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Antibody response to COVID-19 vaccines among workers with a wide range of exposure to per- and polyfluoroalkyl substances

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are a broad class of synthetic chemicals; some are present in most humans in developed countries. Several studies have shown associations between certain PFAS, such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), and reduced antibody concentration after vaccination against diseases such as Tetanus. Recent studies have reported associations between COVID-19 occurrence and exposure to certain types of PFAS. However, studies of antibody concentration after COVID-19 vaccination in relation to PFAS serum concentrations have not been reported. We examined COVID-19 antibody responses to vaccines and PFAS serum concentrations among employees and retirees from two 3M facilities, one of which historically manufactured PFOS, PFOA, and perfluorohexane sulfonic acid (PFHxS).

Participants completed enrollment and follow-up study visits in the Spring of 2021, when vaccines were widely available. In total 415 participants with 757 observations were included in repeated measures analyses. Log-transformed concentrations of anti-spike IgG and neutralizing antibodies were modeled in relation to concentration of PFAS at enrollment after adjusting for antigenic stimulus group (9 groups determined by COVID-19 history and number and type of vaccination) and other variables. The fully adjusted IgG concentration was 3.45 percent lower (95% CI -7.63, 0.26) per 14.5 ng/mL (interquartile range) increase in PFOS; results for neutralizing antibody and PFOS were similar. For PFOA, PFHxS, and perfluorononanoic acid (PFNA), the results were comparable to those for PFOS, though of smaller magnitude. In our study data, the fully adjusted coefficients relating concentration of vaccine-induced antibodies to COVID-19 and interquartile range difference in serum concentration of PFOS, PFOA, PFHxS, and PFNA were inverse but small with confidence intervals that included zero. Our analysis showed that the coefficient for the four PFAS examined in detail was considerably affected by adjustment for antigenic stimulus group.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a broad class of man-made chemicals that have been used since the 1950’s in a wide variety of polymer and surfactant applications (Buck et al., 2011). Because of their widespread use and the long serum half-life in humans of some types of PFAS, most humans in developed countries have detectable serum levels of certain PFAS, including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) (Evich et al., 2022). Human exposure to PFAS occurs primarily through the ingestion of food and water, and

Abbreviations: PFAS, per- and polyfluoroalkyl substances; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFBA, perfluorobutanoic acid; PFBS, perfluorobutanesulfonic acid; PFPeA, perfluoropentanoic acid; PFHxA, perfluorohexanoic acid; PFHpA, perfluorooctanoic acid; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetic acid; EtFOSAA, 2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid; PFOSA, perfluoroctane sulfonamide; NTP, National Toxicology Program; ATSDR, Agency for Toxic Substances and Disease Registry; COVID-19, coronavirus disease 2019; LOQ, limit of quantification; LOD, limit of detection; VNT, virus neutralizing titer; HIV, human immunodeficiency virus; BMI, body mass index; J & J, Johnson & Johnson; IQR, interquartile range; NHANES, National Health and Nutrition Examination Survey; AIC, Akaike information criterion; OLS, ordinary least squares; BKMR, Bayesian kernel machine regression; EPA, Environmental Protection Agency.

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Inhalation of air and dust particles (De Silva et al., 2021; Sunderland et al., 2019). Since the phaseout of perfluorooctanyl-based production and use, serum concentrations of these PFAS measured in the United States (U.S.) general population have declined considerably; however, specific areas with elevated environmental exposure remain, including landfills, areas where aqueous fire-fighting foams were used, and industrial sites involving legacy PFAS use (ATSDR, 2021).

In 2016, the National Toxicology Program (NTP) concluded that exposure to PFOA or PFOS is presumed to be an immune hazard to humans based on a “high level” of evidence in animals and “moderate level” of evidence from studies in humans of reduced antibody response to vaccines (NTP, 2016). The Agency for Toxic Substances and Disease Registry (ATSDR), has also concluded that the available epidemiology studies suggest associations between decreased antibody response to vaccines and PFAS exposure (PFOA, PFOS, perfluorohexane sulfonic acid (PFHxS), and perfluorodecanoic acid (PFDA)) (ATSDR, 2021). NTP found “low confidence” for an association between PFOA or PFOS and infectious disease outcomes and ATSDR did not identify infectious disease as a suggested association with PFAS in its PFAS toxicological profile (ATSDR, 2021; NTP, 2016).

A positive association between COVID-19 occurrence and PFAS was found in a case-control study (Ji et al., 2021) and two ecologic studies of incidence or mortality (Catelan et al., 2021; Nielsen & Joud, 2021). None of these studies evaluated COVID-19 titer suppression after vaccinations. We examined the potential association between COVID-19 antibody responses to vaccines and PFAS serum concentrations, among employees and retirees from two 3M facilities, one of which was a manufacturer of PFAS, including PFOS, PFOA, and PFHxS.

2. Materials and methods

2.1. Study population

A longitudinal study was conducted in a population comprising current and retired employees of 3M facilities in Decatur, Alabama and Menomonie, Wisconsin. These two locations were chosen for their historic PFAS production (Decatur) and non-PFAS production (Menomonie). The Decatur manufacturing site consists of two plants: Specialty Film (film plant) and Specialty Materials (chemical plant) (Olsen et al., 2003). PFAS had limited use at the Decatur film plant and Menomonie plant. Owing to the long serum elimination half-lives of PFOS (half-life ~ 3 to 4 years), PFHxS (half-life ~ 5 to 7 years), and PFOA (half-life ~ 2 to 3 years) (Li et al., 2018; Olsen et al., 2007; Xu et al., 2020), employees at the Decatur facility continue to have elevated serum concentrations compared to the general population. These serum concentrations, however, have markedly declined in Decatur chemical plant employees since 2000 (Olsen et al., 2019). Additional information about the chemical processes used in Decatur are in the supplementary material, section I.

Our original intent was to design the study so that all vaccines were administered at 3M facilities, to give us control over vaccination scheduling, the timing of antibody measurements after vaccination, and the type of vaccine. However, the availability of COVID-19 vaccines at nearby community health care facilities meant such control was not feasible for the target group. Therefore, the study was scheduled around the anticipated period of maximum rate of vaccine administration by community health providers, and logistic considerations. To maximize study power and inclusiveness we invited adults of all ages, regardless of the presence of immunocompromising conditions or number or type of vaccines. We also note that among those in our study group who had not had COVID-19 or a vaccination against it, fewer than 40% percent had detectable levels of IgG and fewer than 11% had detectable neutralizing antibodies. Low levels of antibodies to the SARS-CoV-2 spike protein can be detected in people who have not had COVID-19, due to cross-reactivity with other Coronaviruses (Camerini et al., 2021; Ng et al., 2020); thus, a design including a pre-vaccination antibody concentration would not have been especially helpful.

In the Spring of 2021, as vaccines against COVID-19 were becoming widely available across the U.S., we invited individuals from these two manufacturing facilities to participate in the study to assess antibody responses to the COVID-19 vaccine and PFAS concentrations in serum. Potential participants were told that the study would address antibody response to COVID-19 vaccination. Individuals were eligible to participate if they were at least 18 years of age and an employee or retiree from the Decatur facility or an employee from the Menomonie facility; no other inclusion or exclusion criteria were applied. The study comprised an enrollment and a follow-up visit. Enrollment visits took place in April at the Decatur facility and in May at the Menomonie facility. Follow-up visits were conducted at each site 5–6 weeks later. At each study visit, participants provided a blood sample and completed a self-administered health questionnaire. All questionnaire responses were reviewed by the study staff to ensure completion before the end of the visit. In addition, participants were asked to bring their COVID-19 vaccination card to the enrollment visit for verification of vaccine dosage, type, and date. Each participant was assigned a unique study identification number; questionnaires and blood samples were labeled with this unique number to “blind” the analytical laboratories and those responsible for statistical analysis to the identity of the participants. Serum was aliquoted from each blood sample and stored at or below −20 °C and shipped on dry ice via overnight delivery to the appropriate laboratory.

The work described was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Our study protocol was reviewed and approved by the WCG Institutional Review Board, an integration of several independent IRBs (WCG IRB, 2022). All participants provided written informed consent at the time of enrollment and were notified of their individual test results.

2.2. PFAS analysis

Serum samples from blood collected at the enrollment visit were analyzed for PFAS concentrations by the 3M Strategic Toxicology Laboratory (St. Paul, MN) using solid phase extraction (SPE) and quantitation by liquid chromatography-tandem mass spectrometry (LC-MS/MS) modified from a previously reported method (Ehresman et al., 2007). Serum was measured for 13 PFAS compounds (PFOS, PFOA, PFHxS, perfluorobutanoic acid [PFBA], perfluorobutanesulfonic acid [PFBS], perfluoropentanoic acid [PFPeA], perfluorohexanoic acid [PFHxA], perfluorohexanoic acid [PFHxA], perfluorononanoic acid [PFNNA], PFDA, 2-(N-methyl-perfluoroctane sulfonamido) acetic acid [MeFOSAA], 2-(N-Ethyl-perfluoroctane sulfonamido) acetic acid [EtFOSAA], and perfluoroctane sulfonamide [PFOSA]). All PFAS were quantified in ng/mL. The lower limit of quantification (LOQ) was 0.1 ng/mL. Further details are provided in the supplementary material, section II.

2.3. Neutralizing and IgG antibody assays

Serum concentrations of antibodies against SARS-CoV-2 were measured in samples from both the enrollment and follow-up visits. Anti-spike IgG and neutralizing antibodies are the most frequently used measures of response to COVID-19 vaccines (Gaebler et al., 2021; Levin et al., 2021; Wei et al., 2021). SARS-CoV-2 anti-spike IgG antibodies were quantitatively measured by immunoassay at Quanterix (Billerica, MA) using the Simoa® SARS-CoV-2 IgG assay (Rissin et al., 2020). IgG antibodies were quantified in ng/mL. The lower limit of detection (LOD) of the assay was 15 ng/mL; the upper LOQ was either 224,000 or 280,000 ng/mL depending on the analytical batch. SARS-CoV-2 neutralizing antibodies were quantitatively measured by Imanis Life Sciences (Rochester, MN) using the IMMUNO-COV v2.0™ assay (Van dergaard et al., 2021). Neutralizing antibody concentration was quantified as virus neutralizing titers (VNTs). The lower LOD of the assay was...
32 VNT; the upper LOQ was 9,600 VNT. Further details on the IMMUNO-COV v2.0™ and Simoa® SARS-CoV-2 IgG assays can be found in the supplementary material, section II.

2.4. Covariates

Information collected as part of the enrollment and follow-up questionnaires included date of birth, employment site, race, ethnicity, gender, weight, height, smoking history, any of 12 conditions being treated by a health care professional (including heart disease, asthma, chronic lung disease, diabetes, chronic kidney disease or renal failure, anemia or other blood disorders, human immunodeficiency virus (HIV), hepatitis, immunodeficiency, lupus, rheumatoid arthritis, or organ transplant), splenectomy, cancer, leukemia, or lymphoma treatment in the past 2 years, and corticosteroid or other immunosuppressant drug use in the past 30 days. In addition, COVID-19 information, including dates, was collected on self-reported diagnosis, household exposure, vaccination history, and treatment, including convalescent plasma or antibodies.

For descriptive purposes age was categorized as <45 years, 45–65 years, and ≥65 years. Employment site was classified as Decatur Chemical/Both (included chemical plant only and those who had worked at both the chemical plant and the film plant), Decatur Film, and Menomonie. Race and ethnicity were grouped into three categories: non-Hispanic Black, non-Hispanic White, and other race/ethnicity. Body mass index (BMI, kg/m²) was categorized as normal weight (<25), overweight (25–29.9), and obese (≥30). Individuals who had smoked fewer than 100 cigarettes in their lifetime were considered never smokers, while those who had smoked at least 100 cigarettes were divided into former smokers, smoke some days, or smoke every day. An immunocompromising condition indicator was positive if HIV, immunodeficiency, organ transplant, splenectomy, or cancer in the past 2 years were present. Individuals who did not report an immunocompromising condition but reported taking a corticosteroid in the past 30 days at enrollment or new use at follow-up were dichotomized as corticosteroid use (yes vs. no).

A COVID-19 diagnosis was assigned if the participant reported having tested positive or if they had COVID-19 symptoms at the same time a household member had tested positive for COVID-19; see supplementary material section III for further information on the operational definition of COVID-19 disease and specific measures used. COVID-19 diagnosis and vaccination information were used to create a categorical variable for 12 antigenic stimulus groups: No COVID-19, no vaccine; No COVID-19, one Pfizer; No COVID-19, one Moderna; No COVID-19, Johnson & Johnson (J & J); No COVID-19, two Pfizer; No COVID-19, two Moderna; Yes COVID-19, no vaccine; Yes COVID-19, one Pfizer; Yes COVID-19, one Moderna; Yes COVID-19, J & J; Yes COVID-19, two Pfizer; and Yes COVID-19, two Moderna. In our classification of antigenic stimulus group, we did not count a vaccination unless the blood draw was at least 4 days after the vaccination date. The antigenic stimulus group a participant was assigned to at enrollment could change over time if they reported a vaccination or COVID-19 diagnosis that occurred between enrollment and follow-up. Note that three of these groups were excluded at the analysis stage, as described below.

2.5. Statistical analyses

Excluded from the analyses were observations from participants who were: unvaccinated, treated with convalescent plasma or antibodies, >180 days since vaccination, in an antigenic stimulus group with small cell sizes (n ≤ 5), or missing data for adjustment covariates. As such, the following antigenic stimulus groups were excluded: No COVID-19, no vaccine; Yes COVID-19, no vaccine; Yes COVID-19, J&J. IgG and neutralizing antibody measures below the lower LOD or above the upper LOD were set to the respective LOD/LOQ. Antibody distributions were normalized using a natural log transformation. PFAS with ≥75% of values above the LOQ were examined in models as continuous variables, while those with >10% but <75% were modeled after dichotomizing as above the LOQ (yes/no). For PFAS measures examined continuously, values below the LOQ were re-expressed as the LOQ divided by the square root of 2.

Counts and percentages were used to describe categorical variables, while median and interquartile range (IQR) were used to describe continuous variables. Distributions of measured PFAS, and Spearman correlation coefficients between PFAS were determined. Distribution of select PFAS concentrations were compared to serum PFAS levels in the 2017–2018 National Health and Nutrition Examination Survey (NHANES), the most recent year with available laboratory data. The NHANES program of studies examines a nationally representative sample of children and adults in the U.S.; the data collected are released annually and are publicly available (About the National Health and Nutrition Examination Survey, 2017). These studies include an interview, examination, and laboratory tests. From the NHANES data we examined PFOS, PFOA, PFHxS, and PFNA serum concentrations in adults aged 20 years of age and older. The recommended sample weights were applied, making the results nationally representative.

Observations from participants who were vaccinated before enrollment, or enrollment and follow-up were included in the main analyses. Associations of each PFAS with IgG and neutralizing antibody concentrations were assessed in mixed regression models using an unstructured covariance structure, accounting for the repeated measures study design. A directed acyclic graph was used to identify the minimal adjustment set of potentially confounding factors (Ankan et al., 2021). The identified set was: age, gender, race/ethnicity, site, BMI, and smoking. We also adjusted for immunocompromising conditions, corticosteroid use, antigenic stimulus group, and time since last antigenic stimulus as precision variables (Schisterman et al., 2009). Figure S1 represents our final model. Akaike information criterion (AIC) were used to identify the best fitting models among versions that included the minimal adjustment set and precision variables. For example, we used AIC to determine the number of categories of smoking needed, whether continuous variables such as age required a quadratic term, whether an interaction term between time since last antigenic stimulus and antigenic stimulus group was needed, and whether there was heterogeneity in antibody-PFAS associations across more than two categories of an effect modifier.

After examination of unadjusted associations (model M1), we fit models that were adjusted for age, gender, BMI, site, smoking, immunocompromising conditions at enrollment, corticosteroid use in the absence of an immunocompromising condition, and time since antigenic stimulus (M2); models adjusted for the above plus antigenic stimulus group (M3); and models adjusted for the above plus an interaction between antigenic stimulus group and time since antigenic stimulus (M4, fully adjusted). Operationalization of covariates in the models was as described in section 2.4 and as shown in Table 1, except age and BMI were continuous. PFBS, MeFOSAA, PFBA, PFHpA, and PFDA were analyzed as dichotomous exposures in a similar set of models. For all analyses, time since last antigenic stimulus was represented with a restricted cubic spline with 3 degrees of freedom. The antibody-PFAS associations were also assessed for non-linearity utilizing a restricted cubic spline with 3 degrees of freedom. All coefficients for the antibody-PFAS association were re-expressed as percent difference in antibody measure per IQR difference in PFAS or per detectable level of PFAS (yes/no). Associations per detectable level of PFAS were also examined. To supplement the main analyses, we fit adjusted ordinary least squares (OLS) models for select subgroups of subjects and observations, such as those receiving two Pfizer vaccinations before enrollment, with antibody concentrations measured at baseline.

Multiple imputation was conducted as a sensitivity analysis. Values for IgG and neutralizing antibodies at the upper LOQ were set to missing, and 10 datasets were created via multiple imputation by chained equations that replaced the missing values with concentrations that
Within the function was selected by the algorithm from a wide variety of possibilities (e.g., polynomial, exponential) based on optimization of the model fit. BKMR was implemented using the fully adjusted (M4) model covariate structure accounting for the repeated measures design with 4 chains and 5,000 iterations per chain. The exposure-response function was characterized as an overall association between the mixture of PFAS and IgG or neutralizing antibody concentrations. Interactions among the four PFAS were also explored.

Analyses were conducted in R version 4.2.0 (Vienna, Austria) using RStudio 2022.02.2–485 (Boston, MA) and Stata 17.0 (College Station, TX). R packages used for these analyses include nime (Pinheiro et al., 2022), mice (Buuren and Groothuis-Oudshoorn, 2011), and bkmr (Bobb, 2017); graphs were created with ggplot2 (Wickham, 2016). See Table S1 for a list of all R packages used.

3. Results

Of the 2,338 employees and retirees invited to participate, 25% enrolled in the study (Figure S2). Exclusion of observations from the analysis was primarily because vaccination had not occurred (n = 299 observations). In total, 757 observations of antibody concentrations from 415 vaccinated participants were used in the mixed effects models of antibody concentrations in relation to PFAS concentration.

Among the 415 participants included in our analysis, the highest proportion were aged 45–64 years, male, White, overweight or obese, from the Decatur facility, and had never smoked (Table 1). Approximately 3.9% of participants reported an immunocompromising condition and 16% (n = 67) reported having had COVID-19. The largest antigen stimulus groups (classified according to most recent antigenic stimulus) were: No COVID-19, two Pfizer vaccinations, and No COVID-19, two Moderna vaccinations. In our analytic sample, 96% (399/415) were vaccinated at enrollment. Of these, 65% (258/399) reported receiving a complete series (two doses of Moderna or Pfizer, or one dose of J&J) and 35% (141/399) received an incomplete series (one dose of Moderna or Pfizer).

The highest median PFAS concentration was for PFOS, followed by PFHxS, PFOA, and PFNA; these four compounds were above the LOQ for >75% of participants (Table 2). Compared with NHANES estimates for the U.S. general population aged ≥ 20 years in 2017–2018 (NHANES 2017–2018 Laboratory Data, 2020), median PFOS was higher in the study participants, and the 95th percentile was much greater (Fig. 1). Median PFOA was slightly higher, though among the 415 study participants the 95th percentile was more than 20 ng/mL larger than NHANES. Median PFHxS was about 1 ng/mL higher, whereas the 95th percentile was more than 20 ng/mL larger than NHANES. Median PFNA was ≥ upper LOQ. Associations between antibody concentrations and continuous PFAS were further explored in retirees of the Decatur plant, who were expected to have the highest PFAS exposure levels. Associations in this subgroup were assessed using fully adjusted models (M4). We also conducted exploratory data analyses of potential effect modification by study visit, age, immunocompromising conditions, corticosteroid use, and body mass index, by adding cross-product terms to model structure M4. Within each interaction tested, cross-product terms with a p-value of < 0.10 indicated effect modification, and results were stratified by the modifier of interest.

Finally, Bayesian kernel machine regression (BKMR) was used to examine the joint association of the four PFAS (PFOS, PFHxS, PFOA, PFNA) with the log IgG or neutralizing antibody concentrations (Bobb et al., 2018). In this approach, the four exposure variables contributed to a single hazard function that represented the relation of the mixture to the outcome. The exact representation of each continuous exposure in the mixture and possible interactions among mixture components were included in the function. BKMR was implemented using the fully adjusted (M4) model covariate structure accounting for the repeated measures design with 4 chains and 5,000 iterations per chain. The exposure-response function was characterized as an overall association between the mixture of PFAS and IgG or neutralizing antibody concentrations. Interactions among the four PFAS were also explored.

Analyses were conducted in R version 4.2.0 (Vienna, Austria) using RStudio 2022.02.2–485 (Boston, MA) and Stata 17.0 (College Station, TX). R packages used for these analyses include nime (Pinheiro et al., 2022), mice (Buuren and Groothuis-Oudshoorn, 2011), and bkmr (Bobb, 2017); graphs were created with ggplot2 (Wickham, 2016). See Table S1 for a list of all R packages used.

### Table 1

| Distribution of perfluorooctane sulfonic acid (PFOS) by participant characteristics (n of participants = 415).  
|---|---|---|---|---|---|---|---|---|
| PFOS Concentration | n | % | 25th | 50th | 75th |
|---|---|---|---|---|---|
| **Age** | | | | | | | | |
| <45 years old | 128 | 30.8 | 2.3 | 4.4 | 7.2 |
| 45 to <65 years old | 203 | 48.9 | 3.6 | 7.7 | 15.4 |
| ≥65 and above | 84 | 20.2 | 11.6 | 26.3 | 70.7 |
| **Gender** | | | | | | | | |
| Male | 302 | 72.8 | 4.5 | 9.0 | 22.8 |
| Female | 113 | 27.2 | 2.0 | 3.9 | 8.0 |
| **Race/Ethnicity** | | | | | | | | |
| Non-Hispanic White | 340 | 81.9 | 3.4 | 7.1 | 16.7 |
| Non-Hispanic Black | 37 | 8.9 | 7.8 | 16.7 | 49.8 |
| Other | 38 | 9.2 | 3.5 | 4.9 | 10.4 |
| **BMI** | | | | | | | | |
| Normal Weight | 92 | 22.2 | 3.0 | 4.8 | 11.1 |
| Overweight | 174 | 41.9 | 4.1 | 8.2 | 19.7 |
| Obese | 149 | 35.9 | 3.5 | 8.2 | 22.3 |
| **Site** | | | | | | | | |
| Decatur Chemical/Both | 154 | 37.1 | 7.5 | 19.8 | 51.2 |
| Decatur Film | 95 | 22.9 | 4.5 | 8.9 | 14.8 |
| Menomonie | 166 | 40.0 | 2.2 | 3.9 | 6.6 |
| **Smoking** | | | | | | | | |
| Never | 288 | 69.4 | 3.6 | 7.0 | 17.2 |
| Former | 106 | 25.5 | 4.3 | 10.3 | 28.4 |
| Some Days | 11 | 2.7 | 2.4 | 3.4 | 7.0 |
| Every Day | 10 | 2.4 | 1.9 | 3.1 | 8.0 |
| **Immunocompromising Conditions** | | | | | | | | |
| Yes | 16 | 3.9 | 3.0 | 8.4 | 27.1 |
| No | 399 | 96.1 | 3.6 | 7.4 | 17.9 |
| **Corticosteroid Use** | | | | | | | | |
| Yes | 19 | 4.6 | 3.0 | 7.7 | 23.9 |
| No | 396 | 95.4 | 3.6 | 7.4 | 17.8 |

*a* Age, gender, race/ethnicity, BMI, site, smoking, and immunocompromising conditions are from enrollment. Corticosteroid use and antigen stimulus group data include enrollment and follow-up.

*b* Decatur Chemical/Both includes individuals who reported working at the Chemical plant and both the Chemical and Film plants.

*c* Corticosteroid use in the past 30 days in the absence of immunocompromising conditions.

### Table 2

| Percentiles of serum concentrations of per- and polyfluoroalkyl substances (PFAS), ng/mL (n of participants = 415). |
|---|---|---|---|---|
| | 5 | 25 | 50 | 75 |
| **PFOS** | 99.5 | 1.20 | 3.53 | 7.46 | 18.00 |
| **PFOA** | 99.0 | 0.38 | 0.95 | 1.63 | 4.54 |
| **PFHxS** | 99.5 | 0.32 | 1.07 | 2.20 | 6.09 |
| **PFNA** | 94.5 | 0.07 | 0.23 | 0.35 | 0.56 |
| **PFBS** | 44.5 | 0.07 | 0.07 | 0.20 | 0.75 |
| **PFDA** | 38.0 | 0.07 | 0.07 | 0.18 | 0.31 |
| **EFOSSA** | 4.1 | 0.07 | 0.07 | 0.15 | 0.22 |

Note: Limit of quantification (LOQ) = 0.1, values < LOQ re-expressed as LOQ/√2 = 0.07.
was roughly the same as in NHANES. Because the distribution of PFOS differed from NHANES the most, we showed distributional information for the subgroups in Table 1. Median PFOS concentration was notably higher in the following subgroups: those aged ≥ 65 years, males, non-Hispanic Blacks, and employed at Decatur Chemical/Both or Decatur Film. Antigenic stimulus groups who had COVID-19 tended to have higher median PFOS levels than those who did not. Among the 415 participants, the Spearman correlation among PFOS, PFOA, and PFHxS were 0.72 or higher; the remaining correlations were generally much lower (Table S2).

Among those who had COVID-19 and two vaccinations, the IgG concentrations were similar for Pfizer and Moderna, and both were higher than those in the No COVID-19 groups (Fig. 2a). Among those without COVID-19, the ranking of antibody concentrations (Moderna > Pfizer > J & J) was as expected (Naranbhai et al., 2021). For neutralizing antibodies, a similar pattern was present (Fig. 2b). The Pearson correlation coefficient between the concentration of IgG and neutralizing antibodies at enrollment was 0.82. Among the observations included in the analysis, the proportion of IgG concentrations below the LOD was 0.3% and the proportion above the LOQ was 16.8%. For neutralizing antibodies, the corresponding percentages were: below LOD, 4.8%; above LOQ, 10.7%.

Results from unadjusted and adjusted models examining the percent difference in antibody concentration per IQR difference in PFOS, PFOA, PFHxS, and PFNA are reported in Table 3. The percent difference in IgG concentration per IQR difference in PFOS was attenuated after adjusting for antigenic stimulus group. In the fully adjusted model (M4) IgG concentration was 3.45 percent lower (95% CI –7.03, 0.26) per 14.5 ng/mL (IQR) increase in PFOS. Results for neutralizing antibody and PFOS were similar. For PFOA, PFHxS, and PFNA, for both IgG and neutralizing antibody, the results were comparable to those for PFOS, though of smaller magnitude. There was no indication of effect modification by study visit, age, corticosteroid use, and BMI (Table S3). Effect modification by immunocompromising condition was identified for PFNA exposure – among participants without immunocompromising conditions, IgG concentration was 3.82 percent lower (95% CI –10.22, 3.04) and neutralizing antibody concentration was 3.92 percent lower (95% CI –9.94, 2.50) per 0.33 ng/mL (IQR) increase in PFNA. Due to the small number of participants with immunocompromising conditions (n = 29), estimates were unstable with wide confidence intervals that included zero (data not shown).

The OLS models fit to select subgroups of subjects all showed results that were consistent with those in Table 3, with wider confidence intervals (Tables S4-S8). When we used multiple imputation to replace antibody concentrations that were above the upper LOQ with draws from the upper tail of a simulated distribution (conditional on covariates), the point estimates became slightly more inverse for IgG, and the width of the confidence intervals were larger (Table 3). The results for neutralizing antibody concentration were not substantially different than those from the fully adjusted model. Models based on data from retirees only gave results less inverse than those in Table 3 (data not shown). For the PFAS that were modeled as dichotomous variables, the fully adjusted results indicated inverse associations for some PFAS, but none had precise confidence intervals (Table S9).

When the relation of antibody concentration to the concentration of PFAS in serum was examined using spline models adjusted for other covariates, the model fit was not improved, and the results were
in detail was considerably affected by adjustment for antigenic stimulus group. Our plan for data analysis was based on an a priori directed acyclic graph and additional adjustment for what might be called a \"precision\" variable, antigenic stimulus group, which is known to have a substantial effect on antibody concentration (Naranbhai et al., 2021), and did so in our data (Table S11). A predictor of the outcome that is not related to the exposure is a precision variable. Adjusting for a precision variable can increase the precision of the estimate of interest between an outcome and an exposure (Schisterman et al., 2009). In our study data, antigenic stimulus group was related to PFOS exposure (Table 1), thus making antigenic stimulus group a confounder. In adjusted models of PFAS (data not shown), the generally higher concentration among those who had COVID-19 was no longer present. A greater proportion of participants from the Decatur facility were diagnosed with COVID-19 (19% vs. 13%), and the vaccination types and administration schedules also varied between Decatur and Memomocie (data not shown).

The few studies examining the occurrence of COVID-19 in relation to PFAS exposure suggested a positive association but had a substantial risk of bias. For example, in a case-control study with PFAS measured in urine obtained after disease onset in cases, some urine PFAS concentrations (e.g., PFOA and PFOS) were higher in cases (Ji et al., 2021). The proximal tubule cells in the kidney are an important site for reabsorption of PFAS such as PFOS and PFOA from the glomerular filtrate, and their apical borders are rich in angiotensin converting enzyme 2 (Monteil et al., 2020; Su et al., 2020), which facilitates entry of SARS-CoV-2 into cells. Patients with COVID-19 have been shown to have renal dysfunction and the higher urine PFAS concentrations among cases in the case-control study may have been due to decreased reabsorption in the nephron (Kormann et al., 2020). In the ecologic studies (Catelan et al., 2021; Nielsen & Joud, 2021), it was not possible to establish that exposure to PFAS was greater among the individuals who had or died from COVID-19; the relative incidence (standardized incidence ratio 1.19; 95% CI 1.12, 1.27) or mortality (relative risk 1.6; 90% CI 0.94, 2.51) of the disease in PFAS-exposed communities may have been due to factors other than PFAS.

A number of studies of antibody response to COVID-19 vaccination in relation to PFAS are underway (Read, 2021; Redfield, 2020). The U.S. Environmental Protection Agency (EPA) has recently issued a draft systematic review of antibiotics other than to COVID-19 for consideration by its Scientific Advisory Board and preliminarily concluded that there was an inverse association for PFOA and PFOS in children (US EPA, 2022). Some of the data on antibody concentrations after vaccination in relation to PFAS in adults have provided support for an inverse association (Kielens et al., 2016; Looker et al., 2014; Pilkerton et al., 2018; Shih et al., 2021; Stein, Ge, et al., 2016; Stein, McGovern et al., 2016); however, limited data exist on any given vaccine type. Large differences in Coronavirus antibody concentrations affect risk of Coronavirus disease (Barrow et al., 1990; Bradburne et al., 1967; Callow, 1985). Although the decreases in antibody concentration associated with exposure to PFOS, PFOA, PFHxS, and PFNA in our data were small, and for most people the exposure to PFOS, PFOA, and PFHxS is much less than in our study group, on a population level the presence of these PFAS may hypothetically result in more disease. However, better studies of COVID-19 occurrence and PFAS are needed to evaluate this possibility. A small inverse association between COVID-19 antibody concentration and PFAS was present for PFOA, PFOA, PFHxS, and PFNA. This consistency could reflect similarities in immunomodulation but given the large differences in the range of the PFAS concentrations it might also indicate the influence of a pharmacokinetic bias or reflect the correlations among the PFAS concentrations. Nonetheless, the most inverse association was observed for PFOS, which had the highest median exposure and range.

Our data showed that vaccine-induced antibody levels after the second Moderna vaccine peaked around 21 days after vaccination, whereas Pfizer-induced antibodies appeared to decline gradually after the second vaccination. Other studies have reported an earlier peak of

### Table 3
Percent difference in antibody concentration per interquartile range difference in per- and polyfluoroalkyl substances (PFAS) and 95% confidence interval by degree of adjustment, mixed effect regression models (n of observations = 757).

| IgG Antibody | Neutralizing Antibody |
|--------------|-----------------------|
| %Δ | 95% CI | %Δ | 95% CI |
| PFOS | | |
| M1 (crude) | -8.45 | -12.82 | -7.33 | -11.36 |
| M2 | -3.87 | -3.11 |
| M3 | -3.31 | -6.98 | 0.50 | -2.96 | -6.35 | 0.55 |
| M4 (fully adjusted) | -3.45 | -7.03 | 0.26 | -3.18 | -6.51 | 0.26 |
| Sensitivity analysis with | -3.49 | -7.19 | 0.37 | -3.14 | -6.48 | 0.32 |
| MI | | |
| PFOS | | |
| M1 (crude) | -5.41 | -8.51 | -2.20 | -4.17 | -7.04 | -1.21 |
| M2 | -4.59 | -7.92 | -1.15 | -3.76 | -6.82 | -0.60 |
| M3 | -5.18 | -4.05 | 0.95 | -1.18 | -3.47 | 1.15 |
| M4 (fully adjusted) | -1.95 | -4.35 | 0.51 | -1.55 | -3.79 | 0.74 |
| Sensitivity analysis with | -1.91 | -4.42 | 0.66 | -1.47 | -3.73 | 0.85 |
| MI | | |
| PFHxS | | |
| M1 (crude) | -4.59 | -7.02 | -2.11 | -3.84 | -6.07 | -1.55 |
| M2 | -3.17 | -5.77 | -0.49 | -2.65 | -5.64 | -0.20 |
| M3 | -1.00 | -2.91 | 0.95 | -1.05 | -2.81 | 0.75 |
| M4 (fully adjusted) | -1.39 | -2.35 | 0.51 | -1.31 | -3.04 | 0.45 |
| Sensitivity analysis with | -1.37 | -3.31 | 0.61 | -1.28 | -3.02 | 0.49 |
| MI | | |
| PFNA | | |
| M1 (crude) | -5.32 | -14.52 | 4.87 | -6.37 | -14.67 | 2.75 |
| M2 | -4.79 | -13.94 | 5.35 | -5.67 | -13.97 | 3.43 |
| M3 | -1.64 | -8.41 | 5.62 | -2.33 | -8.55 | 4.31 |
| M4 (fully adjusted) | -2.46 | -9.04 | 4.60 | -2.38 | -8.51 | 4.16 |
| Sensitivity analysis with | -2.53 | -9.38 | 4.83 | -2.39 | -8.55 | 4.18 |

Note: M1: unadjusted; M2: adjusted for age, gender, race, BMI, site, smoking, immunocompromising conditions at enrollment, corticosteroid use in the past 30 days in the absence of immunocompromising conditions, and time since antigenic stimulus group; M3: M2 + antigenic stimulus group; M4: M3 + interaction between antigenic stimulus group and time since antigenic stimulus; Sensitivity analysis with multiple imputation (MI): M4, sensitivity analysis with imputed values for antibody concentrations above and below the LOD/LOQ (see methods). PFOS IQR = 14.47; PFOA IQR = 3.59; PFHxS IQR = 5.02; PFNA IQR = 0.33.

consistent with a linear relationship (Fig. 3). When PFAS were represented as a doubling (log2(PFAS)) in the models, the results were essentially the same as shown in Table 3 (Table S10).

Fitting the BKMR mixture model to the data for PFOS, PFOA, PFHxS, and PFNA indicated that as the percentiles of joint exposure to the overall mixture increased, the relation to antibody concentration was essentially unaffected for both IgG and neutralizing antibody (Figure S3a, S4a). Furthermore, there was no indication of interaction among the four PFAS (Figure S3b, S4b).

### 4. Discussion

In our study data, the fully adjusted coefficients relating concentration of vaccine-induced antibodies to COVID-19 and IQR difference in serum concentration of PFOS, PFOA, PFHxS, and PFNA were inverse but small with confidence intervals that included zero. The statistical power of a study to detect an association is increased by a large variation in the distribution of the independent variable of interest (Freund et al., 1980). The unusually large spread in the distribution of PFOS, PFOA, and PFHxS meant our study had relatively large power to detect a statistically significant association for these compounds. For the PFAS present at lower concentrations (PFBS, MeFOSAA, PFBA, PFHx, PFDA) a number of the associations were inverse but less precise.

Our analysis showed that the coefficient for the four PFAS examined

\[
\Delta = \frac{\text{log}(\text{antibody concentration})}{\text{IQR of PFAS}}
\]

...
Fig. 3. Restricted cubic spline models of the association between serum antibody concentrations and serum concentration of per- and polyfluoroalkyl substances (PFAS), repeated measures. Data are from 415 participants, 757 observations. a. Predicted IgG antibody concentrations across concentrations of perfluorooctanesulfonic acid (PFOS). b. Predicted neutralizing antibody concentrations across concentrations of PFOS. c. Predicted IgG antibody concentrations across concentrations of perfluorooctanoic acid (PFOA). d. Predicted neutralizing antibody concentrations across concentrations of PFOA. e. Predicted IgG antibody concentrations across concentrations of perfluorohexanesulfonic acid (PFHxS). f. Predicted neutralizing antibody concentrations across concentrations of PFHxS. g. Predicted IgG antibody concentrations across concentrations of perfluorononanoic acid (PFNA). h. Predicted neutralizing antibody concentrations across concentrations of PFNA. Models adjusted for age, gender, race, BMI, site, smoking, immunocompromising conditions at enrollment, corticosteroid use in the past 30 days in the absence of immunocompromising conditions, time since antigenic stimulus, antigenic stimulus group, and interaction between antigenic stimulus group and time since antigenic stimulus. Association between linear PFOS and antibody concentrations not significant in adjusted models (p > 0.05, see Table 3); PFAS spline models fit no better than linear models.
antibody concentrations with Pfizer compared with the Moderna after the second vaccination (e.g., Naranbhai et al., 2021). Also, about 100 days after the second Moderna vaccination the antibody concentrations increased. We note that the confidence intervals were wide at that point, and that a sinusoidal pattern superimposed on the overall time course has been reported by others (see Fig. 3c in Wei et al., 2021). It is possible, however, that the increase was due to an unrecognized case of COVID-19.

As noted above, a strength of this study was the relatively wide distribution of serum concentrations of PFOS, PFOA, and PFHxS, which increased statistical power to detect an association. The use of biomarkers for exposure and outcome reduced the possibility that selection or information bias had important effects on the results. The long half-life of the PFAS we focused on (Li et al., 2018; Olsen et al., 2007; Zhang et al., 2013) meant that exposure during a window of susceptibility, if any, was likely captured by the exposure measure, recognizing that the study addressed adult exposure only. Although we were able to examine antibody concentration within a few months of vaccination, we were not able to address potential longer-term associations with PFAS. The truncation of reported antibody concentrations at the upper LOQ could have attenuated associations. Multiple imputation that replaced the truncated values with those sampled from the upper tail of a distribution, however, indicated that the results were not especially sensitive to specific values in the upper tail. As history of COVID-19 illness and diagnosis was self-reported, unrecognized SARS-CoV-2 infection would have led to incorrect antigenic stimulus group classification; how this misclassification may have influenced the results was unclear. In addition, we thank the 3M workers and retirees who participated in this study.

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Appendix A. Supplementary data

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