal models as well as in mammalian and non-mammalian wildlife (reviewed in DeWitt et al., 2012). These immune effects appear to work through numerous pathways including activation of peroxisome proliferator-activated receptor-alpha (PPAR-α), which can be anti-inflammatory (DeWitt et al., 2012) and activation of nuclear factor-kappa B (NF-KB), which can suppress cytokine secretion by immune cells (Corsini et al., 2012). One study of human cord blood gene expression provided support for immune effects mediated through PPAR-δ and NF-KB (Pennings et al., 2015).

Epidemiological evidence of PFAS exposure and immune perturbation is mixed (reviewed in (Chang et al., 2016)). Four studies reported divergent associations between select PFAS and serum IgE levels. In Japan (n=343), prenatal PFOA was negatively associated with cord blood IgE among female infants (Okada et al., 2012). In Taiwan (n=244), cord blood PFOS and PFOA were positively associated with cord blood IgE among male infants (Wang et al., 2011). A Taiwanese case-control study (n=456) reported positive associations between higher PFOS and PFOA exposure and serum IgE among 10 – 15 year old asthmatics; non-asthmatic controls were not tested (Dong et al., 2013). In a cross-sectional NHANES study (n=1,191) of 12 – 19 year olds, there was no adverse association between PFAS exposure and current allergic conditions; children with higher PFOS concentration were less likely to have IgE sensitization to a range of allergens (Stein et al., 2016).

In examinations of clinical disease, the Japanese study found no relation between exposures and allergic disease or otitis media at age 18 months despite the elevated IgE levels at birth (Okada et al., 2012). BraMat (n=99), a sub-cohort of the Norwegian Mother and Child Cohort Study, reported that higher maternal PFAS levels at delivery were associated with increased risk of common cold (PFOA, perfluorononanoate [PFNA]) and gastroenteritis (PFOA, PFHxS) in children up to age 3; no associations were found with allergy or asthma-related outcomes (Granum et al., 2013). A subset of the Danish National Birth Cohort (n=1400) observed no clear pattern between prenatal PFOS and PFOA exposure and risk for infectious disease hospitalizations in childhood (Fei et al., 2010).

There is some epidemiological evidence that select PFAS exposure is inversely associated with vaccine-induced antibody concentration. Most of these investigations have been in children. BraMat reported inverse associations between four prenatal PFAS plasma concentrations and serum antibody concentrations against rubella at age 3 years (Granum et al., 2013). A Faroe Islands birth cohort (n=587) reported inverse associations between prenatal PFOS serum concentration and tetanus and diphtheria toxoids at ages 5 and 7 years (Grandjean et al., 2012). In the NHANES study, higher PFOS, PFOA, and PFHxS serum concentrations were associated with lower levels of mumps and rubella antibody concentrations among children aged 12 – 19 years (Stein et al., 2016).

Two published studies have reported on PFAS exposure and vaccine response among adults (Kielsen et al., 2015; Looker et al., 2014). In a 2010 follow-up to the C8 Health Project adults aged 18 or older (n=411) were vaccinated with inactivated intramuscular trivalent influenza vaccine (Looker et al., 2014). Higher PFOA serum concentration was associated with reduced antibody rise and an increased risk of failed seroconversion. A small (n=12) study in Denmark examined antibody response to a diptheria-tetanus booster among adults aged 23 – 65 years (Kielsen et al., 2015). There was a trend towards reduced antibody rise for both diptheria and tetanus with increasing PFAS serum concentration between days 4 and 10 post-vaccination.

To further explore PFAS's immunotoxic potential we examined immune response to vaccination with FluMist intranasal live attenuated influenza vaccine in relation to PFOS, PFOA, PFHxS, and PFNA serum concentrations among healthy adults using archived materials from a data and biospecimen repository.

2. MATERIALS AND METHODS

2.1 Study Population

From October – December 2010, we recruited 78 healthy adults to receive FluMist intranasal live attenuated influenza vaccine and measured serum and nasal mucosal secretions for numerous immune markers as well as antibody response to FluMist (Barria et al., 2013). Participants were enrolled as a convenience sample from Employee and Student Health Clinics at the Mount Sinai Medical Center in New York, New York. Healthy, nonfebrile individuals aged 18–49 years were eligible. Individuals who reported recent influenza, prior receipt of influenza vaccine during the 2010–2011 season, asthma, concurrent pregnancy, allergy to the vaccine or its components, or chronic medical conditions were excluded. All subjects provided written informed consent and were compensated for their time and effort upon completion of study components. This study was approved by the Mount Sinai Program for the Protection of Human Subjects. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory to analyze de-identified serum samples was determined not to constitute engagement in human subjects research.

2.2 Study Protocol

At the initial study visit (Day 0), subjects provided a blood sample and then were immunized with FluMist (2010–2011 formulation; MedImmune, Gaithersburg, MD) (Barria et al., 2013). Each 2-mL dose contained live attenuated influenza virus reassortants of the 3 strains for the 2010 – 2011 season: A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), B/Brisbane/60/2008. Subjects returned for a first follow-up visit 48 – 72 hours after vaccination (Day 3) and a second follow-up visit at least 30 days after vaccination (Day 30). At both follow-up visits, subjects provided saline nasal washes and blood samples. A nasal wash was not performed at the Day 0 visit to preclude interference with the administration and absorption of the intranasal vaccine. For the nasal wash, 5mL of sterile saline solution was sprayed into each nostril and the expelling fluid was collected in a specimen cup (Noah and Becker, 2000). The expellant was centrifuged at 500x*g* for 10 minutes to remove cells and debris. The resulting cell-free supernatant and serum samples were stored at −80°C until laboratory analysis.

2.3 Measurement of Systemic Antibody Response

Most subjects had high pre-vaccine titers to the H3N2 vaccine component so we focused our analyses on the H1N1 component (Barria et al., 2013). We measured anti-A H1N1 antibody response by hemagglutination-inhibition (HAI) based on World Health Organization standard protocol using Day 0 and Day 30 serum samples (Barria et al., 2013). Briefly, four hemagglutination units of influenza A virus subtype H1N1 were added to serial dilutions of patient sera. HAI titers were determined to the highest dilution displaying hemagglutination activity.

Seroconversion by HAI was defined as at least a 4-fold increase in the antibody response between Day 0 and Day 30. Subjects with pre-vaccine HAI titers of 1:10 or less were categorized as having low baseline antibody titer.

We also measured anti-A H1N1 antibody response by immunohistochemical (IHC) staining using Day 0 and Day 30 serum samples (Barria et al., 2013). Briefly, Madin-Darby canine kidney cell monolayers infected with influenza A virus subtype H1N1 were incubated at different dilutions of patient sera. IHC titers were determined to the highest dilution displaying immunodetection. Seroconversion by IHC was defined as at least a 4-fold increase in the antibody response between Day 0 and Day 30.

2.4 Measurement of Cytokine/Chemokine Response

Measurement of cytokines/chemokines in serum and nasal secretions was performed as described (Kraus et al., 2010) using an 11-plex cytokine panel (Millipore) (Barria et al., 2013). All samples were run in duplicate using a Luminex 200 (Luminex) in accordance with the manufacturer's protocol and were analyzed with Milliplex Analyst software. Granulocyte colony-stimulating factor (G-CSF), interferon-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), interferon-α2 (IFN-α2), interferon-γ (IFN-γ), macrophage inflammatory protein-1a (MIP-1a), granulocyte-monocyte colony-stimulating factor (GM-CSF), interleukin-1B (IL-1B), interleukin-6 (IL-6), and interleukin-12P70 (IL-12P70) were quantified in serum. IP-10, MCP-1, G-CSF, and IFN-α2 were also quantified in nasal secretions. We restricted our analyses to those cytokines with at least 90% of samples above the limit of detection. Accordingly, serum measures of GM-CSF, IL-1B, IL-6, and IL-12P70 and nasal secretion measures of G-CSF and IFN-α2 were not included in statistical analyses. Baseline cytokine concentrations were considered Day 0 for serum and Day 30 for nasal secretions. Nasal cytokine levels are reported to return to baseline levels by day 8 after experimental influenza virus infection (Hayden et al., 1998) and day 9 after live attenuated influenza vaccine (Noah et al., 2011). FluMist-response cytokine concentrations were considered Day 3 for both serum and nasal secretions. Change in cytokine level from baseline to Flumist-response periods was calculated as the difference between Day 3 and Day 0 (serum) or Day 30 (nasal secretions) cytokine levels.

2.5 Measurement of Localized Mucosal Response

Hemagglutinin-specific mucosal immunoglobulin A (mIgA) antibody in nasal secretions was quantified by enzyme-linked immunosorbent assay (ELISA) as previously described (Murphy et al., 1981; Treanor et al., 1999) using as antigen purified recombinant hemagglutinin protein from influenza virus A/California/04/2009 (H1N1) obtained through the National Institutes of Health Biodefense and Emerging Infections Research Resources Repository (Barria et al., 2013). For mIgA analyses, given the length of time required to produce mIgA, baseline is considered Day 3 and FluMist-response is Day 30. Briefly, ELISA titers were calculated using the positive-negative ratio where the end point was the highest dilution with a positive-negative ratio ≥2 (Barria et al., 2013). In the calculation, the optical density of an antigen-coated well (positive) was divided by the optical density of the control well lacking the antigen (negative).

2.6 Measurement of PFAS Exposure Biomarkers

In 2014, the CDC's Division of Laboratory Sciences staff quantified PFOS, PFOA, PFHxS, PFNA, perfluorooctane sulfonamide, 2-(N-ethyl-perfluorooctane sulfonamido) acetate, 2-

(N-methyl-perfluorooctane sulfonamido) acetate, and perfluorodecanoate in surplus Day 0 serum stored at −80°C. In brief, these eight biomarkers were detected and quantified in serum using a modification of the analytic method previously published (Kato et al., 2011a) based on online solid-phase extraction coupled to isotope-dilution high-performance liquid chromatography–tandem mass spectrometry. The limits of detection were 0.2 ng/mL for PFOS and 0.1 ng/mL for the other compounds. All laboratory operations were conducted under the requirements set forth by the Clinical Laboratory Improvement Act of 1988 (United States Congress, 1990) and followed standard quality control procedures. Each analytic batch included reagent blanks and quality control materials, including a blind serum pool every 10^{th} sample. Quality control concentrations were evaluated using standard statistical probability rules. We restricted our investigation to the four PFAS with 100% detectability (PFOS, PFOA, PFHxS, PFNA). PFAS concentrations were categorized into tertiles.

2.7 Statistical Analyses

Descriptive statistics modeled geometric means (95% confidence intervals [CI]) and calculated Pearson correlation coefficients for natural log transformed PFAS concentration by study characteristics. We modeled the geometric mean (95% CI) of the Baseline and FluMist-response immune marker levels, compared the geometric means at both time points using a paired T-Test, and calculated Spearman correlation coefficients comparing concentration distributions at both time points.

To examine the association between PFAS exposure and systemic antibody response to FluMist, we used a modified Poisson approach to estimate relative risk (RR) and 95% confidence intervals (95% CI) with robust error variances (Zou, 2004) for the association between PFOS, PFOA, PFHxS, and PFNA serum concentration tertiles and seroconversion separately for HAI and IHC. We also ran models separately for the full population and the subset with low baseline antibody titers expecting the more naive population to have a greater systemic antibody response to vaccination (Carter and Curran, 2011).

To examine the cross-sectional association between PFAS exposure and baseline cytokine expression, we used linear regression to estimate the mean (95% CI) immune marker concentration in serum or nasal secretions by tertiles of PFOS, PFOA, PFHxS, and PFNA serum concentration.

To examine the association between PFAS exposure and cytokine activation after FluMist, we used linear regression to estimate the mean change (95% CI) in immune marker concentration between baseline and FluMist-response states by tertiles of PFOS, PFOA, PFHxS, and PFNA serum concentration. We ran models separately for the full population and the subset with low baseline antibody titers.

All regression analyses included unadjusted and adjusted models. No covariates qualified as true confounders associated with both exposure and outcome (Rothman and Greenland, 1998), but we included a priori adjustments for age (continuous), sex, and race/ethnicity (non-Hispanic white, Hispanic, other). We present only the adjusted models. We calculated p-values for an ordinal trend across PFAS tertiles and for an overall PFAS effect. Treating

these four PFAS as continuous variables did not notably change the interpretation of the results so all models used a categorical metric of exposure. Statistical analyses were performed using SAS 9.4 (Cary, North Carolina).

3. RESULTS

We included 78 adults with PFAS serum concentrations ranging from geometric mean 0.77 ng/mL (95% CI 0.67, 0.88) for PFNA to 5.22 ng/mL (95% CI 4.52, 6.02) for PFOS (Table 1). Pairwise correlations among PFAS were moderate with all $p<0.001$ (data not shown). The weakest correlation was between PFHxS and PFNA (R=0.42); the strongest was between PFOS and PFNA (R=0.77). The mean age of participants was 30.2 years (standard deviation 7.2; range 21 – 49) and the majority (64%) was female. A sizeable proportion of participants (22%) was Hispanic. Forty-nine percent of participants reported receiving the seasonal or H1N1 epidemic flu vaccine the previous year. Forty percent of all participants, and 34% of participants reporting receipt of a flu vaccine the previous year, exhibited low anti-A H1N1 antibody levels at baseline. The proportions of participants seroconverting after vaccination as determined by HAI (9%) or IHC (25%) were low.

Immune cytokine markers were highly inter-correlated at baseline and FluMist-response states in serum, but not in nasal secretions (Table 2). For the majority of the immune markers there was no difference in geometric mean immune marker concentration between baseline and FluMist-response states based on non-statistically significant Paired T-tests. IP-10, however, was significantly higher during FluMist-response than at baseline for both serum and nasal secretions. Results were comparable for the full population and the population with low baseline anti-A H1N1 titer (data not shown).

The overall associations between PFOS, PFOA, PFHxS, or PFNA and seroconversion were not statistically significant. However, the risk ratios for the association between tertiles of PFAS serum concentration and seroconversion determined by HAI or IHC were generally well above 1.0 for both the full population and the population with low baseline antibody titer (Table 3). For the most part, though, confidence intervals were wide and there was no statistical evidence (p-value for ordinal trend across PFAS ≥ 0.07) of a linear trend between increasing tertile of the four PFAS and likelihood of seroconversion. While the magnitude of the effect estimates varied between the full population and the low baseline anti-A H1N1 subset, there was no discernable pattern of stronger or weaker associations when restricting to those with the low baseline antibody titers.

Similarly, there was little apparent association between tertiles of PFAS concentration and baseline immune profile (Supplemental Table 1). There was no consistent pattern of either increasing or decreasing immune marker concentration by PFOS, PFOA, PFHxS, or PFNA tertile in serum or nasal secretions.

The primary notable association between PFAS serum concentration and response to FluMist vaccination, as measured by adjusted mean change in immune marker between baseline and FluMist-response states, was for the association of PFHxS with IFN-γ and TNF-α in serum (Table 4). Compared to individuals in the lowest tertile of PFHxS, those in

the 2nd (beta=-40, 95% CI −76, −3.7) and 3rd (beta=−40, 95% CI −84, 2.69) tertiles had lower mean IFN-γ in the FluMist-infected state versus the baseline period. There was a smaller, although still consistent, association between PFHxS and TNF-α in serum when comparing the adjusted mean change between baseline and FluMist-response states (2nd tertile beta=−5.3, 95% CI −9.2, −1.3; 3rd tertile beta=−4.8, 95% CI −9.4, −0.10). Restricting to the subset with low baseline anit-A H1N1 titer did not alter the association between PFHxS and IFN-γ and TNF-α (Supplemental Table 2). In general, the restriction to the smaller, naive population did not clarify any associations between the four PFAS serum concentrations and adjusted mean change in immune marker between baseline and FluMist-response states.

4. CONCLUSIONS

In a previous report of this study's immune findings, we observed considerable unexplained variation in baseline immune profile as well as immune response after live virus inoculation (Barria et al., 2013). These variations in baseline immune profile and response to FluMist vaccination do not appear to be related to PFOS, PFOA, PFHxS or PFNA exposure in this small population of healthy adults. We observed no readily discernable or consistent pattern between these four PFAS serum concentrations and baseline cytokine, chemokine, or mIgA concentration, or between PFAS concentration and change in these immune markers between baseline and FluMist-response states. It is unclear whether the association of PFHxS with IFN-γ and TNF-α in serum is meaningful. Similarly, given the low rate of seroconversion the significance of the positive association between PFAS concentration and seroconversion is unclear.

The correlation between baseline and FluMist-response immune markers was considerably weaker in nasal secretions than serum. This difference is expected because the denominator for the markers measured in nasal wash is more variable than for those measured in serum. We included nasal secretions, however, because mucosal (nasal) response has been proposed as a mechanism of action for live attenuated influenza vaccine and may better reflect response to FluMist vaccination than seroconversion (Carter and Curran, 2011). Despite an indication that IP-10 measured at baseline and FluMist-response states reflected different distributions, in multivariable regression models there was no association between exposure to these four PFAS and change in IP-10 after vaccination.

There are only two prior published studies of PFAS exposure and response to vaccination among adults (Kielsen et al., 2015; Looker et al., 2014). One of the studies examined response to influenza vaccine during the same 2010 – 2011 vaccine cycle as our study and reported that elevated PFOA serum concentration was associated with reduced antibody titer rise and increased risk of not achieving a sufficient rise to guarantee long-term protection (Looker et al., 2014). This study, however, used the inactivated intramuscular trivalent influenza vaccine, which generates a more pronounced systemic immune response than FluMist intranasal live attenuated influenza vaccine (Beyer et al., 2002; Ramakrishnan et al., 2012). The study population also had higher serum concentrations of PFOA and PFOS as compared to our population. Additionally, the opposite associations were observed for the H3N2 component of the virus, with no notable effect for the H1N1 component on which we

focused. Our study did not examine anti-A H3N2 because most participants had high pre-vaccine titers to this component, although the previous study also reported higher pre-vaccine titers for anti-A H3N2 than anti-A H1N1. Anti-A H3N2 information for the current study was not available through the data repository. The second study of vaccine response in relation to PFAS exposure among adults observed decreased antibody response after a diphtheria and tetanus booster vaccination, but included only 12 subjects (Kielsen et al., 2015). Three other investigations of selected PFAS exposure and response to vaccination studied children (Grandjean et al., 2012; Granum et al., 2013; Stein et al., 2016), who may be more susceptible to immunotoxicants.

Ours was a rigorous study of immune function in response to FluMist vaccination incorporating multiple measures of immune function at multiple time points. The investigation of the role of PFOS, PFOA, PFHxS, and PFNA exposure on immune response to vaccination was a good use of an existing data and biospecimen repository to address a question of increasing interest. The study, however, was challenged by its small sample size and the many statistical comparisons necessary to explore associations among exposures to four PFAS and 13 immune outcomes. Additionally, FluMist generated a limited systemic response to vaccination. We attempted to address this limited response by examining associations among the population subset with low baseline anti-A H1N1 titer with the expectation that these individuals would be more likely to mount a robust response. The analyses of the more selective population did not notably change our interpretation of the results. Future studies of environmental toxicants and response to vaccination may be better served by focusing on a more immunogenic vaccine than FluMist. This study's findings do not show a reduced immune response to FluMist vaccination among our population of healthy adults based on PFOS, PFOA, PFHxS, or PFNA serum concentrations. Given the study's many limitations, however, it does not rule out impaired vaccine response to other vaccines or vaccine components in either children or adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

CI	confidence interval
ELISA	enzyme-linked immunosorbent assay
G-CSF	granulocyte colony-stimulating factor

GM-CSF	granulocyte-monocyte colony-stimulating factor
HAI	hemagglutination inhibition
IFN-α2	interferon-α2
IFN-γ	interferon-γ
IHC	immunohistochemical
IL-1B	interleukin-1B
IL-6	interleukin-6
IL-12P70	interleukin-12P70
IP-10	interferon-γ-inducible protein 10
MCP-1	monocyte chemoattractant protein-1
mIgA	mucosal immunoglobulin A
MIP-1a	macrophage inflammatory protein-1a
NF-KB	nuclear factor-kappa B
NHANES	National Health and Nutrition Examination Survey
PFAS	perfluoroalkyl substances
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoate
PFOA	perfluorooctanoate
PFOS	perfluorooctane sulfonate
PPAR-α	peroxisome proliferator-activated receptor-alpha
RR	risk ratio
TNF-α	tumor necrosis factor-α

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HIGHLIGHTS

- We measured PFOS, PFOA, PFHxS, and PFNA in serum and then immunized healthy adults with FluMist intranasal live attenuated influenza vaccine
- We examined seroconversion by anti-A H1N1 titers, cytokines, chemokines, and muscosal IgA as markers of immune response to vaccination
- There was no evidence that PFOS, PFOA, PFHxS, or PFNA concentration was associated with a reduced response to vaccination in this population

Table 1

Geometric Mean (95% Confidence Interval) Perfluoroalkyl Substances (ng/mL) by Participant Characteristics, Ages 21 – 49 years, New York City, 2010, n=78

	N	PFOS ng/mL GeoMean (95% CI)	PFOA ng/mL GeoMean (95% CI)	PFHxS ng/mL GeoMean (95% CI)	PFNA ng/mL GeoMean (95% CI)
Overall	78	5.22 (4.52, 6.02)	2.28 (2.03, 2.56)	1.1 (0.897, 1.34)	0.77 (0.673, 0.881)
Age, years					
21 – 30	53	5.53 (4.65, 6.56)	2.39 (2.08, 2.75)	1.18 (0.931, 1.5)	0.814 (0.694, 0.956)
31 – 40	15	3.87 (2.80, 5.35)	1.81 (1.39, 2.36)	0.698 (0.445, 1.09)	0.556 (0.412, 0.751)
41 – 49	10	6.00 (4.04, 8.91)	2.50 (1.81, 3.45)	1.45 (0.836, 2.52)	0.932 (0.645, 1.35)
		p=0.12	p=0.16	p=0.07	p=0.05
Sex					
Male	28	5.94 (4.68, 7.54)	2.44 (2.01, 2.97)	1.58 (1.15, 2.19)	0.753 (0.600, 0.945)
Female	50	4.85 (4.06, 5.80)	2.19 (1.89, 2.53)	0.893 (0.702, 1.14)	0.779 (0.658, 0.924)
		p=0.18	p=0.37	p=0.006	p=0.81
Race/ethnicity					
Non-Hispanic white	40	6.03 (4.95, 7.34)	2.51 (2.14, 2.96)	1.44 (1.10, 1.89)	0.836 (0.690, 1.01)
Hispanic	17	4.73 (3.5, 6.4)	2.09 (1.63, 2.68)	0.85 (0.562, 1.28)	0.713 (0.531, 0.955)
Other	19	4.00 (3.00, 5.32)	1.96 (1.54, 2.48)	0.723 (0.489, 1.07)	0.676 (0.512, 0.892)
		p=0.06	p=0.17	p=0.009	p=0.40
Low Baseline Antibody Titer[a]					
Yes	31	5.36 (4.26, 6.76)	2.26 (1.87, 2.73)	1.37 (0.995, 1.88)	0.717 (0.578, 0.890)
No	46	5.12 (4.24, 6.19)	2.28 (1.95, 2.66)	0.948 (0.730, 1.23)	0.803 (0.672, 0.959)
		p=0.76	p=0.95	p=0.08	p=0.42
Seroconversion Measured by Hemagglutinin Inhibition					
Yes	7	5.29 (3.25, 8.61)	2.89 (1.96, 4.27)	1.35 (0.684, 2.67)	0.844 (0.534, 1.33)
No	70	5.21 (4.47, 6.08)	2.22 (1.96, 2.51)	1.08 (0.868, 1.33)	0.76 (0.658, 0.878)
		p=0.95	p=0.20	p=0.53	p=0.66
Seroconversion Measured by Immunohistochemistry					
Yes	19	5.83 (4.34, 7.82)	2.48 (1.95, 3.15)	1.27 (0.841, 1.92)	0.849 (0.644, 1.12)
No	58	5.03 (4.25, 5.95)	2.21 (1.93, 2.53)	1.05 (0.827, 1.33)	0.742 (0.634, 0.869)

	N	PFOS ng/mL GeoMean (95% CI)	PFOA ng/mL GeoMean (95% CI)	PFHxS ng/mL GeoMean (95% CI)	PFNA ng/mL GeoMean (95% CI)
		p=0.39	p=0.40	p=0.42	p=0.40

PFOS = perfluorooctane sulfonate; PFOA = perfluorooctanoate; PFHxS = perfluorohexane sulfonate; PFNA = perfluorononanoate; GeoMean = geometric mean; 95% CI = 95% confidence interval

[a]anti-A H1N1 hemagglutinin inhibition titers ≤1:10

Table 2

Geometric Mean (95% Confidence Interval) Cytokine, Chemokine, or Immunoglobulin Concentration (pg/mL) at Baseline[a] and FluMist-response[b] States, Ages 21 – 49 years, New York City, 2010, n=77

Immune Marker	Baseline[a] pg/mL		FluMist-response[b] pg/mL			
	N	GeoMean (95% CI)	N	GeoMean (95% CI)	T-test P-value[c]	Correlation Coefficient[d]
Serum						
IFN-α2	72	15.5 (11.8, 20.3)	70	14.6 (10.8, 19.6)	0.09	0.863
IFN-γ	71	40.8 (26.6, 62.7)	73	37.1 (23.8, 57.9)	0.74	0.961
TNF-α	77	11.8 (9.69, 14.4)	77	11.2 (9.22, 13.6)	0.11	0.895
IP-10	77	164 (144, 187)	77	182 (161, 207)	0.01	0.698
MCP-1	77	374 (339, 413)	77	373 (339, 411)	0.93	0.814
MIP-1a	72	41.5 (33.4, 51.4)	70	45.6 (36.4, 57.2)	0.25	0.799
G-CSF	77	35.7 (27.7, 46.0)	77	36.0 (28.2, 46.1)	0.83	0.903
Nasal Secretions						
IP-10	77	423 (340, 526)	77	1330 (1060, 1670)	<0.0001	0.313
MCP-1	77	17.0 (14.8, 19.6)	77	18.5 (16.1, 21.4)	0.29	0.381
mIgA	77	10.4 (8.10, 13.3)	77	11.9 (8.65, 16.3)	0.31	0.433

[a]Baseline is Day 0 for serum; Day 30 for nasal IP-10, MCP-1; Day 3 for mIgA.

[b]FluMist-response is Day 3 for serum; Day 3 for nasal IP1-0, MCP-1; Day 30 for mIgA.

[c]P-value for Paired T-Test comparing geometric mean immune markers between baseline and FluMist-response states.

[d]Spearman correlation coefficient comparing geometric mean immune markers between baseline and FluMist-response states (all correlation p-values <0.001).

GeoMean = geometric mean; 95% CI = 95% confidence interval

Table 3

Adjusted* Risk Ratio (95% Confidence Interval) for Seroconversion by Tertile of Perfluoroalkyl Substances Concentration among Total and Low Baseline Antibody[a] Populations, Ages 21 – 49 years, New York City, 2010 (n=75)

PFAS Tertiles	Seroconversion Measured by Hemagglutinin Inhibition				Seroconversion Measured by Immunohistochemistry			
	Total Population (n=75)		Low Baseline Ab[a] (n=29)		Total Population (n=75)		Low Baseline Ab[a] (n=29)	
	N[b]	RR (95% CI)	N[b]	RR (95% CI)	N[b]	RR (95% CI)	N[b]	RR (95% CI)
PFOS, ng/mL								
1: 0.4 – 4.7	2	1.0	1	1.0	4	1.0	2	1.0
2: 4.8 – 7.1	3	2.6 (0.4, 15.1)	3	6.7 (1.2, 37.9)	7	2.6 (0.9, 7.4)	4	4.5 (1.0, 20.3)
3: 7.2 – 21.4	2	1.3 (0.2, 7.3)	2	1.6 (0.3, 9.7)	8	2.4 (0.9, 6.6)	7	3.1 (1.0, 10.2)
		P-trend=0.81		P-trend=0.81		P-trend=0.12		P-trend=0.13
		P-class=0.63		P-class=0.24		P-class=0.10		P-class=0.11
PFOA, ng/mL								
1: 0.6 – 2.0	1	1.0	1	1.0	7	1.0	4	1.0
2: 2.1 – 2.7	2	2.9 (0.4, 23.3)	2	4.0 (0.3, 47.3)	3	0.6 (0.2, 2.0)	3	1.4 (0.4, 5.1)
3: 2. 8 – 8.1	4	6.8 (1.0, 48.1)	3	5.1 (0.5, 46.8)	9	1.8 (0.7, 4.3)	6	2.5 (0.7, 8.7)
		P-trend=0.07		P-trend=0.17		P-trend=0.27		P-trend=0.12
		P-class=0.16		P-class=0.29		P-class=0.15		P-class=0.28
PFHxS, ng/mL								
1: 0.1 – 0.7	2	1.0	1	1.0	6	1.0	3	1.0
2: 0.8 – 1.3	2	1.2 (0.2, 6.5)	2	1.5 (0.1, 17.0)	6	1.1 (0.4, 2.9)	4	1.1 (0.4, 3.2)
3: 1.5 – 17.9	3	3.1 (0.8, 12.7)	3	2.6 (0.3, 25.0)	7	1.7 (0.6, 4.8)	6	2.0 (0.7, 5.9)
		P-trend=0.22		P-trend=0.37		P-trend=0.34		P-trend=0.17
		P-class=0.45		P-class=0.66		P-class=0.57		P-class=0.29
PFNA, ng/mL								
1: 0.1 – 0.7	1	1.0	1	1.0	6	1.0	4	1.0
2: 0.8 – 1.0	4	6.2 (0.8, 44.8)	4	8.4 (1.5, 45.8)	6	1.6 (0.6, 4.2)	5	2.7 (1.0, 7.5)
3: 1.1 – 2.9	2	2.6 (0.3, 22.7)	1	2.0 (0.2, 24.0)	7	1.5 (0.6, 3.9)	4	2.0 (0.6, 6.3)
		P-trend=0.33		P-trend=0.38		P-trend=0.40		P-trend=0.19
		P-class=0.22		P-class=0.12		P-class=0.56		P-class=0.20

[*] Adjusted for age (continuous), sex, race/ethnicity

[a] anti-A H1N1 hemagglutinin inhibition titers ≤1:10

[b] N represents the number of participants who seroconverted

P-trend = p-value for ordinal trend across PFAS tertiles

P-class = p-value for overall PFAS effect

Ab= antibody; PFAS = perfluoroalkyl substances; PFOS = perfluorooctane sulfonate; PFOA = perfluorooctanoate; PFHxS = perfluorohexane sulfonate; PFNA = perfluorononanoate; RR = risk ratio; 95% CI = 95% confidence interval

Table 4

Adjusted[*] Mean Change in Cytokine, Chemokine, or Immunoglobulin Concentration [pg/mL (95% Confidence Interval)] between Baseline[a] and FluMist-response[b] States by Tertile of Perfluoroalkyl Substances Concentration, Ages 21 – 49 years, New York City, 2010

	Beta (95% CI) for Serum Immune Marker							Beta (95% CI) for Nasal Secretion Immune Marker		
PFAS Tertile	**IFN-α2 (n=65)**	**IFN-γ (n=69)**	**TNF-α (n=75)**	**IP1-0 (n=75)**	**MCP-1 (n=75)**	**MIP-1a (n=69)**	**G-CSF (n=75)**	**IP-10 (n=75)**	**MCP-1 (n=75)**	**mIgA (n=75)**
PFOS, ng/mL										
1: 0.4 – 4.7	0	0	0	0	0	0	0	0	0	0
2: 4.8 – 7.1	8.69 (−23, 40.5)	8.00 (−34, 49.6)	−0.06 (−4.6, 4.45)	12.0 (−44, 67.6)	4.75 (−60, 69.7)	−1.6 (−22, 18.8)	−8.7 (−60, 42.8)	429 (−1309, 2166)	2.34 (−9.2, 13.8)	73.9 (−216, 364)
3: 7.2 – 21.4	−5.6 (−37, 25.7)	9.95 (−28, 47.9)	0.59 (−3.6, 4.81)	−42 (−94, 10.4)	19.0 (−42, 79.8)	−0.51 (−20, 19.2)	−0.36 (−49, 47.9)	−215 (−1841, 1412)	−6.7 (−17, 4.04)	−88 (−359, 183)
	P-trend= 0.67	P-trend= 0.61	P-trend= 0.78	P-trend= 0.11	P-trend= 0.53	P-trend= 0.96	P-trend= 0.99	P-trend= 0.77	P-trend= 0.20	P-trend= 0.49
	P-class= 0.63	P-class= 0.87	P-class= 0.94	P-class= 0.11	P-class= 0.81	P-class= 0.99	P-class= 0.93	P-class= 0.75	P-class= 0.24	P-class= 0.52
PFOA, ng/mL										
1: 0.6 – 2.0	0	0	0	0	0	0	0	0	0	0
2: 2.1 – 2.7	2.54 (−28, 32.9)	−23 (−61, 14.1)	1.28 (−2.8, 5.39)	−3.4 (−55, 48.7)	−9.9 (−70, 49.8)	4.25 (−15, 23.7)	−1.4 (−49, 45.7)	−30 (−1623, 1563)	0.72 (−9.9, 11.3)	−17 (−285, 251)
3: 2. 8 – 8.1	10.9 (−19, 40.4)	−23 (−61, 16.1)	−0.96 (−5.2, 3.26)	−28 (−82, 25.0)	−2.7 (−64, 58.6)	5.09 (−15, 24.8)	−13 (−62, 35.2)	−564 (−2200, 1072)	−6.6 (−18, 4.24)	28.7 (−246, 304)
	P-trend= 0.47	P-trend= 0.25	P-trend= 0.70	P-trend= 0.31	P-trend= 0.92	P-trend= 0.61	P-trend= 0.60	P-trend= 0.51	P-trend= 0.25	P-trend= 0.85
	P-class= 0.75	P-class= 0.40	P-class= 0.59	P-class= 0.54	P-class= 0.95	P-class= 0.86	P-class= 0.85	P-class= 0.76	P-class= 0.37	P-class= 0.95
PFHxS, ng/mL										
1: 0.1 – 0.7	0	0	0	0	0	0	0	0	0	0
2: 0.8 – 1.3	−21 (−51, 8.61)	−40 (−76, 3.7)	−5.3 (−9.2, 1.3)	−32 (−84, 20.1)	−39 (−97, 19.5)	−6.6 (−25, 12.1)	−10 (−56, 35.9)	−691 (−2279, 896)	−.07 (−11, 10.6)	294 (35.2, 553)
3: 1.5 – 17.9	−29 (−64, 5.20)	−40 (−84, 2.69)	−4.8 (−9.4, 0.1)	−15 (−76, 46.9)	20.6 (−48, 89.5)	−5.0 (−28, 18.4)	36.6 (−18, 91.3)	−713 (−2596, 1170)	−3.6 (−16, 9.08)	238 (−69, 545)
	P-trend= 0.09	P-trend= 0.05	P-trend= 0.04	P-trend= 0.59	P-trend= 0.66	P-trend= 0.63	P-trend= 0.23	P-trend= 0.44	P-trend= 0.60	P-trend= 0.11
	P-class= 0.22	P-class= 0.07	P-class= 0.03	P-class= 0.48	P-class= 0.14	P-class= 0.78	P-class= 0.18	P-class= 0.66	P-class= 0.81	P-class= 0.08
PFNA, ng/mL										
1: 0.1 – 0.7	0	0	0	0	0	0	0	0	0	0
2: 0.8 – 1.0	−16 (−46, 14.9)	−17 (−55, 22.0)	1.17 (−3.0, 5.30)	−28 (−81, 24.9)	9.51 (−51, 70.4)	−6.6 (−26, 12.5)	−17 (−65, 30.1)	−683 (−2304, 938)	−7.3 (−18, 3.57)	17.3 (−257, 291)
3: 1.1 – 2.9	−23 (−51, 4.06)	−15 (−50, 20.6)	−2.8 (−6.7, 1.01)	−34 (−84, 15.3)	20.7 (−36, 77.6)	−10 (−28, 7.77)	−38 (−83, 6.01)	328 (−1188, 1844)	0.48 (−9.6, 10.6)	−52 (−308, 204)

	Beta (95% CI) for Serum Immune Marker							Beta (95% CI) for Nasal Secretion Immune Marker		
PFAS Tertile	**IFN-α2 (n=65)**	**IFN-γ (n=69)**	**TNF-α (n=75)**	**IP1-0 (n=75)**	**MCP-1 (n=75)**	**MIP-1a (n=69)**	**G-CSF (n=75)**	**IP-10 (n=75)**	**MCP-1 (n=75)**	**mIgA (n=75)**
	P-trend= 0.10	P-trend= 0.40	P-trend= 0.18	P-trend= 0.17	P-trend= 0.48	P-trend= 0.27	P-trend= 0.09	P-trend= 0.72	P-trend= 0.99	P-trend= 0.71
	P-class= 0.24	P-class= 0.61	P-class= 0.16	P-class= 0.35	P-class= 0.78	P-class= 0.53	P-class= 0.24	P-class= 0.50	P-class= 0.33	P-class= 0.88

*Adjusted for age (continuous), sex, race/ethnicity

[a]Baseline is Day 0 for serum; Day 30 for nasal IP-10, MCP-1; Day 3 for mIgA

[b]FluMist-response is Day 3 for serum; Day 3 for nasal IP-10, MCP-1; Day 30 for mIgA

P-trend = p-value for ordinal trend across PFAS tertiles

P-class = p-value for overall PFAS effect

PFAS = perfluoroalkyl substances; PFOS = perfluorooctane sulfonate; PFOA = perfluorooctanoate; PFHxS = perfluorohexane sulfonate; PFNA = perfluorononanoate; 95% CI = 95% confidence interval